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24-27 JUNE

Stockholmsmässan

An Unveiling of Stem Cell Innovation

POSTER ABSTRACT BOOK

ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

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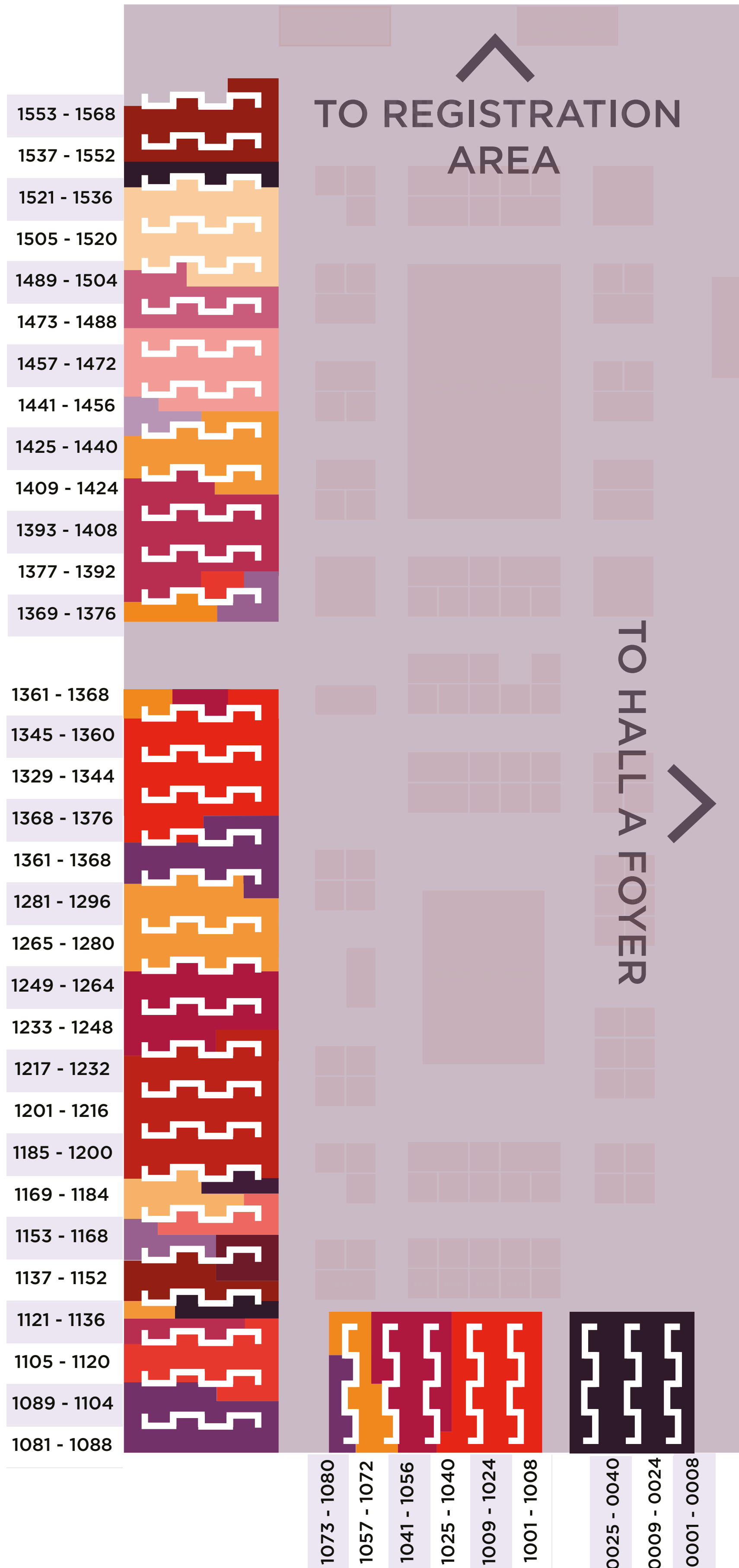

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PRE-CLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL CELLS

W-1001

COMPARISON BETWEEN HEALTHY DONOR-DERIVED BONE MARROW MESENCHYMAL STEM CELLS BM-MSC AND HUMAN DERMAL FIBROBLASTS: IMPACT ON CLINICAL APPLICATIONS OF MSC

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Identification of the differences between these two cell types plays an important role to improve the quality of the therapeutic uses of MSCs. This is the first work to compare the phenotype and functional properties of primary human Dermal fibroblasts HDF to MSC from BM to reflect the impact of the possible contamination of bone marrow derived MSC by fibroblasts. MSC were obtained from the BM of (Healthy donor, n= 5) and compared to primary HDF. Following parameters were used: cell morphology, cell proliferation tests, cell cycle, immunophenotype, pluripotent and differentiation capacity into osteoblastic and adipogenic lineages. Genes expression profile was determined in BM-MSC and HDF by Q-PCR. Results: Both of BM-MSC and HDF cells have homogenous fusiform, fibroblast-like appearance. HDF showed, significantly (p<0.001), high proliferative ability than MSC, which induces a shortening of doubling time (27.3-/+3h vs 44.5-/+ 8h for HDF and BM-MSC, respectively; p<0,0001). The analysis of cell cycle show that HDF cultures contained, significantly p<0.001, two-fold higher cell number at synthesis stage (S) than in BM-MSC cultures. Both cell types share the same immunophenotypic feature for these surfaces markers CD45(-), HLA-DR(-), CD73(+), CD90(+), CD105(+), and CD166(+) but CD146 and CD10 were differently expressed on BM MSC Vs. HDF. Both of cell types have a potential to differentiate into osteoblastic and adipogenic lineages showed by specific stains. That was confirmed by the expression levels of Runx2, PAL and Osterix (osteoblasts); and PPAR-γ2 (adipocytes). However, expression of these genes in osteoblasts and adipocytes derived from HDF remains, significantly p<0.05, two times lower than the expression in osteoblasts and adipocytes obtained in BM-MSC differenced cells. In conclusion, the differentiation potential of BM-MSC can't be used to distinguish between HDF and BM-MSC. The genes expression profile can be used to distinguish between these tow cell types as we found the genes expression in osteoblasts and adipocytes derived from HDF were significantly lower than in BM-MSC. These results may explain the unexpected results of MSC in therapeutic applications. In this current study, new marker was identified to discriminate HDF from BM-MSC in order to improve the quality of the therapeutic uses of MSC.

W-1002

NOVEL SELF-REGULATED VECTOR FOR OPTIMIZATION OF OSTEOGENIC POTENTIAL OF MESENCHYMAL STEM CELLS

Blanca, Vanessa[1], Arroyo, Montserrat1, Abarrategi, Ander[2], García-Castro, Javier[1], **Alfranca, Arantzazu**[1]

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Current therapies for pathologies with extensive defects of bone mass are frequently poorly effective and often have a high economic impact. There is therefore a great interest in the development of therapeutic approaches which overcome these problems. Given their ease of isolation and their ability to undergo osteogenic differentiation, mesenchymal stem cells (MSCs) have been previously employed for this purpose. We have designed a novel tool to optimize osteogenic potential of MSCs, which consists of a lentiviral system which drives self-limited overexpression of osteogenic promoting factor Dlx5. This system comprises a unique bicistronic lentiviral vector, which codes for both Dlx5 and the recombinase Cre, and is flanked by two LoxP sites upon integration into DNA of target cell. The expression of Dlx5 induces osteogenic factor Osterix, which triggers osteogenic differentiation and simultaneously activates the expression of Cre, thus removing provirus from host DNA. MSCs transduced with this lentiviral vector undergo changes in cell morphology and actin cytoskeleton consistent with osteogenic differentiation, and present calcium deposits in vitro as revealed by alizarin red staining. In order to evaluate osteogenic potential of transduced MSCs in vivo, we have included them in ceramic scaffolds placed subcutaneously into mice. MSCs transduced with this vector spontaneously and efficiently form bone tissue in this model, as evidenced by microCT and histological techniques. In summary, we have developed a lentiviral vector with increased biosafety due to its self-regulation design, which efficiently promotes osteogenic differentiation of MSCs in vitro and in vivo. These characteristics make this vector highly suitable for its application in processes which involve bone mass defects.

W-1003

THE VASOPROTECTIVE EFFECT OF EARLY INTRAVENOUS MESENCHYMAL STROMAL CELL DELIVERY AFTER TRAUMATIC SPINAL CORD INJURY

Badner, Anna[1], Vawda, Reaz[2], Mikhail, Mirriam[2], Laliberte, Alex[1], Hong, James[1], Fehlings, Michael[1]

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Vascular disruption is one of the main and immediate consequences of spinal cord injury (SCI) and plays a major role in triggering the

cascade of secondary tissue damage. Early intravenous delivery of mesenchymal stromal cells (MSCs) has been shown to mitigate this progressive secondary vascular pathogenesis and consequently prevent tissue damage in various models of neurologic disease. Yet, the mechanism(s) remain largely unknown. The present study evaluated the vasoprotective effects of early systemic MSCs infusion in a rodent model of traumatic spinal cord injury (SCI). Stromal cells derived from the term human umbilical cord matrix (HUCMCs) as well as human fetal brain (Human Brain Pericytes, HBVPs) reduced vascular permeability (Evans blue assay), hemorrhage (Drabkin's assay) and the lesion volume (quantitative very high resolution ultrasonography) in acute SCI (C7-T1, 35 gram clip injury). Cell infusion (2.5M at 1hour post-SCI) also improved spinal cord vascularity 1 day post-SCI ($p=0.047$ for HUCMCs and $p=0.0178$ for HBVPs), measured by in vivo quantitative Power Doppler ultrasonography. qPCR for the human specific DNA sequence of thymidine kinase showed that only a small proportion of cells are delivered to the spinal cord at 1 day post-SCI (23.96±4.36 human per M rat cells), dissipating at 3 days (13.32±5.29 human per M rat cells) with no detectable human DNA by 7 days. Although the majority of cells were found in the lungs and liver 1 day post-SCI, there was considerable amount of cells (26.44±1.90 human per M rat cells) still in the spleen 7 days post-SCI. In chronic animals (10 weeks post-SCI), both cells types lead to a significant ($p<0.05$) reduction in % lesional tissue (300-600µm rostrocaudal from the epicenter) and significant ($p<0.05$) increase in % white matter (300µm rostrocaudal from the epicenter). This work demonstrates that a less invasive intravenous stromal cell transplantation procedure can effectively reduce secondary vascular damage and lead to chronic tissue preservation in SCI. Despite low cell engraftment in the lesioned spinal cord, the effects may be facilitated through trophic factors secreted from the lungs and/or spleen. This systemic immunomodulatory potential of remotely located cells highlights an exciting therapeutic approach for SCI.

W-1004

TGF-BETA2 DECREASES SURFACE EXPRESSION OF MHC-I ON EQUINE BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS

Berglund, Alix Kay, Schnabel, Lauren V., Long, Julie M., King, Kristin A., Poole, Emma J.

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Bone marrow-derived mesenchymal stromal cells (MSCs) are commonly used for regenerative therapy in the treatment of musculoskeletal disorders. Allogeneic MSCs would allow for immediate treatment of acute injuries, however it has been shown that Major Histocompatibility Complex-I (MHC-1) mismatched MSCs are capable of provoking humoral immune responses in recipients. Previous studies have found transforming growth factor-β2 (TGF-β2) to be present in immunoprivileged locations and to be capable of modulating immune responses. The purpose of this study was to determine if culture of equine MSCs with recombinant human TGF-β2 would decrease MHC-I surface expression. Freshly isolated MSCs were cultured in standard complete media containing 1 ng/ml basic fibroblast growth factor (bFGF), standard complete media containing1 ng/ml bFGF and 5 ng/ml TGF-β2, or complete media with 5 ng/ml TGF-β2 (no bFGF). MSCs from 4 horses were cultured in monolayer for two passages before analysis. MHC-I surface expression for each media group was analyzed by flow cytometry using a monoclonal anti-equine MHC-I antibody. Compared with MSCs cultured with bFGF alone, geometric mean fluorescent intensity decreased by an average of 5.3 fold for MSCs cultured with both bFGF and TGF-β2 and 4.0 fold for MSCs cultured with TGF-β2 alone. MHC-I surface expression was also measured on MSCs from one representative horse using a quantitative cell surface antigen assay. Quantitative MHC-I results from TGF-β2 treated MSCs, dermal fibroblasts, and equine fetal fibroblasts were compared with MSCs cultured with bFGF alone. MSCs cultured in bFGF and TGF-β2 or TGF-β2 alone expressed 2.7 fold lower and 2.38 fold lower MHC-I molecules respectively compared with MSCs cultured with bFGF alone. MSCs cultured with bFGF alone expressed a similar number of MHC-I molecules as dermal fibroblasts, while the number of MHC-I molecules expressed by TGF-β2 treated MSCs were more similar to those expressed by equine fetal fibroblasts, which are considered non-immunogenic. These preliminary results suggest that culture of MSCs with TGF-β2 may shift the immunphenotype of MSCs towards a more immunoprivileged one. Repeated trials with more horses are underway as are tri-lineage differentiation to ensure retention of multipotency of TGF-β2 treated MSCs.

W-1005

ORAL MUCOSAL LAMINA PROPRIA PROGENITOR-CELLS DISPLAY BROAD SPECTRUM ANTIBACTERIAL PROPERTIES VIA THE SECRETION OF OSTEOPROTEGERIN

Board-Davies, Emma[1], Sloan, Alastair[1], Stephens, Phil[1], Davies, Lindsay[2]

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Oral mucosal lamina propria-progenitor cells (OMLP-PCs), a novel population of progenitor cells, demonstrate potent immunosuppressive properties, inhibiting lymphocyte proliferation in a dose and contact-independent manner. This study aims to investigate whether OMLP-PCs additionally possess antibacterial properties. Optimal growth conditions for Gram positive (S.pyogenes: NCTC8198, E.faecalis: NCTC775) and Gram negative (P.aeruginosa: ATCC15692, P.mirabilis: NCTC11938) bacteria were established in RPMI medium supplemented with 0-20% Brain Heart Infusion broth and 10-20% foetal calf serum. Bacteria (150 colony forming units) were cultured for 7-14hrs (established midlog for each individual bacterium) in the presence of OMLP-PCs (25-30 Population Doublings; n=3, 1x105 cells/well) +/- pre-stimulation with interferon γ (IFNγ). The bacterial cultures were spiral plated onto appropriate agar and the number of colonies grown was counted. The antibacterial activity of the conditioned media from the co-cultures was assessed. Media from OMLP-PCs +/- pre-exposure to bacteria were incubated with live bacteria +/- osteoprotegerin (OPG) neutralising antibody for 16hrs. The bacterial cultures were spiral plated onto agar and the number of colonies grown was counted. Genomic and protein levels of OPG were determined using qPCR and ELISA respectively. Bacterial growth was significantly inhibited when co-cultured with OMLP-PCs ($p<0.05$), irrespective of pre-stimulation with IFNγ. Conditioned media from OMLP-PCs significantly decreased the growth of all bacteria irrespective of prior exposure of the cells to bacteria ($p<0.05$). The growth of Gram positive bacteria was partially restored by neutralising OPG. In conclusion, initial data indicates that OMLP-PCs significantly inhibit the growth of bacteria, irrespective of Gram classification. Retention of this antibacterial effect within the conditioned media suggests

soluble factors are mediating this effect. No prior priming of the OMLP-PCs by exposure to bacteria or pre-stimulation with IFNγ was required to exert this antimicrobial effect, demonstrating the constitutive antibacterial nature of these progenitor cells. Neutralising the secreted levels OPG partially restored the growth of Gram positive bacteria.

W-1006

UNRAVELING THE ANGIOGENIC PROPERTIES OF HUMAN DENTAL PULP STEM CELLS IN VITRO AND IN VIVO

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Angiogenesis, the formation of capillaries from pre-existing blood vessels, is a fundamental physiological process in health and disease. Insufficient blood vessel formation is implicated in a wide variety of diseases with devastating consequences such as myocardial infarction and stroke. Within the tooth, precursor cells with mesenchymal stem cell properties can be found, such as dental pulp stem cells (hDPSCs). Since these cells can be easily isolated, cultured and cryopreserved, they may represent an attractive stem cell source for clinical treatments. The purpose of this study is to investigate whether hDPSCs have angiogenic properties and to identify the proteins and molecular pathways involved. The angiogenic secretome of human dental pulp stem cells (hDPSCs) was determined by means of an antibody array and was validated with ELISA and qPCR. Numerous pro- and anti-angiogenic factors such as vascular endothelial growth factor (VEGF), monocyte chemotactic protein-1 (MCP-1), and endostatin were detected. In addition, we studied in vitro the effect of hDPSCs on endothelial cell (HMEC-1) proliferation and migration, key steps of angiogenesis. While hDPSCs were able to significantly HMEC-1 migration in a transwell assay, no effect on HMEC-1 proliferation was observed. Addition of the PI3K-inhibitor LY294002 and the MEK-inhibitor U0126 to the HMEC-1 inhibited this effect, suggesting that both Akt and ERK pathways are involved. Also antibodies against VEGF abolished the chemotactic actions of hDPSCs. Application of only 50,000 hDPSCs for 72 hours onto the chorioallantoic membrane of a chicken embryo, significantly induced blood vessel development. Also in vivo, in the mouse matrigel plug assay, hDPSCs stimulated formation of new blood capillaries. qPCR analysis of matrigel plugs with or without hDPSCs transplanted subcutaneously in mice for 1, 2 or 4 weeks, demonstrated that this angiogenic response was likely to be caused by angiogenic factors (i.e. VEGF) produced by the human stem cells rather than by an upregulation of endogenous mice proteins. In conclusion, hDPSCs are able to induce blood vessel development in a paracrine fashion leading to the conclusion that hDPSCs may be a potent stem cell source for tissue engineering and as a treatment for pathologies correlated with inadequate angiogenesis.

W-1007

CELL THERAPY USING BONE MARROW MESENCHYMAL STEM CELLS (MSC) IN A CANINE MODEL OF CHAGASIC CARDIOMYOPATHY

Campos de Carvalho, Antonio C.[1], Mello, Debora B.[1], Brasil, Guilherme[1], Ramos, Isalira P.[1], Nascimento, Alvaro S.[2], Santos, Danubia S.[1], Cunha, Sandro T.[1], Daliry, Anissa[1], Goldenberg, Regina C.s.[1], Talvani, André[2], Carvalho, Adriana B.[1], Bahia, Maria T.[2]

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We investigated cardiac parameters in Trypanossoma cruzi (T. cruzi)-infected dogs treated with bone marrow mesenchymal stem cells (MSC) in the chronic phase of the disease. Mongrel dogs (4 months age) were infected with 2000 parasites/kg of VL-10 T. cruzi strain. Treatment was performed with 1-6x106 cells/kg of autologous MSC (auto-MSC) or non-infected allogeneic MSC (alo-MSC) from donors with compatible age. Cells were injected in peripheral vein 6-9 months post-infection. Left ventricle ejection fraction (EF%) was assessed before cell treatment, 45 and 90 days post-treatment (dpt). After 180 dpt animals were euthanized and heart samples were collected. Inflammation and fibrosis were evaluated. Transcriptome was performed on RNA from left ventricle samples of Placebo (n=3) and auto-MSC groups (n=4) using microarrays. A -2 to +2 fold change and p<0,05 was considered significant. Placebo-treated animals had a significantly reduced EF% when compared to non-infected animals at all time points analyzed. (Non-infected before: 69.00±3.00; 45 dpt: 70.67±0.577; 90 dpt: 69.67±1.52%, N=3; Placebo before: 49.17±3.54; 45 dpt: 50.33±5,75; 90 dpt: 53.83±9.76%, N=6). Auto-MSC intra-group analysis shows an increase of LVEF in 45 and 90 dpt when comparing the same animals before the treatment (Auto-MSC before: 49.40±10.53; 45 dpt: 66.60±11.67; 90 dpt: 71.00±9.823%, N=5). Values at 45 and 90 dpt were also increased when compared to placebo in the corresponding time points. Interestingly, in alo-MSC group no difference was observed in intra-group analysis or when compared to placebo (alo-MSC before: 60.67±7.47; 45 dpt: 51.83±17.27; 90 dpt: 61.17±13.20%, N=5). Inflammation and fibrosis were present in heart samples in variable degrees. After quantification, using Image-Pro, total heart fibrosis was not different in auto-MSC treated when compared to placebo animals (Placebo 9,013±3,09; auto-MSC 6,510±1,27%, N=3). A total of 27766 genes were analyzed for transcriptome evaluation. We found 8 up-regulated genes and 7 down-regulated genes in transcriptome analysis. We conclude that auto-MSC treatment of chagasic dogs results in an improvement in heart function, but the same is not observed for allogeneic cells.

W-1008

HUMAN ADIPOSE DERIVED PERICYTES INCREASE SURVIVAL IN AN ALS SOD1 MOUSE MODEL BUT ONLY IN AFFECTED MALES

Coatti, Giuliana Castello[1], Frangini, Miriam[1], Gomes, Juliana[1], Valadares, Marcos[1], Assoni, Amanda[1], Pelatti, Mayra[1], De Lima, Antonio Carlos[2], Zatz, Mayana[1]

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Despite contradictory pre-clinical results, therapeutic trials with mesenchymal stromal cells (MSCs) aiming the treatment for Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disease, are currently underway, these cells have a well known paracrine effect, including immunosuppression, neuroprotection and attenuation of oxidative stress which could ammeliorate ALS symptoms. Pericytes represent a more homogeneous cell population, since they are obtained, by FACS sorting enrichment. Besides the secretion of paracrine factors, pericytes have a unique advantage when compared to the other cell types being tested for ALS: they act in the maintenance of the blood brain barrier integrity, an important aspect to avoid the acceleration of symptoms in neurodegenerative diseases. Here we evaluate the therapeutic potential of human MSCs and pericytes derived from the same adipose tissue source in the SOD1 mouse model for ALS (n=19 per group, being 11 females and 8 males). Pericytes and MSCs were weekly injected (one million cells in 100µL in saline solution) intraperitoneally beginning at 8 weeks of age until the mice natural death. Survival of each group (MSC, pericyte or vehicle) was assessed. Since it is known that the progression differs according to gender in SOD1 mice model with a less aggressive and longer survival in females the analyses were performed separately according to gender. No difference on survival was found in the different female groups (p=0,845). Among males no difference was observed between those treated with MSCs or vehicle (p=533). However, male mice treated with pericytes presented a longer survival, comparable to females (p=0,322). Therefore in order to increase the robustness of statistical analysis, all females and males treated with pericytes were assembled in one single group. The difference in survival between this group as compared with males treated with MSCs or vehicle was highly significant (p<0.001).

W-1009

PROSPECTIVE SELECTION OF SURFACE MARKER PROFILES USING SINGLE CELL ANALYTICS TO ENHANCE CELL BASED THERAPIES

Duscher, Dominik[1], Rennert, Robert C.[2], Januszyk, Michael[2], Maan, Zeshaan N.[2], Khong, Sacha[2], Whittam, Alexander J.[2], Hu, Michael S.[2], Walmsley, Graham G.[2], Schmidt, Manfred[3], Huemer, Georg M.[3], Gurtner, Geoffrey G.[2]

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Introduction: Mesenchymal stromal cells derived from adipose tissue (ASCs) have been used clinically to promote wound healing. However, the regenerative capacity of ASCs is impaired in diabetic and aged populations. Exploring cell enrichment strategies to overcome this deficiency, we employed microfluidic single cell transcriptional analysis paired with a novel bioinformatics approach to identify and isolate a subpopulation of ASCs with increased regenerative potential. Methods: Primary ASCs were isolated from human and murine healthy, diabetic and aged adipose tissue, and microfluidic-based single cell transcriptional analysis was employed to characterize the expression of angiogenic, stemness, differentiation and pre-selected surface antigen genes. Clustering analysis was used to identify ASC subpopulations based on transcriptional profiles, and a putatively pro-regenerative subset was prospectively isolated using fluorescence assisted cell sorting (FACS) for assessment of enhanced functionality in vitro and in vivo. Results: We identified a subpopulation of human and murine ASCs characterized by an elevated expression of multiple pro-regenerative genes, which was significantly depleted in ASCs isolated from diabetic and aged samples. Prospective subpopulation isolation using correlative surface markers resulted in prolonged retention of progenitor associated surface antigens, increased cell survival, proliferative capacity and clonogenicity. When applied to an in vivo diabetic wound healing model, this newly defined ASC subpopulation significantly improved healing compared to negatively selected and parent populations, and critically restored normal healing kinetics to diabetic wounds. Conclusion: Functionally distinct ASC subpopulations can be transcriptionally identified and linked to surface marker expression for prospective isolation. Demonstrating the validity of this approach, enrichment of a putatively pro-regenerative ASC subpopulation was found to enhance the regenerative potential of ASC-based therapies in diabetic wounds. Moreover, the depletion of this same functional subset from diabetic and aged ASCs suggests a previously unreported mechanism for the cell dysfunction observed in these settings.

W-1010

OVEREXPRESSION OF MIRNA-92A IN ADIPOSE-DERIVED STROMAL CELLS DOWN-REGULATES THEIR ANGIOGENIC PROPERTIES

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Mesenchymal stem/stromal cells (MSCs) including those from adipose tissue (ADSC) stimulate angiogenesis and regenerative processes in adult tissues. MicroRNAs (miRs), small noncoding RNAs that regulate gene expression by binding to target mRNAs, reducing their stability and/or inhibiting translation, appeared to be important regulators of blood vessel growth. Several miRs, which defined as angio-miRs, were found to be involved in the regulation of angiogenesis and vessel patterning. In this study, we examined the impact of angio-miRs on ADSC angiogenic properties. ADSCs from subcutaneous fat tissue of healthy young donors (n=4) were cultured up to 2-3 passages. ADSC phenotype characterized by flow cytometry was CD90+/CD73+/CD105+/CD45-/CD31- for all samples and these cells were capable of adipogenic and osteogenic differentiation. miR profile in ADSC was analyzed using Illumina microarrays. We found that miR-92a was one of the most abundant angio-miRs (of 17-92 cluster) in all ADSC samples. We transfected ADSC by pre-miR-92a or anti-miR-92a and tested the ability of conditioned medium of transfected cells to stimulate tube formation by HUVECs. ADSC over-expressing miR-92a completely lost the ability to stimulate tubes formation by endothelial cells compared to ADSC transfected by the scramble oligos. However, knocking-out miR-92a by transfection with anti-miR-92a did not increase the ability of ADSC to stimulate tube formation. The expression and secretion of angiogenic factors were analyzed by qRT-PCR and Bio-Plex assay. ADSC transfection by pre-miR-92a or anti-miR-92a led to the coordinated changes of mRNAs level of known miR-92a targets, ITGA5 and MEK4, whereas mRNAs of VEGF, angiogenin and leptin were largely unaffected. Consistently with the observed effect of ADSC conditioned medium on tube formation, the secretion of hepatocyte growth factor (HGF) and angiopoetin-1 was significantly lower in the medium of miR-92a over-expressing cells, however VEGF secretion did not change. We conclude that overexpression of

miR-92a in ADSC suppresses angiogenic properties of these cells by down-regulation of HGF and angiopoetin-1 secretion, therefore miR-92a could be considered as a promising target for the modulation of ADSC angiogenic potential both in vivo and ex vivo.

W-1011

HUMAN FETAL MESENCHYMAL STEM CELLS, THEIR CHARACTERISTICS AND INTERACTIONS WITH ALLOGENEICBLOOD

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Human mesenchymal stem cells (MSC) are currently used in experimental cell therapy. After systemic delivery it has been shown that adult MSC are cleared from the system because of activation of platelets, the complement system and coagulation cascade in the blood (termed Instant Blood Mediated Inflammatory Reaction, IBMIR), but their bioactivity remains by secretion of molecules. The interactions of fetal MSC with blood have not been investigated. MSC were isolated from human 1st trimester fetal livers after legal elective terminations at gestational week 10 after informed consent. The cells were isolated with human pooled AB serum (ABS) or fetal calf serum (FCS), expanded and analyzed at passage 3-4. The ability of MSC to differentiate to adipogenic and osteogenic lineages was determined, and surface marker expression was analyzed by flow cytometry. The MSC were exposed to freshly drawn low anticoagulated human whole blood using a modified Chandler loop system. Human umbilical vein endothelial cells (HUVEC) and NaCl +10 % human plasma served as controls. Blood samples were collected from healthy individuals in an open system by venipuncture. MSC were added to the blood, aliquots were taken before addition of cells and at 5, 15, 30 and 60 minutes for analysis of the MSC effect on the blood status and thrombin-antithrombin complex (TAT) formation. Blood clotting was determined macroscopically. There were no differences in surface marker expression of cells in culture but MSC cultured in FCS differentiated to both adipogenic and osteogenic lineages whereas MSC cultured in ABS did not differentiate into the osteogenic lineage. After addition of MSC the number of free platelets in the blood with MSC cultured in ABS was significantly lower and TAT levels were increased compared to MSC cultured in FCS. There was no reduction in the platelet count and negligible TAT formation with HUVEC or NaCl. Blood clotting was detectable in 7 out of 10 samples with ABS and 3 out of 10 with FCS, while no clotting was detected with HUVEC or NaCl. The count of white blood cells, including lymphocytes and neutrophils, and red blood cells were not affected by addition of any types of cells. In conclusion, fetal MSC cultures expanded in ABS display non-osteogenic differentiation and induce more clotting and TAT formation compared to MSC expanded in FCS.

W-1012

STEERING PROGENITOR CELLS FROM HUMAN ADIPOSE TISSUE ALONG THE CLINICAL HIGHWAY: CLINICAL GRADE MANUFACTURING AND ATMP DEVELOPMENT

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Progenitor cells obtained from human adipose tissue have conquered interest of the scientific and medical communities, as new therapies approach clinical appraisal. On the winding road to human trials, the Stromal Vascular Fraction (SVF) and the subpopulation of stromal/stem cells (ASC) are being tested per se, mostly in inflammatory and/or degenerative conditions, following an autologous approach. The use of self-cells for treatment still raises concerns, such as the need for biopsy which further debilitates patient health, and the risk associated with low quality harvested cells for treatment of specific medical conditions. A pre-validated set of allogeneic cells, qualified for treatment of specific pathologies, would constitute a more efficient therapeutic approach. Thus, a tissue procurement system was established, wherein 31 donor samples were sourced and processed following a proprietary, xeno-free, GMP protocol for isolation of purified SVF and subsequent ASC expansion. Cells underwent thorough quality control, ensuring a platform of cells characterized for viability, immunophenotype and potency. Isolated SVF provided 85.4%±7.1 cell viability, that includes a 12%±5 CD90+CD34+CD73+CD105+ population, while 1.3%±0.8 of cells express these mesenchymal markers excluding CD34 and 15%±6 of the cells express endothelial markers CD45-CD34+CD31+. As the capability to provide validated cells on demand would constitute a valuable asset, a process for xeno-free, GMP cryopreservation of qualified cells was developed and validated regarding maintenance of initial cell characteristics. For proof of concept, certain cells were sub selected for repair of cartilage tissue as one component of an advanced therapy medicinal product (ATMP). In vitro chondrogenesis resulted in a 36x10^4 upregulation of collagen II gene expression, subsequently confirmed by intense immunohistochemical detection of this hyaline matrix component. Regeneration of focal cartilage lesions (4mm diameter) in a rabbit model was significantly improved vs untreated ($p<0.05$), evidenced by O´Driscoll classification. These findings provide intriguing insight regarding the use of adipose progenitor cells as an allogeneic platform to deliver fully validated, conveniently off-the-shelf, pathology-specific stem cell therapy.

W-1013

CULTURE EXPANSION OF UNDIFFERENTIATED HUMAN ENDOMETRIAL MSC USING A SMALL MOLECULE INHIBITOR

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Human endometrial MSC (eMSCs) are a novel source of MSC easily harvested from the highly regenerative lining of the uterus. In

preparation for clinical application, we have developed protocols for eMSC isolation from single cell suspensions using magnetic bead sorting with a perivascular marker, SUSD2 and culture expansion in serum free medium (SFM). Like other MSC, eMSC undergo spontaneous differentiation into fibroblasts during culture expansion decreasing their purity. The aim of this study was to determine if A83-01, a TGFβ-receptor inhibitor with specificity for ALK4/5/7, prevents differentiation of eMSC in culture. SUSD2+ eMSCs were obtained from dissociated endometrial biopsy tissue and cultured in SFM with bFGF and EGF in 5% O2/5%CO2 until passage 5 (P5). P6 cells were incubated with or without A83-01 for 7 days, then analysed for MSC properties. Flow cytometry was used to quantify autofluorescence, examine cell cycle status and assess apoptosis using AnnexinV. A83-01 dose dependently promoted SUSD2+ cell proliferation with maximal effect at 1uM. A83-01 increased the %SUSD2+ cells in P6 cultures (P=0.0016, n=10), with a greater effect on eMSC from older (>40 yr) compared to younger (<40 yr) women. A83-01-treated cells had higher cloning efficiency (p=0.03, n=6), differentiated into mesodermal lineages and expressed MSC phenotypic markers. More A83-01-treated P6 eMSCs were present in the G2/M peak and fewer in the subG0/G1 peak of propidium iodide-stained cells (both p<0.02, n=7) and fewer A83-01-treated cells were expressed AnnexinV (P<0.05 n=6). Fewer A83-01 treated cells were autofluorescent (p=0.001, n=7) or stained with β-galactosidase, senescence markers. These data suggest that A83-01 promotes SUSD2+ cell proliferation and blocks senescence and apoptosis in late passage cultures, with a greater effect on aged eMSC. Decreased SMAD2/3 phosphorylation in A83-01-treated SUSD2+ cells indicates that A83-01 binding to TGFβ receptors blocks downstream signalling leading to apoptosis. Small molecules such as A83-01 that promote eMSC proliferation in the undifferentiated state may provide an approach for the expansion of undifferentiated MSC for use in tissue engineering and cell-based therapies.

W-1014

PREACTIVATION OF HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS WITH TNF-ALPHA AND IL-1-BETA ENHANCES BREAST CANCER SUPPRESSIVE ACTIVITY IN VITRO

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Human umbilical cord mesenchymal stem/stromal cells (hUCMSCs) have been used in cancer therapy for their ability of homing to the tumor site. Although hUCMSCs have antitumor protential, the mechanisms involved in inhibiting cancer cells growth are still unknown. Natural growth environment of hUCMSCs is around the pregnant condition. High levels of cytokines are found during pregnancy, including TNF-α and IL-1β. Previous studies have showed that preactivate human bone marrow derived mesenchymal stem/stromal cells (hBMMSCs) with TNF-α enhances breast tumor-suppressive activity. TNF-α can make hBMMSCs express TNF-related apoptosis-inducing ligand (TRAIL) to suppress breast cancer cells MDA-MB-231 growth. In this study, we found that, unlike the hBMMSCs, hUCMSCs express TRAIL and preactivate hUCMSCs with both TNF-α and IL-1β can upregulate the expression of TRAIL. TNF-α and IL-1β treated hUCMSCs inhibit MDA-MB-231 and MCF-7 breast cancer cells growth. In our study, preactivate with both TNF-α and IL-1β can enhance both TRAIL expression and breast cancer suppressive activity. We suggest that hUCMSCs inhibit breast cancer cells growth by TRAIL signaling pathway.

W-1015

CLINICAL APPLICATION OF HUMAN ADIPOSE DERIVED REGENERATIVE CELLS (ADRCS) IN TISSUE REPAIR OF SEVERE SIDE EFFECTS FOLLOWING CURATIVE RADIOTHERAPY TREATMENT

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Our hospital has the regional responsibility for treating patients with chronic wounds following radiotherapy. The current therapy consists of plastic surgery, and when needed this is combined with hyperbaric oxygen. Recently, it has been shown that injection of ADRCs directly into the chronic wound facilitates tissue healing. The ADRCs, also called the stromal vascular fraction (SVF), from the adipose tissue contains endothelial precursor cells, smooth muscle cells, and adipose derived cells. Although a limited number of patients have been offered this therapy, the results obtained are promising and might be an alternative to the current therapy. ADSCs are easily accessible in large quantities with a minimal invasive, safe and well-established surgical procedure. Normally, one gram of fat contains 300-500 times more stem cells compared to one gram of aspirated bone marrow. ADSCs are an attractive cell source for tissue repair. Recently, we have started to inject SVF cells into the wound area of patients with chronic wounds following curative radiotherapy. SVF cells were isolated from liposuction tissue during surgery using the CelutionTM system, and administered freshly back to the patient. Normally, 200 ml adipose tissue is harvested from the patient prior to SVF isolation. So far, we have treated 3 patients with chronic wounds (two with perineal ulceration and one with intestinal fistulation to the abdominal wall) following curative cancer treatment, including radiotherapy. In the first two patients we observed complete healing of the lesions within 8 weeks, and in the third patient, the intestinal fistula is almost completely healed 6 weeks after injection of SVF cells. In conclusion, our findings show that preparation and injection of SVF cells per-operatively is feasible, and it results in successful healing of chronic wounds.

W-1016

SUPPRESSION OF INTIMAL HYPERPLASIA USING MESENCHYMAL STEM CELLS IN ANIMAL MODEL OF CAROTID BALLOON ANGIOPLASTY

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One of the major cause of the intimal hyperplasia following arterial bypass and balloon angioplasty is restenosis. The aim of this study is to analyze the inhibitory effect of mesenchymal stem cells in animal model of carotid balloon angioplasty. Under the general anesthesia, the rabbit carotid artery was dissected and balloon angioplasty was performed using 2F Fogarty embolectomy catheter. The balloon angioplasty carotid artery was coated with a mixture of 7x106

human cord blood mesenchymal stem cells and fibrin matrix. 2, 4 and 8 weeks after surgery, the carotid artery was harvested and immunofluorescent staining and quantitative real time-PCR were performed. The intimal/media ratio was reduced in the stem cell treated group compare to the non-treated group ($p < 0.05$). The area of re-endothelialization was significantly higher ($p < 0.05$) in the stem treated group than in the non-treated group. Expression of angiogenic genes such as VEGF, PDGF, KDR, Ang-1, and AAMP was increased ($p < 0.05$) in the stem cell treated group relative to the non-treated group. Our study showed that human cord blood mesenchymal stem cells reduce the formation of intimal hyperplasia through the rapid re-endothelialization. This result might be applied to develop stem cell-coated stents, as well as to develop stem cell-contained sheet coat for inhibition of intimal hyperplasia after angioplasty or surgery.

W-1017

ADIPOSE DERIVED STROMAL CELLS CULTURED IN A LOW SERUM ATTENUATED BLEOMYCIN-INDUCED LUNG INJURY WITH LESS COMPLICATION OF THERAPY-RELATED FATAL PULMONARY EMBOLISM

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Adipose derived stromal cells (ASCs) are currently applied to various diseases on clinical trials. Also in our previous animal studies, therapeutic efficacy of cell transfer for inflammatory organ disorders are most impressive for us, in particular, administration of ASCs cultured under low serum condition (LASCs) demonstrated higher potency for Folic acid-induce acute kidney injury and antibody-mediated glomerulonephritis compared to ASCs cultured under the conventional high serum condition (HASCs). Meanwhile, ASC therapy should be carefully considered in clinical application especially for lung injury, because of pulmonary embolism (PE) that is one of a severe complication in cell therapy. In the current study, we investigated both therapeutic potential of LASCs and PE-related deaths in an animal model of acute lung injury. Acute lung injury was induced by transtracheal administration of bleomycin at lethal (5mg/ml) or sub-lethal (2mg/ml) dose in 6 week old C57BL/6 mice. Mouse LASCs, HASCs or PBS as control were intravenously injected at the time of disease induction. Two-weeks mortality and the lung histology on day 7 were evaluated. Pulmonary neutrophil infiltration on day 7 was assessed by esterase tissue stain and cytokine profiles including IL-6, IL-1RA and KGF-1 in lung tissue homogenates were examined by ELISA. For safety evaluation of ASC treatment, PE-related deaths after ASC injection into non-diseased mice were also assessed between LASC and HASC treatment group. A survival rate and a histological change in LASC and HASC groups were significantly attenuated compared with those of PBS control group. ELISA demonstrated reduced pulmonary IL-6 concentration in LASC group. In safety evaluation for PE, all mice were died by rapid intravenous injection of 4.0x106/mouse cells in both experimental groups, but 88.9% and 50.0% survived in LASC and HASC group, respectively. LASC therapy was more potent for acute lung injury and could be used with less complication of PE than HASC. These results suggested LASC have the greater advantage of clinical application than HASC.

W-1018

THERAPEUTIC POTENTIAL OF THE COMPLEX OF MESENCHYMAL STEM CELL CONDITIONED MEDIUM WITH NANODIAMONDS IN THE DOXORUBICIN INDUCED HEART DAMAGE IN RATS

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Cell therapy of patients with a heart damage is a promising way for the treatment of a large number of patients. However, the therapeutic effect in the already completed clinical trials of mesenchymal stem cell (MSC) transplantations does not always satisfy cardiologists. Currently, the therapeutic effect of MSC is referred to their expression of paracrine agents in the damaged regions, which activate regenerative processes, including proliferation of local tissue stem cells. Besides, the condition medium (CM) from the MSC cultures is known to contain a significant amount of paracrine agents, and can have a healing effect on damaged skin and other tissues, when applied locally. Based on these data, we have developed a method for the CM complexation with detonation nanodiamonds, in order to enhance delivery of paracrine agents into the damaged tissues, including the damaged heart tissues. The experiments were carried out on Wistar rats, the heart damage was caused by a doxorubicin administration and the following treatment consisted in either a systemic (intravenous) transplantation of autologous rat MSC or intraperitoneal administration of a suspension of the complex between the CM and detonation nanodiamonds. The control groups were formed from the animals which either were not treated at all (intact control) or were administered a suspension of detonation nanodiamonds alone or the CM alone. The therapeutic effects, estimated by the data of the morphofunctional studies of the cardiac muscle, were approximately equal for the systemic MSC administration and for the CM-detonation nanodiamonds complex administration. Not only the observed effect was significant in comparison with the intact control, but also it noticeably exceeded the effects of the separate CM or detonation nanodiamond administration. Thus, we have developed yet another way of the therapeutic CM application after their complexation with detonation nanodiamonds. Since it is possible to administer such a complex repeatedly for the cardiac muscle damage, we hope that it will result in an enhanced therapeutic effect, which we will study in our further research.

W-1019

IMPACT OF DIABETES ON DERMAL FIBROBLASTS AND KERATINOCYTES: POTENTIAL OF ADIPOSE-DERIVED STEM CELLS IN CELL THERAPY FOR CHRONIC DIABETIC WOUNDS.

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Chronic diabetic wounds are characterized by a specific abnormal inflammatory cell infiltration, a deficiency in several growth

factors, and impairment of angiogenesis, matrix formation, and re-epithelialization. Adipose mesenchymal stem cells (ASC) are then proposed to improve angiogenesis, immunomodulation, and tissue remodelling, but type 2 diabetes state could affect the source of ASC (by chronic inflammation, cell senescence and oxidative stress described in native adipose tissue) and the properties of ASC after implantation in a diabetic wound bed characterized by hypoxia and hyperglycaemia. This study aims to assess, individually or in combination, the in vitro impact of hypoxia (0.1% vs. 5%O2) and hyperglycaemia (4.5mg/L vs. 1mg/L glucose during 24hrs) on ASC (n=8 donors), dermal fibroblasts (DF, n=8 donors) and keratinocytes (Kc, 1 primary lineage) in terms of growth factor release (VEGF, SDF-1α, KGF). Secondly, ASC from diabetic patients were compared to non-diabetic ASC for cell survival and growth factor release in hypoxia and hyperglycaemia. ASC and DF survival rates were not affected by these conditions. The hypoxia (0.1%O2) alone induced a significant increase of VEGF secretion for ASC (+137%, p<0.005) and DF (+228%, p<0.001) in comparison to 5%O2; while no impact of hypoxia on SDF-1α and KGF release was observed. The hyperglycaemia alone induced a significant reduction of DF secretion for VEGF (-56% of secretion in normoglycaemia, p<0.001) and SDF-1α (-93% of secretion in normoglycaemia, p<0.001). When hypoxia was combined to hyperglycaemia (to mimic the conditions of diabetic wounds), DF was significantly affected, by a reduction of SDF-1α (-93% of secretion in 5%O2 and 1mg/L glucose, p<0.001) and KGF (-20% of secretion in 5%O2 and 1mg/L glucose, p<0.05). Interestingly, these diabetic conditions significantly improved VEGF secretion by ASC (+64% of secretion in non-diabetic conditions, p<0.05). DF are highly sensitive in vitro to hypoxia combined with hyperglycaemia, while ASC can survive and function in the hostile environment of chronic diabetic wounds. Based on these properties, one type 2 diabetic patient with severe chronic wounds was treated with a biological dressing made of autologous ASC, and followed for clinical and histological evolutions.

W-1020

INDUCTION OF ANGIOGENESIS BY EXOSOMES DERIVED FROM CLONAL HUMAN EMBRYONIC PROGENITOR CELL LINES

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Pluripotent stem cells are a promising source of cells for tissue repair and regeneration because of their capacity to self-renew as well as differentiate to virtually any cell type. We have developed hundreds of clonal and scalable hESC derived cell lines for rapid scalable production of therapeutic cells on an industrial scale. In recent years, much of the therapeutic effect of transplanted adult stem cells, including cardiosphere derived cells (CDCs), mesenchymal stromal cells (MSCs) and endothelial progenitor cells (EPCs), has been attributed to paracrine effects. These paracrine signaling factors may be particularly effective for treatment of ischemic conditions such as heart disease, peripheral artery disease, stroke and wound healing. Indeed, stem cell secreted vesicles, known as exosomes, have been used successfully in place of the stem cells that secrete them in several animal models of angiogenesis and ischemic tissue repair. We therefore reasoned that clonal hESC derived vascular progenitor cell lines might serve as a scalable and highly pure source of angiogenic exosomes for regenerating damaged tissue following ischemia. We screened exosomes from endothelial, smooth muscle, and pericyte progenitor cell lines and identified several cell lines that produced angiogenic exosomes as determined by endothelial tube formation on Matrigel. One endothelial progenitor line, 30-MV2-6, secreted exosomes that were at least 3 times more potent in the tube forming assay than exosomes from MSCs. We have performed pilot studies demonstrating scalable production of highly pure angiogenic exosomes from the 30-MV2-6 cell line in the GMP compatible Quantum® cell expansion system. We are currently testing clonal progenitor derived exosomes in animal models to identify a preclinical developmental candidate for treatment of peripheral artery disease and other ischemic conditions.

W-1021

MESENCHYMAL STROMAL CELLS GENERATED FROM HUMAN CD271+ BONE MARROW MONONUCLEAR CELLS ARE BETTER "WOUND HEALERS" THAN MESENCHYMAL STROMAL CELLS GENERATED WITH PLASTIC ADHERENCE

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Abundant evidence suggest that Mesenchyl Stromal Cells (MSCs), isolated from different tissue sources, confer benefits in vivo as tissue restorative agents. The goal of this in vitro study was to compare wound healing capacity of MSCs generated from positively selected CD271+ bone marrow mononuclear cells (CD271-MSCs) and MSCs generated by plastic adherence (PA-MSCs). We investigated healing capacity of CD271-MSCs and PA-MSCs of passage 2 and 4 cultured in the presence or absence (control) of growth factors (GF)/cytokines. For that purpose we used an in vitro model of wound healing (CytoSelect™ 24-Well Wound Healing Assay) from the Cell Biolabs company (BioCat GmbH, Heidelberg, Germany). After generation of a "wounded field", the cells were monitored for migration and proliferation into the wounded area and the photographs were taken at 0h, 6h, 12h and 24h. Comparing healing capacity of different passages of CD271-MSCs and PA-MSCs cultured in medium without GF/cytokines, our data indicated that both, CD271-MSCs and PA-MSCs, of passage 4 posses a significantly higher wound healing potential than MSCs of passage 2. CD271-MSCs of both passages compared to PA-MSCs demonstrated significantly higher potential to close the wound 12h and 24h after initiation of the wound healing assay (P=0.03 and P=0.02, respectively). When compared the effect of GF/cytokines on wound healing capacity of both types of MSCs of passage 2, the migration capacity of PA-MSCs after 12h was significantly better compared to control when the cells were stimulated by FGF-2 (P= 0.020), PDGF-BB (P= 0.006), MCP-1 (P= 0.001) and IL-6 (P= 0.033). The PA-MSCs of fourth passage 12h after the treatment with GF/cytokines, showed significant difference compared to control only when the cells were treated with TGF-β (P=0.021). In contrast, compared to control, CD271-MSCs of both passages after 12h and 24h of treatment with GF/cytokines didn't show any significant enhancement in migration capacity. In conclusion, we state that CD271- MSCs of both passages were better in wound healing than PA-MSCs when the cells were untreted with GF/cytokine. In contrast, whereas several growth factors significantly enhance wound healing capacity of PA-MSCs, they do not affect healing capacity of

CD271-MSCs.

W-1022

CHALLENGE OF REGULATING AUTOLOGOUS STEM CELL 'THERAPIES': ANALYSIS OF AUSTRALIAN STAKEHOLDER PERSPECTIVES

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In recent years there has been substantial growth in the number of clinics and companies claiming to offer 'stem cell'- based 'treatments' that make use of each patient's own cells. Proponents of these 'therapies' argue that i) clinical use of such cells or their derivatives represents an extension of medical practice, and ii) that the simplicity and apparent safety of these approaches should enable patients to make their own treatment choices as health consumers. In contrast opponents of the unregulated (and largely scientifically unsupported) autologous 'therapies' are calling for more stringent regulation to address concerns about the safety, lack of efficacy and high cost of these unproven interventions. Caught in between these competing perspectives are patients who, having been exposed to the promise of stem cell science, feel like stem cell therapies are more advanced than they actually are. In Australia the national regulator of medicines and medical devices, the Therapeutic Goods Administration, has launched a public consultation process for input on how to best regulate autologous cell therapies to ensure the inherent risks and opportunities of these technologies are addressed. This paper will analyse public submissions from a diverse range of stakeholders including physicians, commercial bodies, academic stem cell researchers and patients themselves. Our analysis will provide a unique insight into the various positions held on how best to enable clinical stem cell advances while ensuring patient protection and scientific validity, and will more clearly identify the tensions between different stakeholder groups within the Australian health industry. Importantly, the paper will seek to explore the consequence for those seeking treatment in terms of risk if a 'no change' position is adopted, or the question of accessibility if more stringent regulations are introduced. Given that the use of any autologous cell therapy in Australia (no matter the source or its how it is prepared) is broadly excluded from regulatory oversight, our analysis will provide timely and valuable insights into an important policy initiative that has far-reaching implications for clinical translation of stem cell research in Australia.

W-1023

EFFICACY OF STEMPEUCEL®, AN ALLOGENEIC POOLED HUMAN MESENCHYMAL STROMAL CELLS, IN MULTIPLE PRECLINICAL MODELS OF HUMAN DISEASES WITH DIVERSE PATHOPHYSIOLOGY

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Bone marrow derived mesenchymal stromal/stem cells (BMMSC) have been in the forefront of basic and translational research to assess their role in disease modifying processes. BMMSC are known to possess strong immunomodulatotory and anti-inflammatory properties, and in addition, promote angiogenesis and tissue regeneration through the secretion of trophic factors. The ability of BMMSC to affect the functional properties of virtually all types of immune cells by paracrine activity and via cell surface interactions has broadened the scope of using these cells in allogeneic transplantation. However, pre-clinical and clinical studies have shown that the survival period of these cells is limited; spanning from several days to a few weeks. Therefore, it is critical that these cells must be highly potent to elicit a therapeutic effect in a short period of time. Several reports have indicated that BMMSC obtained from different donors differ considerably in regards to expansion potential, immunomodulatory activity and secretome profile, which could impact their large scale expansion and overall biological function. To overcome this problem, we hypothesized that pooling of BMMSC from healthy donors may compensate for potential deficiencies and a pooled BMMSC product could be more potent in comparison to cells derived from a single donor. Stempeucel® is an allogeneic product, manufactured under GMP conditions, comprised of BMMSC pooled from three healthy volunteers. Stempeucel® has been tested in three different disease models in rodents: limb ischemia, osteoarthritis and liver cirrhosis. In order to maximize the therapeutic effect of stempeucel®, appropriate route of administration was used to achieve efficient homing to the injury site. Our findings show that stempeucel® is capable of reducing pain significantly ($p<0.001$) and promotes knee cartilage proteoglycan synthesis in rats, significantly reduces limb necrosis ($p<0.001$) and, improves muscle regeneration ($p<0.001$) and blood flow ($p<0.01$) in the ischemic limb of mice and significantly reduces liver fibrosis ($p<0.05$) and improves liver architecture in rats. Collectively, the study suggests that optimization of MSC technology by pooling several BMMSC samples can significantly enhance the curative potential of these cells against multiple indications.

W-1024

TRANSPLANTATION OF MESENCHYMAL STROMAL CELLS ALLEVIATES MOTOR IMPAIRMENTS AND NEUROPATHOLOGY OF A MOUSE MODEL OF MACHADO-JOSEPH DISEASE

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Machado-Joseph disease (MJD) or Spinocerebellar ataxia type 3 (SCA-3) is the most common SCA worldwide, caused by an expanded CAG repeat in the MJD1 gene, which translates into a polyQ tract within the ataxin-3 protein. Currently, there is no therapy able to modify or delay disease progression. Mesenchymal stromal cells (MSC) are very promising tools for therapy of neurodegenerative disorders and have recently received considerable interest with respect to SCAs. Aligned with these results, clinical trials with MSC are already running and MSC have been reporting to be safe and to delay disease progression in some SCAs, including MJD. However recent studies reported that some patients had regressed to the status prior to the treatment. Preclinical studies in MJD are thus imperative to achieve better outcomes in clinical trials and to assess considerations that are not possible to assess/study in humans. In the present study, we investigated whether stereotaxic transplantation of MSC into the cerebellum (region particularly affected in this model) would induce a neuroprotective effect, rescueing the extremely severe phenotype

of a MJD transgenic mouse model. We found that MSC engrafted in lobules II and III of the cerebellum. Mice treated with MSC showed better performance in both constant and accelerated rotarod as well as in the swimming test, as compared with non-treated mice, at 4 weeks post-transplant. Furthermore, MSC transplantation mitigated the neuropathology associated with MJD motor function impairments, as at 12 weeks after transplantation mice transplanted with MSC revealed a conservation of the molecular layer thickness between lobules II and III, and between lobules III and IV, a tendency for preservation of the granular layer between lobules III and IV and a significant increase of the optical densitometry of calbindin immunoreactivity in lobules II and III, indicating a greater preservation of the Purkinje cells (which are affected in our model) when compared with non-treated mice. Presently we are addressing less invasive routes for MSC administration, which have shown very promising results in preliminary studies. As conclusion, the present study provides evidence that MSC transplantation can alleviate MJD and become an effective candidate for disease-modifying MJD therapies, so far inexistent.

W-1025

TISSUE-ENGINEERED BONE GRAFTS FOR MAXILLOFACIAL SURGERY: FROM BENCH TO BEDSIDE

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Novel effective bone grafts for maxillofacial bone defects reconstruction are in high demand. In our research were used scaffolds of synthetic tricalcium phosphate (TCP) and autologous cells (gingiva derived multipotent mesenchymal stromal cells, MMSC; adipose tissue stromal vascular fraction, SVF) to make different variants of tissue-engineered constructions: TCP+MMSC, TCP+MMSC+fibrin gel and TCP+SVF+fibrin gel. All constructions were tested in vitro to define scaffolds' biocompatibility, cells survival and morphofunctional properties. For in vivo study we used rabbit model of parietal bone defects. Two identical symmetric full-thickness defects 10mm in diameter were made in each of 24 animals. Defects on one side were filled with investigated materials and on opposite side - with same carriers without autologous cells. Animals were sacrificed at 30, 60, 90 and 120 days of experiment. Neither adverse events nor complications have been detected. Results were assessed by microfocus X-ray, computed tomography and histological analysis. Activation of reparative osteogenesis was observed in all experimental groups in comparison to control. These data allowed us to get clinical trial approval (NCT02209311). In this trial tissue-engineered constructions TCP+MMSC transplanted as bone grafts for maxilla alveolar process augmentation as the first stage of dental implants treatment plan. To the moment 4 patients have been enrolled. Neither adverse events nor complications have been detected in the clinical study. In all patients postoperative period was uneventful. According to CT-scan data, 3-6 months after surgery heteromorphic regenerate intimately adjacent to surrounding bone tissue was observed at the site of tissue-engineered bone graft transplantation. Density of regenerate slightly exceeded the parameter of intact trabecular bone. Newly-formed bone tissue was found among the grafts fragments in biopsies. Clinical trial is ongoing. The authors declare no conflict of interest. Part of work related to gingival MMSC obtainment and research was supported by Russian Scientific Foundation (grant #14-25-00166).

W-1026

NEONATAL TRANSPLANTATION OF HUMAN AMNIOTIC FLUID STEM CELLS IMPROVES BONE QUALITY IN A MOUSE MODEL OF OSTEOGENESIS IMPERFECTA

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Osteogenesis Imperfecta (OI) is a congenital bone disorder with prenatal onset affecting 1:10,000 babies. It manifests by brittle bones and no cure is available. Homozygous oim mice carry a mutation in the collagen type I alpha 2 gene. The absence of the α2(I) protein causes collagen type I to be formed of three α1 chains, replacing the normal heterotrimeric Col1(α1)2(α2)1. In addition, most oim pre-osteoblasts fail to differentiate into mature osteoblasts and produce small apatite crystals. As a result, oim mice have brittle bones. Perinatal cell therapy for OI is predicated on the ability of mesenchymal stem cells (MSC) to differentiate into osteoblasts. Human fetal MSC can be isolated from mid-gestation amniotic fluid (AFSC). We injected AFSC in oim neonates (AFSC-oim) and analysed the mice 8 weeks later. Fracture incidence in AFSC-oim (n=28) was lower that in age-matched oim (n=26) in femurs, i.e. 3.6% vs. 33%; and in tibias, i.e. 3.6% vs. 17%. Microcomputed tomography of the tibia (n=9) showed that transplantation did not affect trabecular spacing and number, but trabecular bone pattern factor (TBPF) was 19.9% lower in AFSC-oim (42.79 ± 2.88 mm-1), compared to oim mice (52.80 ± 2.60 mm-1, P<0.01), indicating a better architectural trabecular organisation. Transplanted femurs were stronger, i.e. bending stiffness 26.11 ± 1.39 vs. 42.97 ± 1.68 N/mm, P<0.001; ultimate load 4.64 ± 0.30 vs. 7.51 ± 0.39 N, P<0.001; and yield load 3.03 ± 0.22 vs. 5.22 ± 0.30 N, P<0.001. Total work to fracture was higher in transplanted bones (1.74 ± 0.16 J vs. 0.72 ± 0.07, P<0.0001), indicating that AFSC injection improved overall bone quality. Similarly, work from yield to fracture was higher in AFSC-oim, i.e. 1.49 ± 0.13 vs. 0.49 ± 0.06 J, P<0.0001; reflecting an increase in bone plasticity. Raman spectroscopy revealed that AFSC-oim had higher mineral content, with more mature crystals. AFSC engrafted in bones and differentiated into mature osteoblasts, albeit chimerism remained low, improving ECM quality. This was accompanied by up-regulation of endogenous murine expression of genes involved in ossification, skeletal development and ECM production. We address the manner in which AFSC promote the maturation of resident pre-osteoblasts, either directly or indirectly, to improve mineralisation of the chimeric ECM and bone quality.

W-1027

AGING INFLUENCES THE MESENCHYMAL STEM CELL-DERIVED OLIGODENDROGENIC AND REMYELINATION ACTIVITIES: CONSEQUENCES FOR MULTIPLE SCLEROSIS

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Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) involving oligodendrocyte and myelin loss leading to severe neurological deficits. Mesenchymal stem cells (MSCs) display immunomodulatory and neuroprotective activities and promote oligodendrocyte differentiation of CNS progenitors and are thus interesting candidates for autologous cell therapy in MS. MS frequently has an onset in young adults, and thereafter progresses throughout adult life. It is therefore important to know whether MSCs retain their pro-oligodendrogenic activity with increasing age, especially given the age-related decline in CNS remyelination efficiency. To address this question we isolated neural stem / progenitor cells (NSPCs) and MSCs from young (2 months old) as well as old (17-20 months old) rats and expanded them in vitro. NSPCs were exposed to MSCs-derived conditioned medium (MSC-CM) and oligodendrogenesis was evaluated by immunofluorescence and luciferase assay. We observed that soluble factors derived from aged MSCs display a reduced oligodendrogenic activity on NSPCs. Also, NSPCs obtained from aged donors have a lower oligodendrogenic respond to soluble factors derived from MSCs. Thus, aging decreases both, the MSCs derived oligodendrogenic activity as well as the oligodendrogenic potential of NSPCs. As oligodendroglial progenitor cells (OPCs) represent the main cellular source for myelin repair, we exposed OPCs to young and old MSC-CM. In consistent with the previous findings, we showed that age decreased the MSCs oligodendrogenic effect on OPCs. Finally, as expected only young MSC-CM enhances endogenous remyelination in lysolecithin-induced demyelinated cerebellar slices, while soluble factors derived from old MSCs fail to induce such as increase. In summary, aging reduces the MSC-derived oligodendrogenic activity affecting their remyelination potential. Thus, autologous MSCs are likely to gradually loose pro-regenerative properties throughout disease duration and, therefore, MSC-based cell therapy has to be reconsidered for MS treatment.

W-1028

HETEROGENOUS EXPRESSION OF IMMUNOMODULATORY MOLECULES BY ACTIVATED HUMAN BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS

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Mesenchymal stromal cells (MSCs) possess broad immunoregulatory properties and are being clinically investigated to treat various immune-based disorders. It is widely believed that the immunomodulatory capacity of MSCs is acquired upon encountering an inflammatory milieu in the patient. Here we hypothesize that the response to proinflammatory stimuli may vary between MSC populations derived from different donors and also between different subpopulations within an MSC preparation. To address this hypothesis we determined the expression of various immunomodulatory factors by qRT PCR and flow cytometric analysis in human bone marrow-derived MSC preparations upon exposure to various inflammatory stimuli. TNFα, IFNγ, IL-1β, LPS and PolyIC all induced PD-L2 protein and IDO mRNA expression, where IFNγ was the strongest inducer. In contrast, IL1β was the strongest inducer of Cox-2 and TSG-6 mRNAs, which were both hardly affected by IFNγ stimulation. Furthermore, polyIC was the strongest inducer of PD-L1, ICAM-1 and VCAM-1 protein expression. Overall LPS was the weakest inducer of the various immunomodulatory factors. Although MSCs isolated from various donors showed the same response pattern, the magnitude of the response was donor dependent. In addition, flow cytometric analysis revealed differential upregulation of surface markers within an MSC preparation. Together our data demonstrate that the response of MSC preparations to a proinflammatory environment depends on the specific proinflammatory mediators encountered, on donor origin and even varied between cells within an MSC preparation. Importantly, the finding that MSCs display phenotypic and functional heterogeneity upon activation could be exploited for the development of MSC products with enhanced immunomodulatory capacity.

W-1029

AVOIDING LOWER EXTREMITY AMPUTATION: CELL THERAPY OF CRITICAL ISCHAEMIA OF THE LIMBS (CIL). DESIGN AND INTERIN RESULTS OF FOUR CLINICAL TRIALS.

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Lower extremity amputation (LEA) is a major complication of diabetes mellitus resulting in a poor quality of life and high mortality. Remarkably, USA has the higher incidence in the OECD countries. Since intra-arterial infusion of bone marrow mononuclear cells (BM-MNCs) have been successfully used for the treatment of critical limb ischemia (CIL) in other pathologies we decided to conduct a pilot study (Phase I-IIa) on non re-vascularizable CIL in diabetic patients (NCT-00872326). The fact that in people with diabetes the affected vessels are disperse and of smaller diameter exclude them

form conventional revascularization techniques (shunts, stents, etc) and most frequently LEA is the only therapeutical alternative. Even more, HbA1C > 7% was one of the exclusion criteria in previous cell therapy trials. Subsequently three open, randomized and controlled phase I/II clinical trials under identical conditions were designed to evaluate the safety and the feasibility of intra-arterial clinical application of: i) 2 doses (0.5×106 cells/kg and 1×106 cells/kg of patient weight) of autologous adipose-derived mesenchymal stromal cells (aMSCs) in 30 type 2 diabetic patients with CIL (NCT01257776); ii) 2 doses (0.5×106 cells/kg and 1×106 cells/kg of patient weight) of autologous aMSCs in 30 non-diabetic patients (NCT01745744) and iii) a unique infusion of: 150-250 ×106 of autologous BM-MNCs or 2-7×106 of autologous CD133+ cells or 0.5×106/kg of autologous aMSCs to study insulin resistance, the decrease on insulin needs, and to evaluate the safety, viability and efficiency of the intra-arterial infusion of stem cells in 48 diabetic patients type 2 with CLI (NCT02287974). Time course of clinical effects differed among patients and among each study, but after 12 months of follow-up all patients presented a notable improvement in the Rutherford-Becker classification, the University of Texas diabetic wound scales, and the Ankle-Brachial Index. The clinical outcome was consistent with neovasculogenesis (assessed by digital subtraction angiography).

W-1030

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

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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 intratumorally 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 vitro and 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. Flow cytometry was used to analyze both intracellular and extracellular marker expression and ELISA was used to assess the production of immune modulatory factors. For the survival study, γ-irradiated MSCs (0/2/5/10/15/20 Gy) were injected intratumorally in tumor-bearing mice at day 7 and 17. Our results show that γ-irradiated mouse MSCs (2 Gy) co-cultured with mouse glioma cells affect tumor growth in vitro compared to non-irradiated MSCs. Further analysis reveals that these cells slightly reduce the production of the immune suppressive factors PGE2 and TGFβ1 compared to non-irradiated. In vivo, intratumorally transplanted MSCs enhance the cure rate of GL261 tumor-bearing mice. Groups were compared to the tumor-bearing control using Log-rank test and survival was increased in animals receiving MSCs irradiated with 5 Gy (28.6% cure rate), 10 and 15 Gy (16.7% cure rate) and 2 Gy (14.3% cure rate). No statistical difference was detected when MSCs were irradiated with 20 Gy or non-irradiated (0% cure rate). Our results suggest that low-dose γ-irradiated MSCs affect tumor growth when co-cultured with brain tumor cells and increase the survival of tumor-bearing mice. Low-dose γ-irradiated MSCs may represent a favorable alternative approach in cancer therapy. This strategy is straightforward and can be easily combined with other therapies such as immunotherapy.

W-1031

TRANSPLANTATION OF PLACENTAL ADHERENT MULTIPOTENT CELLS DOES NOT AFFECT MID/LATE TUMOR PROGRESSION IN DIMETHYLHYDRAZINE-INDUCED COLON CARCINOGENESIS IN RATS

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Colorectal cancer remains one of the most common type of cancer. Therefore, finding of new approaches for colon cancer treatment remains an actual problem, since the diagnoses are not discovered in the early stages. Recently, the placenta-derived cells have become a focus of interest as a potential source for cancer treatment, because it has been concluded that the placenta is not a favorable niche for cancer cells. The purpose of study was to determine the effects of intravenous rat placental adherent multipotent cells (PAMC) transplantation into rats with dimethylhydrazine (DMH)-induced colorectal mid/late-stage tumors. Experimental colon carcinogenesis was induced in male albino Wistar rats by injecting DMH at 20 mg/kg of body weight (b wt) once a week for 20 consecutive weeks. The administration of PAMC was performed when each rat exhibited at least one adenocarcinoma. We demonstrate the placenta-derived cell cultures combined unique feathers of both mesenchymal and trophoblast stem cells. Rat PAMC, like mesenchymal stem cells, expressed CD90, CD29, CD44, were negative for the hematopoietic marker CD45, and had the capacity to differentiate into adipogenic and osteogenic lineages. In contrast, PAMC could potentially belong to trophoblast progenitors due to their expression of CDX2 and ID2 and lack of expression of TPBPA, PRL3B, NANOG. In first pilot study the number and size of colon lesions decreased, but not significantly. However, it was observed the correlation between dose of injected cells and the number and size of tissue lesions (r=-0.691 for the number of lesions [p=0.013] and r=-0.794 [p=0.002] for the size of tissue lesions, n=12). We assume that transplantation of more than 1.2×106 rPAMCs/kg b wt could halt subsequent tumor progression. In second study the dose of injected cells was average 2.2×106 PAMCs/kg b wt (n=30), but no affect on tumor growth were observed. In addition, the percentage of aberrant crypt foci (first step in malignant changes) among all lesions per rat decreased and percentage of tumors increased after PAMC administration, what might suggest about stimulation of carcinogenesis. Furthermore, the survival rate of PAMC-treated group was lower than in control group. This study identified that PAMC did not effect on mid/late

tumor growth.

W-1032

PURIFICATION OF HUMAN ADIPOSE-DERIVED STEM CELLS FROM FAT TISSUES USING PLGA/SILK SCREEN HYBRID MEMBRANES

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The purification of human adipose-derived stem cells (hADSCs) from human adipose tissue cells (stromal vascular fraction) was investigated using membrane filtration through poly(lactide-co-glycolic acid)/silk screen hybrid membranes. Membrane filtration methods are attractive in regenerative medicine because they reduce the time required to purify hADSCs (i.e., less than 30 min) compared with conventional culture methods, which require 5-12 days. hADSCs expressing the mesenchymal stem cell markers CD44, CD73, and CD90 were concentrated in the permeation solution from the hybrid membranes. Expression of the surface markers CD44, CD73, and CD90 on the cells in the permeation solution from the hybrid membranes, which were obtained using 18 mL of feed solution containing 1 x10^6 cells, was statistically significantly higher than that of the primary adipose tissue cells, indicating that the hADSCs can be purified in the permeation solution by the membrane filtration method. Cells expressing the stem cell-associated marker CD34 could be successfully isolated in the permeation solution, whereas CD34+ cells could not be purified by the conventional culture method. The hADSCs in the permeation solution demonstrated a superior capacity for osteogenic differentiation based on their alkali phosphatase activity, their osterix gene expression, and the results of mineralization analysis by Alizarin Red S and von Kossa staining compared with the cells from the suspension of human adipose tissue. These results suggest that the hADSCs capable of osteogenic differentiation preferentially permeate through the hybrid membranes.

W-1033

EX-VIVO INDUCED REGULATORY HUMAN/MURINE MESENCHYMAL STEMCELLS AS IMMUNE MODULATORS.

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Over the past decade there has been a growing interest in utilizing mesenchymal stem cells (MSCs) as an immune-regulatory agent for prevention and treatment of various immune disorders including graft-versus-host disease (GVHD), transplanted organ rejection and autoimmune diseases. However, the high diversity in the results from clinical trials using MSCs for such disorders emphasizes the need for MSCs to be "professionalized" ex-vivo to a more defined regulatory phenotype before administering to patients. To this aim, we have established an ex-vivo immunomodulatory triple combination treatment (TCT) for MSCs, using IFNγ, TGFβ and kynurenine. We show that pre-treated MSCs acquire an immunomodulatory phenotype, have improved regulatory functions, and up-regulate the expression of iNOS, IDO, COX2, HO-1, LIF and PD-L1. We define the pathway of kynurenine induced AhR activation in MSCs and how it contributes to the up-regulation of COX2 expression and IL-6 down-regulation. The combination of reduced IL-6 secretion with enhanced LIF expression leads to the inhibition of Th17 differentiation in co-culture of TCT MSCs and lymphocytes. To test the immunomodulatory function of TCT MSCs in vivo, we used the cells as GVHD prophylaxis in a GVHD mouse model. TCT MSCs administration significantly decreased GVHD score and improved mouse survival. Importantly, single administration could attenuate disease symptoms for more than three weeks. Based on these results, we suggest considering TCT MSCs as an improved cell therapy for systemic diseases with an underlying inflammatory and immunologic etiology.

MESENCHYMAL STEM CELL DIFFERENTIATION

W-1034

EFFECTS OF NON-STEROIDAL DRUGS (NSAIDS) ALLEVIATES BASAL EXPRESSION OF GROWTH FACTOR - ASSOCIATED GENES IN PERIODONTAL LIGAMENT STEM CELLS (PDLSCS)

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Periodontal ligament stem cells (PDLSCs) are useful source for periodontal regeneration due to its unique characteristic of self-renewing and promotion of cell differentiation. Non-steroidal anti-inflammatory drugs (NSAIDs)(aspirin and acetaminophen) are use as analgesics agent and capable to slow the progression of periodontal disease. Periodontal disease patient who is taking aspirin and acetaminophen and stem cell treatment may be affect the natural growth factor in stem cell tissue which may disturb their roles to repair the tissue damage. This growth factor is one of the biological mediators that play and important role in tissue regeneration, proliferation and differentiation. The present study was undertaken to investigate the expression level of growth factor-associated genes in PDLSCs after exposure to both drugs. Mesenchymal stem cell (MSCs) derived from periodontal ligaments tissue were isolated and characterized. MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay was used to determine the deleterious effect of analgesic agent on PDLSCs within therapeutic range of periodontal disease treatment. In addition, growth factor associated genes profiles were assessed using PCR array. Stem cells isolated from periodontal ligament cultures were capable to differentiate to trilineage differentiation and expressed specific phenotype profile of human MSCs. The proliferation rate of PDLSCs is decreased significantly after exposure to aspirin and acetaminophen in MTT assay. Based on the PCR results, several group of growth factor associated genes revealed higher propensity towards angiogenesis and osteogenic differentiation in PDLSCs after exposure to aspirin. These were including bone morphogenetic

proteins (BMP2 and BMP10), fibroblast growth factors (FGF2, FGF7, and FGF14), vascular endothelial growth factors (VEGFA and VEGFC) and interleukins (IL2, IL4 and IL10) that involved in tissue regeneration. Genes including BMP1, BMP8, DKK1, NDP, NTF3, SLCOA12, TGFB1 and VEGFA were highly expressed after exposure to acetaminophen. Overall, the identification of up-regulated growth factor associated genes in PDLSCs after exposure to both drugs mainly related to angiogenesis and osteogenic differentiation.

W-1035

SELECTION OF LINEAGE GUIDING METABOLITES IN STEM CELL CULTURES

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This study reports the use of self assembled peptide (FFS) hydrogels and cellular metabolomics to identify a number of innate molecules that are integral to the metabolic processes which drive cellular differentiation. Current methods for achieving this wholly depend on the use of chemical induction media which generally comprise synthetic molecules or attempting to match the physical environment to that of targeted tissue types. By culturing pericytes on FFS hydrogels with varied mechanical qualities, these cells were induced to undergo neuronal (2 kPa), chondrogenic (15 kPa) and osteogenic (40kPa) differentiation. Because the system relies solely on mechanical tuning of a uniform substrate, alterations in cell behaviour by way of total metabolism could be used to mine for selective compounds of interest by way of patterned depletion. When the selected metabolites (ceramide, lysophosphatidic acid and cholesterol sulphate respectively) were reintroduced into stem cells cultures, they were observed as having the ability to direct differentiation in their own right and with similar efficacy to routinely used induction media. Interestingly, it was also observed that these metabolites, when introduced in vitro, have distinct effects on stem cell behaviour dependent on whether the cells are amenable to them. That is, metabolic functions are innately coupled to the pericytes physical and morphological states. This approach shows that simple metabolites provide an alternative means to direct stem cell differentiation and that materials can be used to identify them simply and quickly which has clear implications for stem cell drug discovery.

W-1036

EFFECTS OF TSH ON HUMAN MESENCHYMAL STEM CELLS

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There is emerging information about functional consequences of thyroid stimulating hormone-thyroid stimulating hormone receptor (TSH-TSHR) interactions in mesenchymal stem cell biology. Therefore, we investigated effects of TSH-TSHR interactions on gene expression of human mesenchymal stem cells. Whole-genome microarray analysis was performed on human mesenchymal stem cells that were isolated and described in our laboratory as well as human mesenchymal stem cells that were isolated and certified by the American Type Culture Collection (ATCC). We found that expression of various genes was altered as a result of TSH treatment. We concluded that TSH might be a crucial hormone for some biochemical pathways in self-renewal, differentiation, and maintenance of human mesenchymal stem cells.

W-1037

IDENTIFICATION AND SPECIFICATION OF THE SKELETAL STEM CELL

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How are skeletal tissues derived from skeletal stem cells? Here, we map bone, cartilage, and stromal development from a population of highly pure, post- natal skeletal stem cells (mouse skeletal stem cells, mSSCs) to their downstream progenitors of bone, cartilage, and stromal tissue. We then investigated the transcriptome of the stem/progenitor cells for unique gene-expression patterns that would indicate potential regulators of mSSC lineage commitment. We demonstrate that mSSC niche factors can be potent inducers of osteogenesis, and several specific combinations of recombinant mSSC niche factors can activate mSSC genetic programs in situ, even in nonskeletal tissues, resulting in de novo formation of cartilage or bone and bone marrow stroma. Inducing mSSC formation with soluble factors and subsequently regulating the mSSC niche to specify its differentiation toward bone, cartilage, or stromal cells could represent a paradigm shift in the therapeutic regeneration of skeletal tissues.

W-1038

DERIVATION OF MALE GERM CELLS FROM RAM BONE MARROW MESENCHYMAL STEM CELLS BY THREE DIFFERENT METHODS AND EVALUATION OF THEIR FATE AFTER TRANSPLANTATION INTO THE TESTIS

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Mesenchymal stem cells (MSCs) have the capacity to differentiate into many cell types including germ cells (GCs). This unique characteristic together with the other distinctive properties like low immunogenicity, high proliferative capacity and etc have labeled MSCs as a good option for use in cell therapy. This research, for the first time, has evaluated the fate of in vitro MSC-derived GCs generated by three different induction methods and compared them after transplantation into testes of rams. Three treatment groups of passage-3 ram bone marrow (BM)-MSCs were considered in this study for generation of male GCs, 1) 14-day treatment with 10 µM retinoic acid (RA; RA14), 2) 21-day treatment with 10 µM RA (RA21) and 3) 21-day treatment with 10 ng/ml transforming growth factor beta-1 (TGFb1). After confirmation of the existence of germ-like cells in the culture by real-time reverse transcription polymerase chain reaction (real-time RT-PCR) for VASA, PIWIL2, INTEGRIN beta 1 (ITGb1), OCT4, DAZL, and ACROSIN (ACR) and immunocytochemistry for PGP9.5, the treated cells were labeled with PKH26 and transplanted into the testes of ram lambs. After two months, the rams were castrated and rams' testes underwent histological evaluations. Results showed that some cells from all three treatment groups differentiated into germ-like cells. Histological evaluations revealed that in vitro-derived GCs from all treatment groups survived in the testes. Some of these GCs homed at the basement membrane of seminiferous tubules and formed colonies. The homed cells and cell colonies were similar to testicular native spermatogonia and expressed PGP9.5. TGFb1 exhibited the highest efficiency for in vitro production of GCs, as well as the highest capability for homing and colony formation in the testes. RA21 was less efficient than TGFb1, particularly in colony formation. RA14 was the weakest group. No further differentiation of the transplanted GCs was observed. From our results, it could be concluded that a 21-day treatment period of BM-MSCs with TGFb1 is the most efficient method for in vitro generation of spermatogonia-like cells that survive, home and form colonies in the testes. More studies should be performed to establish a standard in vitro system for production of GCs from stem cells and possibly treat male infertility.

W-1039

PURIFICATION OF HUMAN ADIPOSE-DERIVED STEM CELLS FROM FAT TISSUE WITH HIGH PLURIPOTENCY BY STEM CELL MIGRATION FROM SYNTHETIC POROUS MEMBRANES

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Human adipose-derived stem cells (hADSCs) exhibit heterogeneous characteristics, indicating various genotypes and differentiation abilities. The isolated hADSCs can possess different purity levels and divergent properties depending on the purification methods used. In general, hADSCs are isolated from liposuction-derived adipose tissue, digested by collagenase, centrifuged, and then cultivated in cell culture dishes for at least one passage to purify hADSCs (the "culture method") by using strong adhesion characteristics of hADSCs on dishes. hADSCs are defined by the expression of specific cell surface markers of mesenchymal stem cells (MSCs), such as CD29, CD44, CD73, CD90, CD105, and CD166. hADSCs can be also isolated from and/or characterized by these surface markers using Fluorescence Activated Cell Sorting (FACS). However, it is extremely difficult to obtain hADSCs that express all of the above surface markers with greater than 90% purity in both culture method and FACS. In most cases, first-passage hADSCs have been approximately 60-80% positive for these MSC markers in previous studies, indicating that hADSCs are not a homogeneous cell population. The clinical application of hADSCs requires an easy and xeno-free method able to purify hADSCs with high purity and high pluripotency. We propose novel hybrid membrane migration method that offers to purify hADSCs from fat tissue solution with extremely high purity and pluripotency in this study. A primary fat tissue solution was permeated through the porous membranes having pore size from 8 to 25 µm and the membranes were incubated in cell culture medium for 15-18 days. The porous membranes used in this study are poly(lactide-co-glycolic acid)/silk screen hybrid membranes as well as commercially available polyurethane, nitrocellulose and nylon net filter membranes. The migrated cells from membranes showed approximately 98% of mesenchymal stem cell markers and exhibited higher expression of pluripotent genes such as Oct4, Sox2 and Nanog than hADSCs purified by conventional culture method. hADSCs with high purity and high pluripotency should be useful in cell therapy as well as the cell source to be reprogrammed into hiPSCs with high efficiency by using less number of transduction genes of pluripotency in stem cell therapy.

W-1040

BIOLOGICAL SUBSTRATES TO PREVENT DEDIFFERENTIATION OF OSTEOGENICALLY INDUCED MESENCHYMAL STEM CELLS

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Prior to transplantation, mesenchymal stem/stromal cells (MSCs) can be induced toward the osteoblastic phenotype using a cocktail of soluble supplements. However, the optimal induction duration

is unknown and there is little evidence of differentiated MSCs directly participating in bone formation, suggesting that MSCs may revert to an undifferentiated phenotype upon transplantation. Cell-secreted decellularized extracellular matrices (DMs) represent a promising strategy to confer bioactivity and direct cell fate through the presentation of a complex and physiologically relevant milieu. Therefore, we examined the effect of induction duration on cementing the osteoblastic phenotype of MSCs, as well as the capacity of biomimetic DMs to preserve the phenotype upon withdrawal of the induction stimulus. We show that increasing the duration of induction does not preserve the osteoblastic phenotype after the removal of the osteogenic stimulus. Regardless of induction duration, ranging up to 6 weeks, MSCs exhibited up to a 5-fold reduction in osteoblastic markers within 24 hours following stimulus withdrawal. Osteogenically induced MSCs retained their ability to produce oil droplets despite ample mineral production and changes in cell morphology characteristic of osteoblastic differentiation. We further show that seeding osteogenically induced MSCs on DMs sustains the osteoblastic phenotype of MSCs by preserving up to 2-fold more calcium deposition than tissue culture plastic. DMs sustain the phenotype in MSCs at least partially by increasing actin cytoskeletal tension via the ROCK II pathway. MSCs on DMs also secreted 25% more vascular endothelial growth factor (VEGF) secretion, a crucial endogenous proangiogenic factor that is abrogated during MSC osteogenic differentiation and is identified as the primary contribution of MSCs to tissue repair. These results underscore the rationale for deploying MSCs into a bone defect site using biomaterial platforms such as DMs to preserve the in vitro-acquired osteoblastic phenotype to accelerate the process of bone repair.

W-1041

MESENCHYMAL STROMAL/STEM CELLS PROLIFERATE IN PERIVASCULAR NICHES WITHIN INFLAMMATORY AND DEMYELINATING REGIONS OF MULTIPLE SCLEROSIS BRAINS

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Multiple sclerosis (MS) is a chronic neuroinflammatory disease characterized by focal inflammatory demyelinating lesions and neurodegeneration in the central nervous system (CNS). So far, the causes of MS remain unknown, but autoimmune mechanisms are considered crucial. Recently, a cell type with multiple differentiation and regeneration potency, with similarities of mesenchymal stromal cells (MSCs) and pericytes was described in the perivascular niches of the human brain. In view of the abundant vascular alterations in the CNS of MS patients, in addition to the known regenerative and immunomodulatory properties of MSCs, we aimed to explore the phenotype, distribution and proliferative activity of perivascular cells co-expressing markers for MSCs and pericytes in brains from MS and healthy controls (HC). Serial sections of formalin-fixed, paraffin embedded autopsy brain tissue blocks from 11 MS and 10 HC were immunohistochemically stained with a panel of markers specific for MSCs and pericytes; CD271, CD73, CD146, PDGFRβ, Ebf2, α-SMA and Ki67. Stained slides were scanned and quantified. Ten active, 20 chronic active, four inactive lesions, and 12 non-lesion regions were identified in the MS tissues using detailed neuropathological examination including CD68, CD8, CD4 and Luxol fast blue myelin staining. All MSC/pericyte markers studied were detected in the perivascular niche around blood vessels in the brain of MS and HCs. An increased density of cells expressing the marker signature were observed across all the MS tissues compared to HC ($p<0.0001$). Dynamic variations in marker expression patterns were found in lesions with different activity. Active and chronic active plaques, respectively, displayed a significantly increased density of proliferating perivascular cells expressing all markers, in particular CD73, CD271 and PDGFR-β, compared to HC. By contrast, a different profile composed of cells with reduced levels of CD271 and CD146 was found in chronic inactive plaques. We report of an increased accumulation of MSCs/pericytes with proliferative activity within vascular niches in areas of ongoing inflammation and demyelination activity in MS brains. Our findings support a role for perivascular MSCs in inflammation and tissue repair in MS.

W-1042

STK40 REPRESSES ADIPOGENESIS THROUGH TRANSLATIONAL CONTROL OF C/EBP PROTEINS

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A better understanding of molecular regulation in adipogenesis may help develop efficient strategies to cope with obesity-related diseases. Here, we report CCAAT/enhancer binding protein (C/EBP) β and δ, two critical pro-adipogenic transcription factors, are controlled at a translational level by serine/threonine kinase 40 (Stk40). Genetic knockout (KO) or knockdown (KD) of Stk40 leads to increased protein levels of C/EBP proteins and adipocyte differentiation in mouse embryonic fibroblasts (MEFs), fetal liver stromal cells, and mesenchymal stem cells (MSCs). In contrast, overexpression of Stk40 abolishes the enhanced C/EBP protein translation and adipogenesis observed in Stk40-KO/KD cells. Functionally, knockdown of C/EBPβ eliminates the enhanced adipogenic differentiation in Stk40-KO/KD cells substantially. Mechanistically, deletion of Stk40 enhances phosphorylation of eIF4E-binding protein 1, leading to increased eIF4E-dependent translation of C/EBPβ and C/EBPδ. Knockdown of eIF4E in MSCs decreases translation of C/EBP proteins. Moreover, Stk40-KO fetal livers display an increased adipogenic program and aberrant lipid/steroid metabolism. Collectively, our study uncovers a new repressor of C/EBP protein translation as well as adipogenesis and provides new insights into the molecular mechanism underpinning the adipogenic program.

W-1043

DEVELOPMENT OF DMSO-FREE CRYOPRESERVATION SOLUTION FOR HUMAN-DERIVED MESENCHYMAL STEM/STROMAL CELLS

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Cryoprotective agents (CPA) such as dimethyl sulfoxide

(DMSO), glycerol, and propylene glycol have been used for the cryopreservation of cells and tissues. While DMSO is the most effective CPA, it also has been shown to be highly cytotoxic and can affect cell viability after thawing. Moreover, amid growing concerns that DMSO may compromise the potency of mesenchymal stem/stromal cells (MSCs) and its differentiation potential, a DMSO-free cryopreservation solution is highly desired. In this study, we demonstrate the effectiveness of using a novel DMSO-free cryopreservation solution on human adipose-derived, human bone marrow-derived, and human umbilical cord-derived mesenchymal stem/stromal cells (AD-MSCs, BM-MSCs, and UC-MSCs) as compared to traditional DMSO cryopreservation solutions (10% DMSO). For this study, all three MSCs were frozen at equal cell density in DMSO and DMSO-free solution. After storage in liquid nitrogen, the MSCs were thawed and cultured through two passages and assessed for morphology, cell viability, cell marker expression, and differentiation potential. This study found comparable before and after cryopreservation performance characteristics. No significant difference was noted for cell viability and the expression of CD105 and CD90. Initial differences in cell morphology can be seen with superior quality found in cells cryopreserved in DMSO-free solution. Additionally, MSCs frozen in DMSO-free solution retained its differentiation potential. Overall, this study demonstrated a novel alternative to the traditional DMSO cryopreservation solution that can be used on human-derived mesenchymal stem/stromal cells.

W-1044

SITE- AND TIME-SPECIFIC EMERGENCE OF ADIPOGENIC COMPETENCE IN SKELETAL STEM CELLS DETERMINES THE NATURAL HISTORY OF FIBROUS DYSPLASIA OF BONE

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Fibrous Dysplasia of bone is a human disease caused by point mutations in GNAS, encoding for Gs-alpha, and leading to excess cAMP. The mutations arise in the inner cell mass, and can be modeled by heterotopic transplantation of affected skeletal progenitors in SCID/bg mice.. We generated mouse models of FD by expressing the mutation either constitutively or as targeted to osteoblasts. Mice that constitutively express GsaR201C develop an exact replica of the human disease over time, while mice with osteoblast-targeted mutation develop a phenocopy of high bone mass disorders caused by dysregulated Wnt signaling in humans. Detailed analysis of FD lesion development in mice revealed deposition of osteomalacic and over-remodeled bone that deforms and fractures. Bone lesions arise from a unique process of transient brownization of bone marrow (BM) adipocytes triggered by excess cAMP and characterized by morphological and molecular changes (UCP-1, PGC1a) defining BAT. Newly brownized BM adipocytes remodel to form aberrant osteoblasts that ectopically express adipocyte genes. Among these, Matrix Gla Protein (expressed in adipocytes but not in osteoblasts) is a potent inhibitor of mineralization, explaining the characteristic osteomalacia of FD bone both in humans and in mice. Importantly, reprogramming of BM adipocytes to aberrant osteoblasts via BAT occurs in an obligate site and time specific pattern, directly dictated by emergence of adipogenesis at specific times and sites in mouse BM. Sites of yellow marrow such as tail vertebrae are the first to be affected, and onset of disease coincides with onset of BM adipogenesis. Of note, BM adipogenesis is a postnatal event, and prenatal development is normal in FD mice. Mutant mouse ES cells formed normal cartilage in vitro, and normal ossification in a transplantation model. These data demonstrate the significance of site and time specific emergence of differentiation potential in BM skeletal progenitors; reveal that BM adipocytes are brownized by excess cAMP, like extramedullary beige/brown fat; demonstrate a unique path to FD strictly dependent on the existence and properties of skeletal stem cells and its dependent system of time and site specific lineages.

W-1045

THE EFFECT OF LONG TERM CULTURE UNDER LOW OXYGEN TENSION OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELL

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Mesenchymal stem cells (MSCs) play an important role in tissue repair regeneration and can be used to promote engraftment prevent graft-versus-host disease. MSCs can be isolated from many tissues in the body such as bone marrow, placenta, umbilical cord blood and adipose tissue. Due to the small number of MSCs at isolation, in vitro culture of MSCs expansion is therefore needed for effective therapeutic use. However, MSCs have been found to have limited expansion capacity as it reached the senescence stage as quick as normal somatic cell. A culture protocol to improve cell proliferation and inhibit cell senescence is needed to be explored. Here we aimed to study the effect of long-term culture of MSCs in hypoxic condition (5% O2 concentration) on gene expression, cell proliferation and differentiation potential of human bone marrow-derived mesenchymal stem cell (hBMSC). hBMSC was isolated from healthy subjects and cultured under normoxic condition for 4 passages before started culture under hypoxic condition up to 60 days. The result of this study showed that hypoxic condition can promote cell proliferation and enhance differentiation capability of hBMSC towards adipogenic and osteogenic. However, cell morphology was not different between normoxic and hypoxic condition both in early and late passages. In both culture conditions, hBMSC are larger at late passages. Significantly up-regulated of hypoxic genes, hypoxia-mediated effect 1 alpha (HIF-1), was found in hBMSC cultured under hypoxic condition, but not for those cultured in normoxic. This suggests that there is an inhibition of cell senescence after long-term cultured in hypoxic condition. The telomere length has been also measured. No telomere shortening at passage 7 and 11 in hBMSC under hypoxic condition were observed. Altogether, our results demonstrate the benefit of culturing hBMSC under hypoxic condition on cell expansion, proliferation and differentiation.

W-1046

PROTEOMICS OF STIMULATED MESENCHYMAL STEM CELLS - INTERFERON GAMMA ACTIVATION, SERUM STARVATION AND HYDROGEN PEROXIDE EXPOSURE

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Introduction: Mesenchymal stem cells (MSC) have been shown to have therapeutic properties and have been evaluated in several clinical trials. Preconditioning of MSC, such as hydrogen peroxide (H2O2) exposure or interferon gamma (IFN-g) activation have been demonstrated to enhance the therapeutic efficacy. The aim of this study was to determine how the proteome of MCS are altered upon different stimulations including H2O2, IFN-g and serum starvation (SS). Methods: Bone marrow derived human MSC were cultured under four different conditions; H2O2, SS, IFN-g and control condition (Ctrl). The proteome was analysed by LC-MS/MS with the application of an exclusion lists. GO Term finder was used to analyse the associated functions and compartments of the MSC proteome. Results: In total, 2850, 2885, 2726 and 2853 proteins were identified in Ctrl, H2O2, SS and IFN-g respectively. The use of exclusion lists allowed the identification of approximately 8% additional proteins in each condition. MSC markers such as CD73, CD90, CD105, CD44 and CD166 were identified in all samples, while all samples lacked CD14, CD19, CD34, CD11b, CD45, CD79a and MHC class II, except for the IFN-g activated cells that express MHC class II. In total 2050 proteins were identified in all four samples, while 160, 235, 140 and 256 proteins were unique for the Ctrl, H2O2, SS and IFN-g cells respectively. When MSC were cultured with H202, mitochondrial proteins associated with the electron transport chain and cellular respiration were down regulated. MSC activated with IFN-g up regulated proteins associated with antigen processing and presentation and immune response. Furthermore, MSC that had been cultured under serum starvation upregulated proteins associated with sterol and cholesterol biosynthesis. Conclusion: When MSC are activated (IFN-g) or stressed (H2O2 and SS) the expression of several proteins are alter and their proteome is changed, which may be important for their therapeutic efficacy.

W-1047

THE FATE OF SYSTEMICALLY ADMINISTRATED ALLOGENEIC MESENCHYMAL STEM CELLS IN MICE WITH FEMORAL FRACTURE

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Bone marrow mesenchymal stem cells (MSCs) are multipotent stem cells which have innate ability to self-renew and differentiate into multiple cell types. MSCs have been used widely in tissue engineering and cell therapy applications. However, the fate of allogeneic MSCs in vivo following their administration was not well studied. The aim of this study is to investigate the fate of systemically administrated allogeneic MSCs in mouse fracture healing using in vivo imaging system and immunostaining. Open femoral fracture with internal fixation was established in 30 FVB mice, whom were assigned into 3 groups receiving: PBS injection; MSCs systemic injection; and MSCs local injection. 5x105 Luc-MSCs isolated from the luciferase transgenic mouse were injected at 4 days after fracture. All animals were terminated at 5 weeks after fracture; examinations included bioluminescence based in vivo imaging, Micro-CT, mechanical testing, histology, immunohistochemistry and double immunofluorescence staining. For Luc-MSCs local injection, the signal from Luc-MSCs at the fracture site lasted 12-14 days. For Luc-MSCs systemic injection, Luc-MSCs were trapped in lungs for 8-9 days, and then gradually released to the fracture site. BMD, BV/TV in MSCs injection groups were significantly higher than these in the PBS group, but no difference was found between MSCs injection groups. Ultimate load and E-modulus were significantly higher in the MSCs injection groups than these in the PBS group, but no difference was found between the MSCs local and systemic injection group. Double immunostaining demonstrated that the MSCs local injection group had more Luc-positive cells with higher apoptotic rate at the fracture site than the MSCs systemic injection group. Both Luc-MSCs and Luc-osteoblasts were present in the callus in the MSCs injection groups at 5 weeks after fracture, and contributed to the new bone formation. Less than 3% injected luc-cells remained at the fracture site in MSCs injection groups at 5 weeks following the fracture, and the rest of injected Luc-cells were dead. These findings provide critical information to implement the development of MSC-based therapies and expedite clinical utility.

W-1048

PAMIDRONATE NEGATIVELY REGULATES THE OSTEOGENESIS IN MSCS OF FIBROUS HAMARTOMA IN CONGENITAL PSEUDARTHROSIS OF THE TIBIA

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Neurofibromatosis type 1 (NF1) is a commonly occurring genetic disorder in children. Mutation in the NF1 gene has its implication in poor osteoblastic capabilities. We hypothesised that pamidronate will enhance the osteoblastic potential of the mesenchymal stem cells (MSCs) derived from lipofibromatosis tissue of children with congenital pseudarthrosis tibia (CPT) associated with NF1. In this study, normal BMSCs and CPT MSCs were obtained from three patients undergoing salvage surgeries/bone grafting and those undergoing excision of the hamartoma and corrective surgeries respectively. The effects of pamidronate (0, 10 nM, 100 nM and 1 μM) on cell proliferation, toxicity and differentiation potential were assessed and the outcome was measured by staining and gene expression. Our outcome showed that CPT MSCs had more proliferation rate as compared to normal BMSCs. All 3 doses of pamidronate did not cause any toxicity to the cells in both the groups. The differentiation potential of CPT MSCs was lower compared to the control MSCs. This was quantitated by gene expression analysis. Therefore, supplementation with pamidronate alone will not aid in bone formation in patients diagnosed with CPT. An additional stimulus is required to enhance bone formation.

W-1049

PLATELET POOR PLASMA INDUCES BONE/CEMENTUM DIFFERENTIATION IN HUMAN PERIODONTAL LIGAMENT STEM CELLS

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The main goal of periodontal regeneration involves stem cell proliferation, migration and differentiation, guided by biomolecules. Platelet rich plasma (PRP) is a blood fraction used in regenerative medicine as a source of growth factors. Here, we characterized the remaining plasmatic fraction of PRP separation, the Platelet poor plasma (PPP). We identified proteins involved in periodontal regeneration in PPP fractions. Then, we analyzed the effect of PPP over bone/cementum differentiation of Human Periodontal Ligament Stem cells (HPLSC) and compared this effect with PRP incubation. Primary cultures of HPL were obtained from explants, from third impacted molars under approved guidelines set by the Ethics Committee of the Faculty of Medicine, Pontificia Universidad Catolica de Chile. Cells were characterized by flow cytometry, immunofluorescence and their differentiation potential into osteogenic, adipogenic and chondrogenic lineages. PRP and PPP fractions were collected from young and healthy volunteers using the Biomet GPS III system. We analyze protein levels in both fractions with a 44 Membrane-Based Antibody Array and we quantified levels of PDGF isoforms, EGF and FGF-2 by ELISA. Then, HPL cells were treated during 14 or 21 days with different concentrations of PPP or PRP (2.5 or 5 or 10%) in the presence of osteogenic factors. Finally, calcium deposit was quantified (µg/mL). We used FBS (2.5 or 5 or 10%) as a control. We obtained HPL primary cultures with mesenchymal stem properties. By flow cytometry cells were positive for CD105, CD90, CD73 (>98%) and negative for CD34, CD45, CD11b, CD14, CD79a. HPLSC were able to differentiate into the bone/cementum, adipose, and cartilage lineages. PPP protein profile was very similar to PRP showing high levels of growth factors: PDGF-AA, BB, EGF and IGFBP 2 and 6. HPLSC treated with PPP or PRP during 21 days in the presence of osteogenic factors, evidenced calcium deposits even in low concentrations (2.5%). This suggests an inductor effect of PPP in bone/cementum differentiation process. PPP treatment induce bone/cementum differentiation of HPLSC as good as PRP in an In Vitro model. PPP fraction constitutes an alternative source of growth factors thal could be used in future periodontal regeneration therapies.
Fondecyt 11121294 and 1130618.

W-1050

HUMAN EXTRA OCULAR MUSCLE DERIVED MESENCHYMAL STEM CELLS POSSESS MULTI-LINEAGE DIFFERENTIATION POTENTIAL

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Human adult stem cells are multipotent in nature and are used extensively for cell therapy. They can be cultured in vitro without losing their differentiation capacity and are less tumorigenic than embryonic stem cells. In this study, we isolated adherent multipotent mesenchymal stem cells (EOM-MSC) from extra ocular muscle tissue which was excised during corrective strabismus surgery and were compared with bone marrow derived MSC. Morphology and mitochondrial distribution was observed through phase contrast/ fluorescence microscopy. Differentiation of EOM-MSC into adipocytes, osteocytes were determined by Oil Red O, alkaline phosphatase staining respectively and neuronal differentiation was monitored by GFAP staining. Changes in gene expression in undifferentiated and differentiated cells were monitored by real time PCR analysis. Cell surface marker expression profile was analysed through flow cytometry. We found that EOM-MSC was similar in morphology, growth and phenotypic properties to bone marrow derived MSC but expressed higher levels of NESTIN, OCT4, NANOG and SOX2 in undifferentiated state. These cells also expressed embryonic surface marker SSEA-4 and their intracellular mitochondrial distribution pattern was similar to stem cells. EOM-MSC differentiated into adipocytes and osteocytes and also highly into neuronal cells. Our results show that multipotent stem cells are present in EOM and could be easily isolated. These cells with high neuronal differentiation capacity, with low adipogenic and osteogenic differentiation ability would be suitable in regenerative therapy for neurodegenerative diseases.

W-1051

IMPAIRED FUNCTION OF BONE MARROW STROMAL CELLS IN SYSTEMIC MASTOCYTOSIS

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Patients with systemic mastocytosis (SM) have a wide variety of problems, including skeletal abnormalities. More than 90% of patients with SM carry the somatic D816V activating mutation in the KIT gene in mast cells and we wondered if the function of bone marrow stromal cells (BMSCs; also known as MSCs or mesenchymal stem cells) might be affected by the invasion of bone marrow by mutant mast cells. We determined that BMSCs from SM patients do not have a mutation in c-kit, but they do proliferate poorly. In addition, while osteogenic differentiation of the BMSCs seems to be deficient, their adipogenic potential appears to be increased. Since the hematopoietic supportive abilities of BMSCs are also important, we studied the engraftment in NSG mice of human CD34+ hematopoietic progenitors, after being co-cultured with BMSCs of healthy volunteers vs. BMSCs derived from patients with SM. BMSCs derived from the bone marrow of patients with SM could not support hematopoiesis to the extent that healthy BMSCs do. Finally, we performed an expression analysis and found significant differences between healthy and SM derived BMSCs in the expression of genes with a variety of functions, including WNT signaling, ossification, and bone remodeling. We suggest that some of the symptoms associated

with SM might be driven by epigenetic changes in BMSCs caused by dysfunctional mast cells in the bone marrow of the patients.

W-1052

EXPLORING THE CARDIOMYOGENIC DIFFERENTIATION POTENTIAL OF HUMAN MESENCHYMAL STEM CELL DERIVED FROM BONE MARROW AND ADIPOSE TISSUE

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Human Mesenchymal Stem Cells (MSCs) are multipotent & self renewing cells which can be isolated from various tissues. They are being used in the treatment of many diseases. Clinical trials are also going on based on their immune privilege, paracrine properties & absence of ethical concern. Bone marrow is most common source of MSCs & shows differentiation into cardiomyocytes like cells. However, isolation of bone marrow is a painful procedure. Therefore, we looked for a different source of tissue for the same. Adipose tissue (AT) is easy to isolate and is considered waste in surgeries. Therefore, we planned to isolate MSC from AT (Ad-MSC) & compare its cardiomyogenic differentiation potential with bone marrow MSC (BM-MSC). We used 5-Aza (6uM) as inducer in the study. Study was initiated after ethical approval from Institute & Stem Cell Ethics Committee. Cryopreserved BM-MSCs were revived and expanded in vitro in DMEM-LG with 10% FBS. MSCs from Adipose tissue were isolated by Explant method with success rate >90%. 3rd passage cells were used for differentiation after their characterization by surface marker (CD105, CD29, CD90, CD73 and HLA I & II, CD34/45) studies. 60-65% confluent cells were induced with 5-Aza (6um) for 24 hours. Next day, media was replaced with DMEM-LG +10% FBS & observed for 30 days with regular media change. After Induction, cells were observed for morphological changes. After 4-5 days, cells started flattening & acquiring bincleation. Post induction, cells were processed & characterized for markers like Myosin Light Chain-2v (Mlc-2v), Connexin 43 and cardiac Troponin I (cTnI) by RT-PCR and Mlc-2v & cTnI by IF (n=3). qPCR was done for Mlc-2v , SerCa2, Ryr2 & Transcription factors (TFs). Ad-MSC show similar percentage positivity for surface markers as BM-MSCs. After differentiation cells showed positivity for Mlc-2v, CA, Cx4, cTnI by RT-PCR and by IF showed the expression of cTnI and Mlc-2v which was comparable in Ad-MSC and BM-MSC treated with 5-Aza. qPCR and WB results showed similar expression of Mlc-2v, SerCa2 & Ryr2 and (TFs) GATA4, Nkx2-5, Baf60C and Tbx5 in both groups. These results show that Ad-MSC is a good source of MSC with high yield and cardiomyogenic potential comparable to BM-MSC. Further experiments including functionality of Aza induced cardiomyocytes, will substantiate to the above pool of data.

W-1053

YAP MECHANOSENSOR AS A CRUCIAL DETERMINANT IN THE DIFFERENTIATION OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS

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Extracellular matrix (ECM) provides a dynamic environment acting as critical regulator of cell behavior and stem cell fate decision. ECM composition and mechanics are known to contribute to organ development, tissue remodeling, while participating in the onset and progression of degenerative diseases. Yes-associated protein (YAP) and WW domain-containing transcription regulator protein 1 (WWTR1 or TAZ), the key effectors of the Hippo Pathway, have been proposed as cell mechanosensors converting the mechanical cues arising from the ECM into biochemical intracellular signals. This pathway is also known to play a crucial role in contact inhibition, by mediating the cadherin-catenin activity in vitro and in vivo. Additionally, YAP/TAZ proteins have been indicated as regulators of adult mesodermal progenitor differentiation, through the direct interaction with the transcription factors RUNX-2 and PPAR Here, we establish a direct correlation between the signals arising from the ECM and YAP activity in determining adipose mesenchymal stem cell (AD-MSC) fate decision. By taking advantage of micropatterned surfaces coated with different components of ECM or proteins involved in cell-cell communication, we mimic cell-matrix and cell-cell interaction at the single cell level and highlight the crucial role of YAP in determining AD-MSC shape, polarity and fate in response to ECM composition and mechanics. We demonstrate that YAP activity is directly dependent upon the availability of cell-ECM or cell-cell binding sites and determines the mechanical pertinence of AD-MSCs and their fate. In turn, we unveil the role of the cadherin/catenin axis as a positive regulator of the Hippo pathway in AD-MSCs, inhibiting YAP nuclear localization, preventing cell spreading and shaping, while modulating focal adhesion maturation, cytoskeletal assembly, and force distribution. Taken together, our results show that molecular and mechanical signals concur to a selective modulation of the Hippo pathway to regulate the fate of AD-MSCs and shape a central role of YAP/TAZ proteins in bridging between the extracellular milieu and cell function.

W-1054

GROWTH PROPERTIES OF STEM CELLS GROWN ON A MESENCHYMAL STEM CELL SPECIFIC EXTRACELLULAR MATRIX

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Previous studies have demonstrated that mesenchymal stem cells (MSCs) grown on a MSC derived extracellular matrix (ECM) double more quickly and better retain their stemness when compared to cells grown on tissue culture plastic. To evaluate the biological

characteristics of MSCs grown on this matrix we first measured their cell motility. Cells grown on ECM had both faster instantaneous velocity and higher average velocity. In addition, cells grown on ECM had greater linear velocity when compared to those grown on tissue culture plastic. We next evaluated gene expression in these two cell populations. Of a set of genes associated with stemness in MSCs, only IL10 had statistically higher expression in cells grown on the ECM. We followed this result by investigating genes associated with immune response and autoimmunity. In this screen, expression of CCR7, CD14, CSF1, CXCL6, IL1R1, LTB and TLR3 were all significantly elevated in cells grown on the ECM. These data were analyzed using Ingenuity Pathway Analysis to identify upstream pathway activation indicative of this expression. This analysis suggested that MSCs grown on ECM have higher expression or activation of MAP3K7 (TAK1) and TANK, two proteins involved in NFκB signaling. To determine if other stem cell types could be maintained on the MSC derived ECM, we cultured baboon induced pluripotent stem cells (biPSCs) on ECM. These cells were manually passaged onto the ECM to which they readily attached and proliferated. However the ECM was not able to maintain the undifferentiated state as seen when cultured on matrigel. biPSCs cultured on MSC derived ECM began differentiating within the first passage. Neural rosettes were readily identified in the differentiating areas and further characterization will be necessary to determine if the differentiation was random or directed toward a specific germ layer or cell lineage. These data demonstrate that culture of MSCs on ECM affected the motility and gene expression of these cells. Similar culture of bIPSCs did not result in improved maintenance of these pluripotent stem cells but instead led to differentiation.

W-1055

DEVELOPMENT OF LOCAL ACTING BIOLOGICS FOR COMBINED STEM CELL AND GENE THERAPY TO TREAT ARTHRITIS

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation, joint destruction, and an overall decrease in quality of life. In RA, the excessive production of tumour necrosis factor-α (TNF-α) enhances immune cell proliferation, and the release of other pro-inflammatory cytokines that collectively contribute to joint destruction. Current therapies approved for treating RA focus on systemically inhibiting TNF-α; however, this results in dangerous side-effects such as an increased susceptibility to tuberculosis and neoplastic disease. The purpose of my project is to develop a cell therapy that uses local-acting TNF-α inhibitors to reduce joint inflammation and treat RA in the absence of serious systemic side-effects. Recently, our lab has developed novel biologics known as TNF-α "sticky traps." These molecules are termed sticky because they harbour heparin binding domains (HBDs) that allow them to bind the extracellular matrix (ECM) where they are injected as well as to local TNF-α. Using our lab's established protein production protocol, I have purified large quantities of TNF-α sticky trap protein to evaluate its neutralization ability in vitro. Furthermore, I have established compact bone-derived mesenchymal stem cell (CB-MSCs) lines that inducibly express this local-acting biologic. In addition to their regenerative potential, these cells possess immunomodulatory properties that could help ameliorate joint inflammation; making them an attractive vehicle for arthritis cell therapy. In vitro TNF-α neutralization assays have illustrated that this biologic is functional as a purified protein as well as when produced from engineered CB-MSCs. Additionally, I am planning to perform intra-articular injections of these engineered CB-MSCs into an RA mouse model to try and treat arthritis-related symptoms. Further in vitro and in vivo characterization of the engineered CB-MSCs is required although we hypothesize that the TNF-α sticky-trap will neutralize TNF-α in diseased mouse joints and consequently reduce joint inflammation in the absence of systemic side-effects.

W-1056

COMPARISON OF EXTRACELLULAR MATRIX FORMATION AND OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS DERIVED FROM HUMAN ADIPOSE TISSUE AND BONE MARROW.

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The two mesenchymal stem cell (MSC) populations that have gained most attention in relation to bone tissue engineering are adipose tissue (AT) MSCs and bone marrow (BM) MSCs. The purpose of this study was to compare the effect of medium composition on extracellular matrix (ECM) formation and osteogenic differentiation of the two cell types. AT-MSCs (n=3) were isolated from liposuction material and BM-MSCs (n=3) were obtained from BM aspirates (All donors provided informed consent) The cells were cultured in growth medium (GM) consisting of DMEM/F12, 10% human platelet lysate plasma, 2 IU/ml heparin and 1% pen/strep. Osteogenic medium (OM) consisted of GM with 150 μM ascorbic acid and 10mM β-glycerophosphate or OM with 10 nM dexamethasone (dOM). Cells were seeded at 8.9x10^4 cells/cm2 on PCL nanofiber scaffolds 2 days before start of experiment. Live-dead staining indicated excellent cell survival and viability under all conditions after 21 days. Staining for collagen type 1 (COL1) was only detected intracellular when cultured in GM. In OM COL1 was seen in the ECM in both cell types. However, it was stronger and denser in AT-MSCs compared to BM-MSCs. Moreover, COL1 was also detected intracellular in BM-MSCs but not in AT-MSCs. When cultured in dOM both cell types had a dense network of COL1 in the ECM. However, the orientation of the COL1 fibres was altered in dOM compared to OM cultures. The COL1 fibres appeared laminar organized in the OM whereas they were randomly oriented in dOM cultures. Osteogenic markers were evaluated by qRT-PCR at day 0, 10 and 21. AT-MSCs had significantly higher COL1 expression at day 10 compare to BM-MSCs. Expression of ALPL was higher in BM-MSCs at day 10, while AT-MSCs cultured in dOM expressed significantly more ALPL at day 21 compared to the other groups. The late osteogenic markers, BGLAP and BSP were both expressed in BM-MSCs. The expression of BGLAP became down-regulated in dOM while BSP was up-regulated. The AT-MSCs expressed neither BGLAP nor BSP during osteogenic differentiation. In conclusion, AT-MSCs have a better ability to form an extracellular COL1 network and have higher expression of early osteogenic markers, whereas BM-MSCs have higher expression of late osteogenic markers. This indicates that AT-MSCs are more immature compared to BM-MSCs, with respect to osteogenic differentiation.

W-1057

EFFECTS OF LASER THERAPY ON THE OSTEOGENIC DIFFERENTIATION OF DENTAL PULP STEM CELLS

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Dental Pulp Stem cells (DPSCs), has been used in bone tissue engineering studies. Several studies have shown that low level laser irradiation has beneficial effects in bone regeneration. The aim of this study was to examine the "in vitro" effects of low level laser irradiation at different power densities and irradiation time (5 J/cm2 for 5 seconds , 10 J/cm2 for 10 seconds, 20 J/cm2 for 20 seconds) during DPSCs osteogenic differentiation. DPSCs were obtained from deciduous teeth of 10 Cleft Lip and Palate patients and these DPSC strains were characterized through flow cytometry analysis. Appropriate osteogenic medium was used during twenty one days and the DPSC strains were exposed to a 660 nm and 20 mW laser every day in three groups of power density and irradiation time (group 1 : 5 J/cm2 for 5 seconds , group 10 J/cm2 for 10 seconds, Group 3: 20 J/cm2 for 20 seconds) . Control group did not receive irradiation. Cell differentiation was evaluated by alizarin red and the quantification of calcium deposition was measured by enzyme-linked immunosorbent assay (ELISA) 21 days after the osteogenic differentiation beginning. All 10 DPSC strain have positive reaction for mesenchymal stem cell antigens (CD29, CD90, CD105, CD73, CD166), and negative reaction for hematopoietic (CD45 e CD34) and endothelial cell markers (CD31). After induction to osteogenic differentiation under appropriate cell culture conditions all 10 DPSC strains produced calcium deposition. It was observed significant difference between the non-irradiated group and the others three irradiated groups. Laser therapy significantly stimulated DPSCs differentiation independently of laser power density. The optimal laser power density was 20 J/cm2 to increase the osteogenic differentiation. In conclusion, the use of low level laser irradiation during osteogenic DPSC differentiation has beneficial effects because it increased the osteogenic potential of those cells. Our findings suggest that this process may provide a novel approach for the preconditioning of DPSCs in vitro prior to transplantation.

W-1058

THE PARACRINE FACTORS FROM HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS OF VERTEBRAL BODY ENHANCE INTERVERTEBRAL DISC REGENERATION.

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Several in vivo studies have reported that mesenchymal stem cell (MSC) implantation into degenerate intervertebral discs (IVD) leads to regeneration of disc cells, although the exact mechanisms are not understood. Here, we investigate the different regulation in interaction between human MSCs and degenerate disc cells from both nucleus pulposus (NP) and annulus fibrosus (AF) during co-culture. Human MSCs and degenerate disc cells were directly or indirectly co-cultured. The stimulatory effect in cellular proliferation between MSCs and degenerated disc cells were assessed by cck-8 assay during co-culture or culture of cell-conditioned medium. Collagen synthesis of extracellular matrix (ECM) and RNA expressions of selected genes including ECM and proinflammatory cytokine were then evaluated by sircol collagen assay or quantitative real-time PCR after co-culture. In addition, trophic effect was exams concerning on the growth factor genes to understand underlying mechanism on the biologic changes of cells. MSCs and degenerated disc cells proliferation was increased in co-culture system compared to monolayer culture or in conditioned medium of each cell. During co-culture with NP cells, mRNA expression of extracellular matrix for aggrecan, versican, SOX9, type II and VI collagen in MSCs was significantly increased, whereas MSCs increased in mRNA expression for type Vcollagen in co-cultured with AF cells. In addition, co-cultured degenerate disc cells had accumulated more total ECM collagen than did monoculture cells. During co-culture, MSCs down regulated expression of pro-inflammatory cytokine genes of degenerate disc cell involved in degradation of ECM molecules. In association with trophic effect of MSC to degenerates disc cells, up regulation of growth factors mRNA expression was shown in MSCs co-cultured with NP or AF cells. Importantly for MSC-based therapies for repair of the degenerate IVD, these data suggest that cellular interactions between MSCs and degenerate IVD may be enhancing IVD regeneration. This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (2012M3A9C6049862).

W-1059

THE EFFECT OF ENDOPLASMIC RETICULUM STRESS ON HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS

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The Endoplasmic Reticulum (ER) maintains cellular homeostasis by regulating protein synthesis and folding. ER homeostasis can be distrupted by accumulation of unfolded proteins or surplus protein production and may result in "ER stress", causing series of complex signal pathways in order to restore ER homeostasis. Mesenchymal Stem Cells (MSCs) are multipotent cells that can be isolated from bone marrow (BM) and differentiate into fat, bone and cartilage cells. Here, we modulated ER stress to assess its effect on proliferation and differentiation of MSCs using inducers of ER stress, Thapsigargin (TG) and Tunicamycine (TM) and inhibitors of ER stress, TUDCA and 4-PBA. Cell proliferation was measured real time for 10 days (xCELLigence, Roche). For differentiation assays, MSCs were maintained in differentiation media supplemented with different concentrations of TG, TM, TUDCA and 4-PBA for 3 weeks. Semiquantitative analysis of differentiation was performed using spectrophotometric comparison of Oil Red O for adipogenic differentiation or Calcium for osteogenic differentiation. Apoptosis and cell cycle analyses were done using a FACSARIA (BectonDickinson). Treatment with TG resulted in complete inhibition of proliferation (50-400 nM), as apparent by cell cycle arrest in G0/

G1 phase and increased apoptosis, whereas treatment with TM resulted in a dose dependent suppression of proliferation with cell cycle arrest and increased apoptosis at doses of ≥200 nM. Treatment with TUDCA did not affect cell proliferation (50-200uM), whereas 4-PBA displayed a dose-dependent inhibition of proliferation (0,1-5 mM), without a clear effect on cell cycle or apoptosis. TG treatment resulted in inhibition of adipogenesis and osteogenesis, whereas TM treatment resulted in a shift from adipogenic to osteogenic differentiation. TUDCA decreased adipogenesis, but increased osteogenesis, whereas 4-PBA caused an increase in both adipogenesis and osteogenesis. Effects of TG and TM could not be reversed by either TUDCA or PBA. Most, likely because 4-PBA and TUDCA affect different ER stress signaling pathways from ER stress inducers TG and TM. In conclusion, modulation of ER stress using different chemicals can affect proliferation and differentiation of MSCs.

W-1060

BONE MARROW MESENCHYMAL STEM CELLS DERIVED OLIGODENDROCYTE PROMOTE AXON REMYELINATION

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Adult bone marrow is likely to be an abundant, accessible and safety source for autologous stem cell transplantation. Evidences that grafted neural stem/neural precursor cells (NSCs) provide a promising approach to central nervous system (CNS) regeneration. Purpose of this study was to generate a purified population of oligodendrocyte progenitor cells (OPCs) from the NSC which is derived from bone marrow pluripotent stem cells. After sorted by magnetic bead, Sox2 positive BM-MSC was treated with series induced medium contained growth factors and small molecules. Induced neural stem cells (iNSCs) were identified by RT-PCR and immunostaining with CD133 and Nestin markers. Neuronal signal could be detected by Multi-electrode arrays (MEAs) on differentiated neurons from MSC-derived NSC. Oligodendrocyte differentiated from iNSCs were transplanted into an axonal injured model with intracerebral hemorrhage, behavioral tests and immunostaining showed that grafted cells could improve function of injured axon with remyelination. Our findings might enable to get autologous cell derived NSCs for the therapy of axonal degenerative disease in clinic.

MESENCHYMAL CELL LINEAGE ANALYSIS

W-1061

THERAPEUTIC POTENTIAL OF CANINE MESENCHYMAL STEM CELL DERIVED FROM ADIPOSE TISSUE: ARE THERE EFFECTS OF TISSUE-HARVESTING TISSUE?

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Adipose-derived mesenchymal stem cells (MSCs) are attractive for cell therapy and tissue engineering because of their multipotency and ease of isolation from many animal species. Another aspect of their therapeutic potential includes paracrine effect through secretion of trophics, vasculogenics and immunomodulatories factors that modulate the molecular composition of the environment to evoke responses from resident cells. In this study we have compared the in vitro therapeutic potential of canine adipose tissue derived mesenchymal stem cells from subcutaneous (S-MSCs) and from omental fat (O-MSCs). Both cell types were isolated from subcutaneous and omental adipose tissue during elective ovariohisterectomy from female dogs of different breeds, clinically healthy and normal corporal condition and characterized their phenotype, cell doubling time, colony-forming unit fibroblast (CFU-F) abundance, immunophenotypic properties, and multilineage differentiation potential. The gene expression of trophics, vasculogenics and immunomodulatories citoquines was evaluated. In vitro vasculogenesis was followed by tube formation assay and in vitro immunomodulation was assessed by Inhibition of CD4+ T- cell proliferation assay. The result obtained revealed that Independent of age and body weight of dogs is always possible to obtain omental adipose tissue. Not so, subcutaneous. Furthermore, S-MSCs and O-MSCs derived had same morphology, immunophenotype, potential proliferation and differentiation. The CFU-F/g obtained data showed that the anatomical origin of the adipose tissue has an evident effect in the abundance of MSCs, omental fat was more abundant than subcutaneous. Real-time polymerase chain reaction analysis revealed high levels of hepatocytes growth factors (HGF) and Indoleamine 2, 3-dioxygenase (IDO) in S-MSCs. However, the in vitro vasculogenic and immunomodulatory assays showed equivalent results trough of paracrine affects in both types of cells. Thus, our data suggest that despite the differences in gene expression, the S-MSCs and O-MSCs have an equivalent in vitro therapeutics potential. However the greater abundance of O-MSCs can be a useful feature for certain conditions as patients with low body fat or high number of cells in a short time requirements.

W-1062

EQUINE MESENCHYMAL STEM/STROMAL CELLS PUTATIVELY DERIVE FROM PERIVASCULAR STEM CELLS

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Other than humans, the horse is likely the species where Mesenchymal Stem/Stromal Cells (MSCs) have the highest potential for regenerative medicine. High demand for cellular therapies for horses has resulted in a rapid increase in the number of specialised veterinary companies and clinics which now drive a multimillion dollar business worldwide. Equine MSCs are identified according to ISCT guidelines, namely, expression of the surface markers, CD105, CD73, CD90, CD44, CD29, and lack of hematopoietic markers. However, MSC preparations obtained from equine tissues are heterogeneous and contain only a small proportion of stem cells which precise identity is not known. Recently, perivascular cells expressing CD146, NG2, PDGFRβ and αSMA were identified as a native source of MSCs in multiple human tissues, and it was shown that Perivascular Stem Cells (PVSCs) obtained from adipose may provide a superior source for regenerative therapy. The aim of the

present study was to gain an understanding of the nature of equine MSCs by determining whether 1) these cells express PVSC markers and 2) whether the expression of these markers is maintained during culture. Adipose samples were obtained from 4 adult horses and analysed by immunohistochemistry (IHC). Both MSC (CD73, CD44, CD29) and PVSC (CD146, NG2, αSMA) markers were present and had a perivascular location in adipose samples. In addition, MSCs were obtained by culturing adipose stromal vascular fractions. Flow cytometry and qPCR analyses at passage 3 showed that the expression of MSC (CD105, CD73, CD90, CD44, CD29) and PVSC (CD146, NG2 and PDGFRb) markers was indeed maintained in the cultured MSCs; levels of CD146 transcript increased ($p<0.05$) during culture whereas expression of other markers did not change. In contrast, CD144 and CD45 transcripts decreased ($p<0.01$) to very low or undetectable levels, respectively. Importantly, dual-antibody IHC and flow cytometry indicated that PVSC (CD146, NG2, αSMA) and MSC (CD105, CD44, CD29) markers co-localised both in adipose tissue and in cultured MSCs, indicating that MSCs obtained from adipose tissue extracts originate from perivascular cells and maintain their immunophenotype in culture. In view of these findings, we suggest that, in addition to MSC markers, PVSC markers should be used when characterising equine MSCs.

W-1063

CHARACTERIZATION OF MESENCHYMAL STEM CELLS SECRETOME FROM VARIOUS TISSUES

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It is currently believed that the predominant mechanism by which mesenchymal stromal cells (MSCs) function could be related to their paracrine activity, creating a microenvironment with trophic signals. However, in vivo studies have shown conflicting results with MSCs obtained from different tissues. Therefore, evaluating the secretome of these cells is of great interest. Towards this end, the aim of this project is to analyze the proteins from MSCs conditioned medium obtained from different sources (bone marrow, uterine tubes and umbilical cord). The MSCs were characterized by flow cytometry for specific membrane markers and by in vitro differentiation into adipocytes, chondrocytes, and osteoblasts. The protein profile of the conditioned media was analyzed by bidimensional liquid nanochromatography coupled to tandem mass spectrometry. The samples were digested in solution with trypsin, processed by a nanoACQUITY system equiped with a capture column "Symmetry C18" (20 nm × 180 mm, 5 mM) and an analytical column "BEH C18" (75 nm × 250 mm, 1.7 mM) coupled to a mass spectrometer "Q-Exactive" (Thermo Scientific). Spectral data were obtained by full-acquisition mode MS / dd-MS2 (Top15). The acquired data were processed by MaxQuant software. Differentially expressed proteins were observed in MSCs secretome from different sources. MSCs from the bone marrow and uterine tubes showed enrichment of pathways related to cellular maintenance, organization and morphology, while MSCs from umbilical cord tissue showed enriched pathways related to cell proliferation, signaling and interaction. In short, the observed variation in content and pathways enrichment between MSCs from different sources indicates different functional potentials which may be of utmost importance before selecting the most appropriate source aiming different therapies.

W-1064

HIGH GLUCOSE INHIBITS E-CADHERIN EXPRESSION THROUGH NOTCH, SNAIL, AND POLYCOMB REPRESSIVE COMPLEX SIGNALING PATHWAY, WHICH CONTRIBUTES TO HIGH MIGRATORY ACTIVITY OF HUMAN MESENCHYMAL STEM CELLS

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Nutrient resources such as glucose, fatty acids, or certain amino acids, and their transport have been implicated as important regulators of stem cell behaviors. Among nutrient resources, glucose plays important roles in stem cell fate determination and embryonic development. Stem cells have the ability to migrate towards sites of injury, which is essential to maximize their therapeutic benefits. However, it is still not known how high glucose contributes to regulation of stem cell migration. Thus, we investigate the mechanism underlying the effect of high glucose (25mM) on regulation of human mesenchymal stem cell (hMSC) migration and related signaling pathways. Reactive oxygen species (ROS) generation by high glucose treatment promoted the activation of c-Jun N-terminal kinase (JNK), which regulated γ-secretase that can cleave Notch proteins, followed by nuclear translocation of Notch intracellular domain (NICD). The inhibition of the Notch pathway suppressed high glucose-induced Akt activation and cell migration. These activities were blocked by N-acetyl-L-cysteine (ROS inhibitor), SP 600125 (JNK inhibitor), L-685,458 (γ-secretase inhibitor), LY 294002 (PI3K inhibitor), wortmannin (PI3K inhibitor), or an Akt inhibitor. High glucose also enhanced GSK3β phosphorylation and β-catenin activation. Furthermore, the Polycomb repressive complex 2 (PRC2) subunit Ezh2 interacted with β-catenin to promote its translocation into the nucleus. High glucose-induced GSK3β activity also increased levels of cytoplasmic Snail, and subsequently Snail translocated into the nucleus. Our results show that Snail is required for Ezh2 recruitment and for the subsequent its association with other PRC2 components, Suz12 and Eed, to the E-box of the E-cadherin promoter (CDH-1) under high glucose conditions, which in turn causes repression of E-cadherin. In conclusion, this study implicates high glucose stimulates the migration of hMSCs through E-cadherin repression via Notch, Snail, and PRC2 signaling pathways.

W-1065

COMPARATIVE CHARACTERISTICS OF MESENCHYMAL STEM CELLS FROM CLONAL POPULATIONS AT DIFFERENT SITES OF RAT LARYNX

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Various cell types of laryngeal subsites have been isolated and suggested for the candidate of laryngeal tissue-resident stem/progenitor cells, but it still remains to elucidate the stem cell characteristics and their biological roles in laryngeal tissue regeneration. This study was conducted to investigate the comparative mesenchymal stem cells (MSC) characteristics of

clonal populations from rat laryngeal mucosa (LM), vocal fold lamina propria (LP), and macula flava (MF) comparing with bone-marrow (BM)-MSCs. Single cell-derived laryngeal clonal cells were isolated by the subfracitionation culturing method following microdissection of epiglottic LM, vocal fold LP, and MF of rat larynx. Several clonal populations from three laryngeal subsites were chosen and expanded in vitro. MSC characteristics of self-renewal, marker expression, differentiation potential, and immunosuppressive activity as well as laryngeal tissue-specific marker expression were investigated. LM- and LP-resident clonal cells showed fibroblast-like features but MF-resident clonal cells showed stellate cell morphology and different expression of GFAP and desmin. All laryngeal clonal cell populations showed MSC-like characeteristics in terms of their proliferative activities, marker expression and differentiation potential into fat, bone, and cartilage cell lineages with some different potential in each clones. They also possessed immnomodulatory properties as comparable with BM-MSCs. Regarding fibroblastic differentiation, they showed the capability of differentiation into FSP-expressing vocal fold fibroblasts. These results suggest that there are laryngeal tissue-specific stem cells in different subsites of larynx with some different MSC characteristics and tissue-proper properties. Further investigation needs to be performed to investigate their biological roles in laryngeal tissue regeneration and clinical implications.

W-1066

ROLE OF NOTCH SIGNALING IN GLYCOLYSIS REGULATION UNDER HYPOXIC CONDITIONS

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Human adipose-derived multilineage progenitor cells (hADMPCs) 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 hADMPCs. Some somatic stem cells including hADMPCs 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 hADMPCs. These effects were mediated by Notch signaling pathway. Hypoxia significantly increased the level of activated Notch1 and expression of its downstream gene, HES1. Furthermore, hypoxia markedly increased glucose consumption and lactate production of hADMPCs, which decreased back to normoxic levels upon treatment with a γ-secretase inhibitor. We also found that HES1 was involved in induction of GLUT3, TPI, and PGK1 in addition to reduction of TIGAR and SCO2 expression. In addition, we also found that HES1 was involved in the induction of GLUT3 expression through NF-κB signaling. Finally, 2-deoxy-glucose, an inhibitor of glycolysis, attenuates the proliferation rate of hADMPCs, whereas the aerobic respiration block by NaN3 did not decrease the proliferation; rather, it increased proliferation at a low concentration, which may support our data indicating that the metabolic switch from mitochondrial respiration to glycolysis provides a growth advantage to hADMPCs. These results clearly suggest that Notch signaling regulates glycolysis under hypoxic conditions and thus likely affects the cell lifespan via glycolysis.

W-1067

IN VITRO COMPARATIVE STUDY OF STEM CELL CHARACTERISTICS OF HUMAN PERIODONTAL LIGAMENT STEM CELLS UNDER SERUM-FREE CULTURE CONDITIONS

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Previously, we isolated and characterized periodontal ligament stem cells (PDLSCs) derived from human wisdom teeth using fetal bovine serum-containing growth medium (FBS+GM). PDLSCs showed higher proliferative potential than bone marrow-derived stem cells and they possessed identical multilineage differentiation capabilities. However, it is difficult to use FBS+GM for clinical applications due to immune rejection or infection caused by FBS. In the present study, we developed FBS-free culture medium, MSC-T4, and examined whether the new media can maintain and stimulate the proliferative potential and the multidifferentiation capacity of PDLSCs. PDL tissues were gently separated from the extracted human wisdom tooth of a healthy volunteer aged 27 years and were digested with a solution of 3 mg/mL collagenase type I and 4 mg/mL dispase. Isolated cells were cultured in either DMEM/F12 containing 15% FBS (FBS+GM) or MSC-T4 up to passage 3 before use. PDLSCs were subjected to a proliferative potential test, RT-PCR, flow cytometry, and multidifferentiation induction into osteogenic, adipogenic, chondrogenic, and neurogenic lineages. MSC-T4 cells exhibited significantly greater growth than FBS+GM cells ($p < 0.01$). Cell cycle analysis also revealed that MSC-T4 cells contained a significantly higher percentage of cells in the G_2/M phase (20.3 ± 0.8%) than the FBS+GM cells (9.7 ± 1.0%), while MSC-T4 cells contained a significantly lower percentage of cells in the G0/G1 phase (65.3 ± 1.5%) than the FBS+GM cells (81.4 ± 0.7%). Both media-cultured PDLSCs were positive for CD44, CD90, and CD105, as determined by flow cytometry analysis, and expressed osteogenic (Vimentin, Runx2, and Type I collagen), neurogenic (Nestin), and stem cell marker genes (Nanog, Oct3/4, and Sox2), as determined by RT-PCR. MSC-T4 cells showed an equivalent multidifferentiation capability to that of FBS+GM cells. These results suggest that MSC-T4 culture medium is more efficient for the maintenance of the stem cell phenotype and multidifferentiation potential and further promotes stem cell proliferation compared with FBS+GM. This newly developed culture medium might facilitate the ex vivo expansion of stem cells with multipotency in clinical applications.

W-1068

PRETREATMENT WITH DEFEROXAMINE AND/OR FGF-2 INCREASES VEGF SECRETION OF HUMAN PERIODONTAL LIGAMENT STEM CELLS

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The survival of stem cell transplants in vivo is dependent on a sufficient blood vessel growth (i.e. angiogenesis) for the supply of oxygen and vital nutrients. Recently, adult stem cells were detected in dental tissues such as the periodontal ligament (PDL). These human periodontal ligament stem cells (hPDLSC) have been shown to possess a self-renewal capacity and a (mesodermal) multi-lineage differentiation potential. Furthermore, hPDLSC can easily be isolated from human teeth and have been shown to modulate angiogenesis, and are therefore considered to be promising candidates for regenerative purposes. The objective of this study is to further improve these intrinsic properties of hPDLSC by pharmacological pretreatment in vitro. With this pretreatment we aim to upregulate the secretion of angiogenic factors thereby enhancing the effects of hPDLSC on endothelial cell proliferation, migration and tube formation, key events of angiogenesis. hPDLSC were pretreated with fibroblast growth factor-2 (FGF-2) for 72 hours and/or with deferoxamine (DFX), an iron chelator, for 24 hours. An antibody array was used to detect changes in the angiogenic expression profile of pretreated hPDLSC compared to nontreated hPDLSC and revealed an increased secretion of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) in pretreated hPDLSC. ELISA data confirmed these findings and illustrated that the VEGF secretion was still elevated 48 hours after removal of DFX. The factors secreted by both control and pretreated hPDLSC elicited an increase in endothelial cell migration, whereas there was no effect on endothelial cell proliferation. Currently, the effects of pretreated hPDLSC on tube formation are being investigated as well as their ability to induce in vivo angiogenesis in the chicken chorioallantoic membrane assay. Eventually, improving the angiogenic properties of these stem cells could aid the development of cell-based therapies for pathologies associated with insufficient angiogenesis such as stroke and myocardial infarction.

W-1069

EQUINE ENDOMETRIAL MESENCHYMAL STROMAL/STEM CELLS: ISOLATION, CULTURE AND GENE EXPRESSION PROFILES

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Equine mesenchymal stromal cells (MSCs) are mainly harvested from bone marrow or adipose tissue requiring surgical procedures. While human endometrium is known to harbor mesenchymal precursor cells, the presence of MSCs in equine endometrium, a dynamic tissue undergoing regenerative changes during estrus cycles and puerperium, has not been investigated. This study reports for the first time the isolation, culture and characterization of MSCs from equine endometrium which may provide a convenient source for veterinary regenerative therapies. Endometrial tissue (n=4 horses) was digested using collagenase I and red blood cells were excluded from the resultant single cell solution using a density gradient. CD324 (E-cadherin)-bound magnetic beads were utilized to separate epithelial (CD324+) from stromal (CD324-) cell fractions. The two fractions were separately cultured in DMEM/F-12 containing 10% fetal bovine serum and gene expression was analyzed later. Both isolated cell fractions, CD324+ and CD324-, were plastic-adherent and grew well under standard MSC culture conditions. The CD324+ fraction primarily contained epithelial cells which rapidly formed cobblestone-like clusters, whereas the CD324- fraction contained mainly fibroblast-like cells which grew fast and quickly became confluent. Epithelial clusters in CD324+ cell lines were overtaken by fibroblasts and eventually disappeared after passage three. Consistent with morphological differences, qPCR analyses revealed higher expression of the epithelial markers, CD324 and CD227, in the cultured CD324+ fraction at passage one ($p<0.05$), and a subsequent decrease in the expression of both markers in the two cultured cell fractions. Both cultures of CD324+ and CD324- fractions at passage one had detectable mRNA levels of MSC (CD29, CD73, CD90, CD105)and perivascular (CD146, NG2) markers, with relatively higher expression of CD105 and lower expression of CD29 in CD324- cells than in CD324+ cells ($p<0.05$). In conclusion, we showed the presence of putative MSCs in equine endometrium. We successfully isolated and cultured these cells, which might in the future provide a suitable source for regenerative therapy. A set of surface marker antibodies was also established which will be used in further studies to characterize equine endometrial MSCs.

W-1070

A NOVEL AND FASTER CULTURE SYSTEM TO HIGHLY ENRICH MOUSE MESENCHYMAL STEM AND PROGENITOR CELLS USING MESENCULT™ PROLIFERATION MEDIUM WITH MESENPURE™

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Cultures of mesenchymal stem and progenitor cells (MSCs) isolated from mouse bone marrow (BM) or compact bone (CB) typically contain unwanted hematopoietic cells. We developed MesenCult™ Proliferation Medium with MesenPure™ to inhibit hematopoietic cell growth and expand primary BM- and CB-MSCs. Cells were seeded (P0) and then maintained for multiple passages in MesenCult™ Proliferation Medium with MesenPure™ or without (control). At each passage, cells were inspected and analyzed by flow cytometry for expression of CD45, Sca1, and CD29. In CFU-F assays, primary cells (P0) were plated at 2.5-10 x 105 cells/cm2, maintained for 14 days and the resulting colonies stained with Toluidine Blue for counting. The range of CFU-F in BM cultures containing MesenPure™ was ~42% higher than in control cultures without MesenPure™: 47-303 vs 30-230 per 106 cells (n=3). Similar results were obtained with CB cultures with and without MesenPure™: 77-640 vs 51-450 per 106 cells (n=3). Flow cytometric analysis of P0 BM cultures revealed that CD45+ cells were reduced from 91% to 29% in cultures containing MesenPure™ compared to

controls. The CD45- fraction increased from 9% to 71% in the presence of MesenPure™, with a concomitant increase in CD45- / CD29+/ Sca1+ MSCs from 75% to 96%. Similar results were obtained in CB cultures: CD45+ cells decreased from 93% to 1%, the CD45- fraction increased from 7% to 96%, and CD45-/ CD29+/ Sca1+ MSCs increased from 80% to 96% in cultures containing MesenPure™ compared to controls. The benefit of MSC enrichment was demonstrated by the ability to induce efficient osteogenic and adipogenic differentiation from cells maintained for 2 passages in MesenPure™ and seeded at only 4 x 104 cells/cm2. Similar differentiation of control cells required seeding at 3-fold higher plating density. These results show that addition of MesenPure™ to cultures of BM- and CB-derived MSCs rapidly depletes unwanted CD45+ cells and improves overall MSC expansion and differentiation.

W-1071

IL-8 IS ABLE TO COMPENSATE THE REDUCED MIGRATORY ACTIVITY OF MSC AFTER SM EXPOSURE

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Mesenchymal stem cells (MSC) are important for the regeneration of wounded skin. Essential requirement for a MSC driven wound regeneration is an active migration into the wounded area. Is the skin wounded for example by the bi-alkylating agent sulfur mustard (SM), patients show chronic wounds with an impaired wound healing for weeks till month. As for patients with chronic wound healing disorders is known that migratory capacity of MSC is altered we therefore wanted to investigate how SM affects MSC and if these findings might constitute a better understanding of the effect of sulfur mustard gas with respect to skin wounds. One aspect we analyzed in the effect of SM exposure to the secretome of MSC. While using slide-chip arrays we ascertain the semi-quantitative secretion of 275 cytokines. Out of these we observed the strongest impact from SM to the secretion of IL-8, which was significant reduced. Beside that we also observed a significant reduction of the migratory activity of MSC under influence of this alkylating agent in a dose dependent manner using a modified Boyden Chamber system. As IL-8 is known to enhance the migratory activity of MSC we supposed a direct link between the inhibitory effect of SM onto MSC migration and the secretion of IL-8. To proof that question we repeated the SM exposure experiments while adding additional IL-8. Additional IL-8 was able to repeal the inhibitory effect of SM onto MSC migration. These findings demonstrate a direct dependence from the anti-migratory effect of SM to MSC via IL-8. These results can indicate potential therapeutic opportunities for using IL-8 to reduce chronic wound healing after exposure of SM.

W-1072

SEVERE LOSS OF HETEROGENEITY IN MSC EXPLANT CULTURES AFTER PROLONGED IN VITRO EXPANSION

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Cultured umbilical cord (UC) tissue pieces can give rise to mesenchymal stem cell (MSC) cultures. These MSC explant cultures grow continuously out of UC pieces, have multi-lineage differentiation capacity and typically express the surface markers CD73, CD90 and CD105. Moreover, MSC explant cultures show no typical signs of senescence directly after initiation and can provide a constant source of MSCs for cell therapies. However, the initiated MSC explant cultures age during the obligatory in vitro expansion and clonal selection may occur. Clones optimally adapted to the cultivation conditions rather than clones with the desired clinical features may become dominant at the time of transplantation. This selection process is poorly understood and may explain the varying treatment benefits observed among patients and applications. To address clonal dynamics in MSC cultures, UC pieces (n=15) were transduced with stably integrating lentiviral vectors, coding for one of three different fluorescent proteins and a highly variable barcoding region. This allowed us to follow the clonal development by flow cytometry and to identify each transduced cell and their progeny by Ion Torrent deep sequencing. We analyzed 63 MSC explant samples at various time points to monitor the clonal composition of serial explant cultures over time. We observed a general reduction in clonal complexity during prolonged in vitro culture. Sequencing results implied that clonal selection already occurred in early passages, reflected by detection of less than 10 barcodes, possibly suggesting the presence of even fewer clones. In some cases, the identical dominant barcodes were observed in different explant cultures initiated by the same UC piece, supporting the idea that only a limited number of founder cells were present in the original UC samples. Our results show that MSC cultures are subjected to a strong selection pressure during the necessary in vitro expansion phase and are composed of only a few clones. This highlights the need for improved characterization of expansion techniques related to the heterogeneity of clinically applied MSC. The in vitro selection pressure influences the characteristics and performance of the transplanted cells and may account for differences observed in clinical trial outcomes.

W-1073

GALECTIN 3 INHIBITION IN CARDIAC MESENCHYMAL STEM CELLS REMARKABLY IMPAIRS ITS PROLIFERATION, CELL CYCLE AND IMMUNOMODULATORY PROFILE

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Galectin 3 is a soluble lectin involved in a variety of processes, both physiological or pathological, including cell proliferation and apoptosis. The role of this molecule in the regulation of cardiac fibrosis and remodeling and its contribution to the development and progression of heart failure has been recently described. Since cardiac mesenchymal stem cells (CMSC) express galectin 3, we evaluated the effects of galectin-3 inhibition in CMSC. Cardiac mesenchymal stem cells (CMSC) were obtained from cardiac tissue of adult mice by partially digestion with collagenase type I and culture as explants. Cells migrated from the cardiac tissue and attached to the surface of the culture flask, displaying polymorphic and fibroblast like morphological characteristics. Cell population displayed cell surface markers and had differentiation potential into adipocytes, osteocytes

and chondrocytes characteristic of mesenchymal stem cells. The knockdown for galectin 3 was achieved by lentiviral transduction with shRNA. To confirm the knockdown, qRT-PCR as well as immunofluorescence demonstrated a low expression of galectin 3 in comparison with mock cells. Similarly, the expression of collagen I was drastically reduced by galectin 3 knockdown. Assessment by 3H-thymidine incorporation assay showed a decreased proliferative rate of CMSC knockdown for galectin 3, which was in accordance with the cell cycle analysis performed by flow cytometry, showing a reduced division cell ratio. Moreover, analysis by qRT-PCR showed a reduction of cyclin D1 gene expression in CMSC knockdown for galectin 3. Importantly, these cells have a reduced inhibitory activity in lymphoproliferation assay and TGFβ1 production when compared to mock cells. Knockdown of galectin 3 decreased cell proliferation, altered the cell cycle and reduced the immunomodulatory capacity of CMSC, indicating the importance of galectin 3 for mesenchymal stem cell functions.

HEMATOPOIETIC CELLS

W-1075

A HUMANIZED BONE MARROW NICHE MODEL IN MICE TO STUDY THE INTERACTIONS STROMA-HSC IN BOTH PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

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In adult, hematopoietic stem cells (HSCs) primarily reside in the bone marrow (BM) cavity, where they interact with different types of stromal cells. In parallel, leukemia stem cells (LSC) are also in contact with BM niche in order to grow and expand. Both HSC and LSC cannot be maintained ex vivo without the addition of a stroma support, indicating that they are dependent on their microenvironment for their survival/maintenance. Therefore, the study of niche components and interactions is crucial in order to better understand how they regulate HSCs, both in physiological or pathological conditions. In a previous study we reported an implant based on BMP-2 and human Mesenchymal stem cells (hMSCs) which yields subcutaneous (s.c.) ossicle formation in 15 days with "humanized" tissue formed by chimeric human-mouse bone, BM, cartilage, adipose and fibrous tissues. Based on this, here we report in vivo assays in order to study the relevance of human factors within the stromal component for in vivo HSC and LSC maintenance/engraftment. Using non-irradiated NSG mice, hHSC were co-implanted with hMSC/BMP-2 in a 3D gelfoam scaffold s.c in the back of NSG mice. Control scaffolds were also implanted. Samples were harvested at different time points and processed for FACS or histology studies. For leukemia the ML1 cell line was used as a rapid AML engraftment model. First, hMSC/BMP-2 scaffolds were s.c. implanted into NSG followed 20 days later by the i.v injection of ML1-GFP-Luciferase cells and sequential ossicle-engraftment was monitored by bioluminescence. Finally ossicles and femur samples were harvested and processed for histology or FACS studies. Our results indicate that this humanized-ossicle approach allows hHSC engraftment and maintenance. Moreover, leukemic cells were able to engraft in these ossicles, in contact with human bone, adipose and fibroblastic cells. In conclusion, here we described a tool to study in vivo stroma-hHSC interaction/cross-talk mechanisms. It is based on an initial hMSC differentiation, which forms humanized mature bone tissue in mouse. hHSC or AML cells are able to engraft within it and interact with the human cells present there, providing a new vision of human stroma-HSC interactions.

W-1076

IDENTIFICATION OF CRITICAL FACTORS AFFECTING MICROGLIA RECONSTITUTION FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR DESIGNING THERAPEUTIC APPROACHES

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A critical need exists to enhance and fasten microglia turnover with donor cells following Hematopoietic Cell Transplantation (HCT) in order to anticipate the time of clinical benefit and improve the efficacy of the transplant in severe diseases like Lysosomal Storage Disorders (LSDs). However, obtaining microglia reconstitution is a challenging goal, particularly when clinically predictive settings are employed. Since very little is known on the modalities of microglia turnover in physiological and pathological conditions, we investigated in deep this phenomenon and showed that microglia reconstitution after HSCT is i) driven by a fraction of the transplanted HSC pool homing to the brain short-term after infusion and ii) positively affected by the use of a conditioning regimen capable of ablating functionally-defined brain-resident myeloid precursors. In order to identify and characterize the fraction of early hematopoietic brain immigrants capable of microglia reconstitution in pre-conditioned LSD brains we are testing the capability of different cell populations that differ for stemness and commitment to contribute to microgliosis, both in the murine and in the human setting. In particular, in the murine setting the populations under testing are identified within c-kit+Sca1+ Lin- (KSL) cells according to the differential expression of the markers CD150 and CD48, while in the human setting we studied the potential of differentially labelled CD34+CD38- HSCs and more committed progenitors to repopulate the brain myeloid compartment of immunodeficient mice. Importantly, we observed that early stem/progenitor cells rather than more committed elements mostly contribute to microgliosis after HSCT in both settings. Moreover, in order to attempt characterizing the bona fide microglia progenitors ablated by the conditioning regimen, we are studying the transcriptional profile of different fractions of the brain myeloid compartment of naïve and conditioned and transplanted mice and, in parallel, analysing specific markers which could be enriched in bona fide myeloid/microglia progenitors. These two approaches could provide important insights for a more precise definition of the nature and function in vivo of brain myeloid progenitors.

W-1077

INTERACTION OF TUMOR CELLS WITH THE BONE MARROW MICROENVIRONMENT

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The bone marrow microenvironment, including mesenchymal stromal cells (MSC), supports self-renewal and differentiation of hematopoietic stem and progenitor cells (HSPC). The supportive functions of the niche could also be relevant for its neoplastic transformation. Dissemination of breast cancer cells into the marrow has been described even in early stages of the disease. We investigated the modulation of the niche post invasion by breast cancer cells. In vitro coculture models of breast cancer cell lines- MDA-MB231 and MCF-7 with primary MSCs derived from bone marrow of healthy donors were utilized in the study. Hypoxic culture conditions (0.5% oxygen), mimicking the niche environment, were used in the experiments. The presence of breast cancer cells competitively reduced HSPC adhesion to MSCs (88% by MDA-MB 231 cells; $p<0.005$ and 73% by MCF-7 cells; $p<0.005$). Atomic force microcopy based single -cell force spectroscopy studies demonstrated higher binding force between tumor cells and MSCs, as compared to HSPCs. MDA-MB231 and MCF-7 cells express high levels of Intercellular adhesion molecule-1(ICAM-1), which has been correlated with metastasis and tumor relapse.ICAM-1 knockdown/blocking studies showed that tumor cell mediated reduction in HSPC adhesion to MSCs was mediated via homophilic interaction between ICAM-1 expressed on tumor cells and MSCs . A cytokine array showed altered growth factor profile in MSCs upon invasion by tumor cells [basic fibroblast growth factor (bFGF) and platelet derived growth factor-beta (PDGF-BB) showed a 2.2 fold upregulation and 0.5 fold downregulation in tumor cells- MSC cocultures, respectively]. Based on the array, upregulation of bFGF coupled with PI3K-Akt activation was found to mediate increased proliferation of MSCs and breast cancer cells in cocultures. Interestingly, breast cancer cells caused a reduction of osteogenic differentiation of MSCs by downregulation of PDGF-BB. Organotypic cocultures of breast cancer cells and MSCs also led to reduced osteogenic differentiation of MSCs. Furthermore, breast cancer cells caused a reduction in the cobblestone area forming cells in long-term HSPC-MSC cocultures. Our findings describe the mechanisms regulating neoplastic transformation of the niche by tumor cells via interference with the stromal compartment.

W-1078

ACTIVATION OF MEDIAL HOXA GENES IS REQUIRED FOR ESTABLISHMENT OF "STEMNESS" IN HUMAN HSCS

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The ability of hematopoietic stem cells (HSCs) to self-renew, generate all lineages of the blood and reconstitute the hematopoietic system, enables their use in treating diseases of the blood and immune system. The shortage of HSCs from bone marrow and cord blood could potentially be overcome by generating HSCs from pluripotent cells. However, lack of understanding of the regulatory mechanisms governing HSC function has prevented their in vitro generation. Using a two-step human ESC differentiation protocol during which CD34+ cells from embryoid bodies were co-cultured on OP9-M2 stroma to mimic the environments where HSCs develop and expand, we derived hematopoietic cells with the CD34+CD38-CD90+CD45+GPI-80+ human HSC immunophenotype. However, although ESC-derived cells differentiated into definitive erythroid, myeloid, and T-cells, they displayed impaired self-renewal and engraftment ability compared to hematopoietic stem/progenitor cells (HSPCs) isolated from the fetal liver (FL). Microarray analysis of immunophenotypic HSPCs from ESC-derived cells and different stages of development revealed successful transition of ESC-HSPCs from the hemogenic endothelium towards a fetal-liver like HSPCs with the upregulation of genes critical for HSPC function. However, despite the close correlation between ESC-HSPCs and FL-HSPCs, the medial HOXA genes were not activated in ESC-derived cells, similar to immature HSPCs from the 1st trimester placenta. Knockdown of HOXA5 or HOXA7 in FL-HSPCs recapitulated the self-renewal defects observed in ESC-HSPCs. The regulator of HOXA genes, MLL1, was expressed, but not bound to HOXA genes in ESC-derived cells, suggesting the inability to recruit MLL1 to HOXA genes prevents their activation during ESC differentiation. We identified a region of 89% sequence homology to the mouse lincRNA Mistral, which in mice recruits MLL1 to HOXA6/HOXA7 genes, in a similar location in the human genome. Moreover, there was high expression of putative human MISTRAL in FL-HSCs but not in their differentiated progeny or ESC-derived hematopoietic cells. These data identify insufficiency of HOXA gene expression is a developmental barrier for generating HSCs and nominate MISTRAL as a novel component of the fetal HSC regulatory machinery that conveys "stemness" in hematopoietic cells.

W-1079

MONITORING AND MODIFICATION OF HEDGEHOG SIGNALLING IN HUMAN EMBRYONIC HAEMATOPOIESIS

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The first definitive haematopoietic stem cells (dHSCs) emerge in the ventral domain (AoV) of the dorsal aorta (Ao) in both the mouse and human embryo. In the mouse it was reported that hedgehog (Hh) proteins, expressed ventrally in the gut and dorsally in the notochord, are important effectors of early HSC induction. However, studies of the haematopoietic differentiation of human and mouse pluripotent stem cells (PSCs) have thus far provided conflicting evidence for the role of Hh signalling in the production of early haematopoietic progenitor cells (HPCs). Here we aimed to evaluate the role of Hh signalling in early human haematopoiesis, both in the embryo itself and during haematopoietic differentiation of hPSCs. Ventral (AoV) and dorsal (AoD) domains of Ao were dissected from Carnegie Stages 15 and 16 (when dHSCs are first

detected) human embryos, which were obtained following elective medical termination of pregnancy. At both stages the expression of Hh signalling genes (SHH, GLI1, PTCH1) by RT-qPCR was markedly higher in AoD than AoV. Hence, we report for the first time that Hh signalling exhibits dorso-ventral polarity in the human embryo around the stage of dHSC emergence. To study the role of the Hh pathway during haematopoietic differentiation in vitro, we used an established serum-free, feeder-free differentiation protocol, which allows efficient generation of HPCs. Over the 10 days differentiation, there was no significant change in the expression of Hh signalling genes. However, by sorting specific populations at day 6, we noted that the expression of GLI1 and PTCH1 was consistently higher in the haematopoietic (CD43+) rather than non-haematopoietic (CD43-CD34+/-) cells. We attempted to modulate Hh signalling by addition of a Hh agonist and antagonist during the first 3 days or throughout the 10 day protocol. However, no consistent effect was observed on the total cell count, nor on production of HPCs, as assessed by the CFU-C assay and surface marker expression, which is at odds with published work using an embryoid body differentiation system. Hence further research is required to unravel the complex role of Hh signalling during the initiation of haematopoiesis both in vivo and in vitro. We believe this could facilitate the derivation of an efficient differentiation protocol for the production of dHSCs in the future.

W-1080

LONG-TERM-REPOPULATING HEMATOPOIETIC STEM CELLS ARE DISPENSABLE IN STEADY STATE BUT ESSENTIAL FOR STRESS HEMATOPOIESIS

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The small population of self-renewing bone marrow hematopoietic stem cells, defined by their unique ability to long-term repopulate lethally irradiated recipients upon transplantation (LT-HSCs), are thought to maintain the hematopoietic system for the life of the organism by continuously producing more differentiated cell populations. We report a mouse model allowing inducible Cre/loxP-mediated depletion of LT-HSCs in vivo. Upon depletion of LT-HSC we observed, that this population did not recover for at least half a year. Unexpectedly, absence of LT-HSCs did not compromise steady state production of blood cells, demonstrating that the self-renewal capacity of short-term-HSCs and progenitor cells has been underrated in the past. Hematopoiesis rapidly collapsed, however, in LT-HSC-depleted animals in response to treatment with 5-fluorouracil. We conclude that LT-HSCs are not required to maintain adult hematopoiesis under steady state conditions, but rather represent a reserve population for situations of extreme hematopoietic stress.

W-1081

IDENTIFICATION OF KEY REGULATORS OF SYMMETRIC VERSUS ASYMMETRIC CELL DIVISIONS DURING HEMATOPOIETIC LINEAGE SPECIFICATION

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Hematopoietic stem cells (HSCs) contain lifelong potentials to self-renew and to create progenitors of all mature blood cells. According to the current view, HSC homeostasis is controlled by both, HSC-niches as well as asymmetric cell divisions. Our previous studies linked the process of asymmetric cell division of human hematopoietic stem and progenitor cells (HSPCs) to the expression kinetics of the stem cell surrogate antigen Prominin 1/CD133. Furthermore, by characterizing human HPSCs subpopulations by means of their CD133 surface expression we gained evidence that CD133+ multipotent progenitors (MPPs) create CD133+ lymphomyeloid (LMPP) and CD133low erythromyeloid (EMP) daughter cells. The LMPP lineage was shown to contain lymphoid and neutrophil potentials, while the EMP lineage mainly creates eosinophils and basophils as well as erythrocytes and megakaryocytes. Regarding lineage specification, we showed for the first time that under conventional culture conditions almost all MPPs divide asymmetrically to create a set of LMPP and EMP daughter cells, resulting in a loss of MPPs after the first cell division. Thus, our data suggest that under conventional culture conditions asymmetric cell divisions are rather lineage instructive than self-renewing. Now, aiming to identify key factors regulating the MPP division mode, we study whether conditions reported to promote HSC/MPP expansion interfere with the outcome and symmetry of the HSC/MPP cell division. In this context we co-cultured human HSCs/MPPs with various murine and primary human stromal lines (human bona fide MSCs) and surprisingly observed that LMPPs are maintained and expanded but not MPPs. This contrary finding can be attributed to the former experimental definition of multipotent cells based on the classical model of hematopoiesis, according to which cells with dual lymphocyte and granulocyte (conventionally neutrophil) potentials can insufficiently be considered as multipotent. Currently, we test other culture conditions reported to expand human HSCs/MPPs in vitro. After confirming any of these conditions as HSC/MPP expansion condition, we will analyze its impact on the division mode of HSCs/MPPs using multi-parametric flow cytometry, live-cell imaging and functional differentiation assays at the single cell level.

W-1082

PTPN13 AND BETA-CATENIN LEVELS ARE IMPORTANT TO REGULATE HEMATOPOIETIC STEM CELLS QUIESCENCE

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Contrary to other processes that are mainly restricted to embryonic development, the differentiation of hematopoietic stem cells

(HSCs) into the different blood lineages occurs along the life of the individual. The relevance of HSCs in regenerative medicine is remarkable, and the possibility of expanding HSCs in vitro, preserving their multipotency, would be a milestone in this regard. Therefore, understanding the orchestration of the multiple intercellular and intracellular signaling events that control HSCs quiescence and self-renewal in vivo should help to attain this goal. Beta-catenin is an intriguing protein, since it shows a role as a cell adhesion molecule, or as the nuclear effector of the canonical Wnt signaling. Despite the importance of Wnt/beta-catenin for hematopoiesis in Xenopus, the role of beta-catenin in mammalian hematopoiesis remains highly controversial. We have recently shown that the protein tyrosine phosphatase PTPN13 regulates beta-catenin stability and transcriptional activity during in vitro megakaryopoiesis. In the present work we studied how the downregulation of PTPN13 or beta-catenin affects in vivo hematopoiesis. Lethally irradiated mice transplanted with Lin- bone marrow cells in which PTPN13 or beta-catenin had been silenced showed a significant increase of long-term (LT) and short-term (ST) HSCs. A decrease in cycling cells was also found, together with an increase in quiescence. Cell adhesion to the bone was enhanced by PTPN13 or beta-catenin downregulation, which could be explained by the upregulation of several genes coding for integrins and several cadherins we observed. We also show that PTPN13 or beta-catenin levels are regulated by different cytokines that are involved in the regulation of HSCs, such as Wnt, TPO, CXCL-12 or SCF. Our data are consistent with the notion that the levels of PTPN13 and beta-catenin must be strictly regulated by extracellular signaling to regulate HSC attachment to the niche and the balance between proliferation and quiescence. Low levels of these two proteins induced by extracellular signaling would favor a stronger attachment to the niche, which would help to preserve HSC quiescence.

W-1083

MOLECULAR SIGNATURES OF HETEROGENEOUS STEM CELL POPULATIONS ARE RESOLVED BY LINKING SINGLE CELL FUNCTIONAL ASSAYS TO SINGLE CELL GENE EXPRESSION

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The discovery of considerable heterogeneity in the self-renewal durability of adult hematopoietic stem cells (HSCs) has challenged our understanding of the molecules involved in population maintenance throughout life. Gene expression studies in bulk populations are difficult to interpret since multiple HSC subtypes are present and HSC purity is typically less than 50% of the input cell population. Numerous groups have therefore turned to studying gene expression profiles of single HSCs, but again these studies are limited by the purity of the input fraction and an inability to directly ascribe a molecular program to HSCs with durable self-renewal. Here we combine single cell functional assays with flow cytometric index sorting and single-cell gene expression assays to gain the first insight into the gene expression program of murine HSCs that possess durable self-renewal. Analysis of over 90,000 Q-RT-PCR reactions from single stem and progenitor cell populations allowed us to identify the molecular overlap from four commonly used HSC isolation strategies. Using the different functional purities in a weighting algorithm, we were able to cluster individual cells based on the expected proportion of functional HSCs in each fraction. We were then able to further resolve the gene expression programs of phenotypic and functional HSCs by linking single cell RNA-sequencing analysis to single cell transplantation assays. These analyses showed that HSCs lacking durable self-renewal were transcriptionally primed for proliferation and differentiation. Finally, we used the individual cell surface marker profiles of the single HSCs to further resolve the functional purity of HSCs and confirmed these predictions by single cell transplantation assays. In conclusion, combining index sorting with single cell functional and transcriptional analyses can is a powerful approach that can be used in any stem cell system that has single cell functional assays and sets the stage for linking key molecules with defined cellular functions.

W-1084

STATISTICAL RELATIONSHIP BETWEEN LEVELS OF AUTOLOGOUS BONE MARROW-DERIVED CD34+ CELLS AND CLINICAL STATUS OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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ALS is a fatal disease characterized by a progressive degeneration of motoneurons. Several studies described that degenerative diseases develop following an imbalance between cellular loss and tissue renewal. Our objective was to investigate the relation between bone marrow (BM) CD34+ cells and clinical status of patients undergoing stem cell therapy. An open-label pilot study in 85 patients with ALS was performed and the clinical status assessed with ALSFRS-R. The BM was harvested from the iliac crest and infused intrathecally after an on-site cell separation with density centrifugation. Samples of BM were analyzed for CD34+ cells content according to the ISHAGE guidelines and the vitality assessed with the 7-AAD dye. Earlier deaths were found higher in patients with more than 100 CD34+ cells/µl (6,1 ± 3,1 vs. 8,4 ± 5,7 months; p=0,021). We found a relation between between high vitality and high ALSFRS-R in the follow-up (p=0,009). Patients with more than 80% of vitality had more time passed between diagnosis and treatment (26,7 ± 31,4 vs. 21,92 ± 17,3 months; p=0,049), had lower ALSFRS-R before treatment (28,5 ± 13,8 vs. 30,2 ± 9,5; p=0,035), higher ALSFRS-R after treatment (31,6 ± 13,2 vs. 28,8 ± 7,5; p=0,041) and died later (6,8 ± 5,5 vs. 5,6 ± 2,5 months; p=0,049). Early deaths were related with high levels of CD34+ cells/µl. The patients with more vitality even they had more years passed since the diagnosis and worse ALSFRS-R respond better to treatment, denoting the importance of vitality for autologous transplant. The patients that had more advance clinical stage had apparently better BM conformation with more CD34+ cells/µl, however we can assume that the problem of the SCs in these patients is not the number available but the capacity to respond to an injury. The patients with advanced disease, regardless the treatment are probably producing a strong signal to the BM that makes a highly active SCs production, however the lack of migrating capacity or functional deficiency of these SCs could cause the imbalance in tissue renewal, these should be confirm in the future by

sampling SCs levels in peripheral blood. These findings also support the rationale of our method while an infusion of SCs directly in the affected organs in intent to overcome the lack of migration capacity.

W-1085

ZEB2 IS REQUIRED FOR MULTILINEAGE DIFFERENTIATION OF ADULT MURINE HEMATOPOIETIC CELLS

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The differentiation of hematopoietic stem cells (HSC) into specialized blood cells is tightly controlled by a complex network of transcription factors (TF). The zinc finger E-Box binding TF Zeb2 is known to govern the epithelial to mesenchymal transition (EMT) during embryonic development and tumor progression and metastasis in an adult organism. Our previous work showed that deletion of Zeb2 within hematopoietic compartment resulted in early embryonic lethality due to differentiation and migration defects. More recently, Zeb2 has been identified as a novel oncogene in early T-cell precursor leukemia (ETP-ALL). In order to identify the role of Zeb2 in adult hematopoiesis, the Mx1-Cre based inducible Zeb2 knockout model was used. We found drastic reduction of B-lymphocytes, monocytes, platelets and erythrocytes in peripheral blood while accumulation of hematopoietic stem and progenitor cells in the bone marrow after Zeb2 deletion. In addition, we found partial differentiation blocks within different hematopoietic lineages at various stages of maturation. The most prominent differentiation block was observed within B lymphopoiesis at the prepro-B to pro-B cell transition. To identify critical Zeb2 target genes in B cell development, we analyzed the expression level of key components of B-cell specific genes in sorted prepro-B cells by qPCR. The early B-cell receptor IL-7r as well as fingerprint transcription factors such as Runx1, IKAROS, E2A, EBF1 and PAX5 were markedly reduced in Zeb2-null cells. Since all of those genes can cooperate with or be regulated by epigenetic regulators, we further examined methylation associated genes, including key members of Mi-2/NuRD complex and polycomb repressive complex 2 (PRC-2). Interestingly, the expression level of EZH2, SUZ12, as well as LSD1 and Mi2-β were significantly reduced in Zeb-deficient prepro-B cells, suggesting the B cell differentiation block due to altered epigenetic regulation. In summary, Zeb2 is essential for multilineage differentiation at different stages of hematopoiesis, which is a consequence of defective epigenetic regulation.

W-1086

OPTIMIZATION OF THE CULTURE CONDITIONS FOR THE DEVELOPMENT OF GENE THERAPY OF HEREDITARY PULMONARY ALVEOLAR PROTEINOSIS

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Pulmonary Alveolar Proteinosis (PAP) is a group of rare lung disorders characterized by the inability of alveolar macrophages to remove surfactant from the lungs due to impaired GM-CSF receptor (GM-CSFR) signaling. GM-CSFR is encoded by the CSF2RA/B genes, either of which can lead to hereditary PAP (hPAP) when mutated. Our group has recently established the foundation for both cell and gene therapy approaches as viable treatment options for PAP (Suzuki et. al., Nature, 2014). Direct installation of normal or gene corrected macrophages into the lungs of mice with hPAP resulting from GM-CSFRB deletion have sustained physiological correction of disease. The current study is focused on the development of conditions for the GMP manufacture of macrophages for clinical application. CD34+ cells from human BM or mobilized PB were analyzed for in vitro expansion and differentiation towards myeloid precursors and macrophages. We compared 3 different GMP grade medias, of which Stemline outperformed the others with >5-fold expansion over X-VIVO10 and >2-fold over SCGM in media supplemented with KIT, FLT3L, GM-CSF, and IL-6 (K/F/GM/6). In protocol 1, M-CSF (M) was added after 2 weeks of expansion. In protocol 2, cells were moved after one week of expansion into M-CSF and IL-1β (M/1β) media for differentiation. Differentiation was consistently higher at two weeks in M/1β compared to K/F/GM/6/M. Six BM samples were also obtained from 3 hPAP patients with CSF2RA mutations and CD34+ cells were isolated and transduced with a CSF2RA-GFP lentivirus resulting in 25% transduction efficiency. CSF2RA transduced cells had increased expansion compared to mock with 16.6 fold and 3.3-fold expansion respectively. Expanded transduced cells were able to differentiate into myeloid effector cells as evidenced by expression of CD11b and CD14. The cells were functional with an increased phagocytosis of E. coli beads (44 to 65%) compared to control cells (22%), and restored GM-CSF signaling through Stat5. Together, these data demonstrate the feasibility of expanding CD34+ cells from normal or gene corrected hPAP patients, and highlight the potential of this approach as a treatment for hPAP. Current studies are evaluating the in vivo potential of these cells to functionally correct hPAP in an immune deficient mouse model of hPAP.

W-1087

MAKING ANTI CANCER EFFECTOR CELLS DERIVED FROM IPSCS BY T-CELL RECEPTOR TRANSFER

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Adoptive T-cell transfer is potentially a very effective therapeutic strategy for cancer. However, previous studies about cancer immunotherapy revealed that in vitro expanded T-cells fall into exhausted state and have limited therapeutic effect. One way to overcome this obstacle is to use induced pluripotent stem cells (iPSCs) as the cell source for making effector T-cells. We have reported that differentiating iPS cells established from antigen specific monoclonal CD8 T cells (T-iPSCs) enables us to grow large amount of young CD8 T cells that are comparable to original CD8 cells in antigen specificity. The problem is it takes too much time at least 6 months to finally get the antigen specific CD8 T-cells derived from iPSCs. The other way of alternative immunotherapy is to transfer T-Cell Receptor (TCR) genes isolated from rare tumor-specific lymphocyte into peripheral T-cells. However the transferred TCR gene often makes inappropriate pairing between the endogenous TCR, resulting in limited therapeutic effect and potentially harmful unpredicted adverse effect. As a solution for these hurdles, we propose the strategy to combine TCR therapy and genome edited iPSCs technology. We transduced a tumor gene product specific TCR into iPSCs derived Hematopoietic cells. After 2weeks of coculture

with OP9 DLL1 mouse stroma cells, TCR transduced cells started to express CD3-TCR complex on cell surface and could react to transduced TCR specific Vβantibody. Next we established RAG2 (Recombination Activating Gene 2) knockout iPSC line. RAG2 KO iPSC derived hematopoietic cells could differentiate into T-cell without producing newly rearranged endogenous TCR. We have the hypothesis that by transducing tumor specific TCR into RAG2 KO iPSC We should be able to make effector T-cells free from TCR miss pairing and relatively in a short time. This strategy can be a more effective and safer way of future adoptive immunotherapy.

W-1088

SERUM FREE FEEDER FREE ERYTHROID DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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Erythroid differentiation of human pluripotent stem cells (hPSCs; (hESC and hiPSC)) has long been hampered by major bottlenecks including poor differentiation efficiency, low yield achieved, primitive character of the cells produced and frailty of the cells obtained in feeder free (FF) and serum free conditions. To overcome these hurdles, we have developed a multistep differentiation protocol which combines cytokines, and more recently, small molecules to mimic the early stages of development and successfully used this method on more than 10 different PSC lines grown in FF conditions. The changing cytokine formula within each step keeps the differentiation specifically directed towards hemato- erythroid fate and the small molecules (IBMX, Inhibitor VIII, StemRegenin (SR1) and Pluripotin (SC1)) increase the yield and robustness of the produced cells. The differentiation efficiency supports near complete conversion of PSC into hematopoietic progenitor cells (HPC) without any selection, with 90-100% of cells positive for CD34 by day 7 and 95-100% of cells positive for CD43 by day 10 of the protocol. Moreover, this differentiation is accompanied by extensive expansion with a single PSC producing up to 150 HPC by day 10 of the protocol. The later phase of the protocol focuses on erythroid differentiation and yield between 500 and 1500 orthochromatic normoblast per day 10 HPC by the day 31 of the protocol where 95 to 100 % of the cells are CD235a positive, again without any cell selection.
Overall the protocol produces 50,000 to 200,000 erythroid cells per PSC in GMP compatible, FF serum free conditions, and the cells exhibit a definitive foetal hematopoietic type with 90-95% foetal globin and variable proportion of embryonic and adult globins (1 to 10% for each). The presence of small molecules during the differentiation protocol also increases the proportion of adult globins and decreases the proportion of embryonic globin. In conclusion this efficient differentiation protocol allowing the production of large amount of erythroid cells can be a considerable asset to try to tackle the enucleation issue for which resolution has so far proven elusive. The early stages of the differentiation protocol could also serve as a starting point to optimise the production of long-term reconstituting hematopoietic stem cells from hPSCs.

W-1089

TERATOMA FORMATION ASSAY IN MICE- TOWARDS A HUMAN HEMATOPOIESIS IN VIVO MODEL

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Transplantation of hematopoietic stem cells (HSC) represents a promising treatment option for many immune diseases. Although protocols are being constantly improved there are still problems with graft versus host disease and often matching donors are not available. The discovery of induced pluripotent stem cells (iPSC) raised great prospects for cell therapies using corrected patient iPSC. Although the in vitro differentiation of iPSC into transplantable HSCs could not be achieved yet, repopulating HSCs can be isolated from teratoma bearing mice. We aim to employ teratoma formation in mice as an in vivo model for hematopoiesis in order to transfer findings to in vitro protocols for HSC generation. Therefore, we established a reproducible teratoma assay with CD34+ derived hiPSC. We evaluated histogenesis with different culture conditions before injection, several injected cell numbers and co-injections with OP9 cells. CD34+ derived hiPSC and supporting cell lines were subcutaneously injected into the flanks of NSG mice. Tissue samples were examined by labelling cell type specific surface markers in Flow Cytometry (FC) and Immunohistochemistry (IHC). The average time for developing teratoma was 80 days. Despite the fact that teratoma formation, when induced with OP9 coinjection, resulted in more solid tissue than with hiPSC alone, the efficiency and duration for teratoma development appeared to be unrelated to coinjected cells or total cell quantitiy.
The number of cells expressing hematopoietic markers in teratoma was overall constant whereas the composition of teratoma regarding endodermal and mesodermal proportions varied within groups.
Cell clusters expressing CD90 and CD34 were localized next to mesodermal descendants, potentially representing early hematopoietic cells. Endpoint analysis of spleen, bone marrow, liver and peripheral blood revealed the absence of the adult hematopoietic marker CD45, suggesting that successful migration and homing of hHSC did not occur in this basic experimental setting. We established a reproducible protocol for subcutaneous teratoma formation with CD34+ derived hiPSC lines in NSG mice. Necessary refinement of this hematopoiesis model could be achieved by including human cytokines

W-1090

BONE MARROW MACROPHAGES REGULATE PHARMACOLOGICAL MOBILISATION OF MESENCHYMAL STEM/PROGENITOR CELLS

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Mesenchymal stem/progenitor cells (MSPCs) in the bone marrow (BM) are closely associated with tissue-resident macrophages (MΦs), and through their interaction regulate retention of haematopoietic stem/progenitor cells (HSPCs). We previously demonstrated that

pre-treatment of mice with Vascular Endothelial Growth Factor (VEGF) or β3-Adrenoceptor agonist (β3-AR) over 4 days primed MSPCs for mobilisation from the BM to the peripheral blood in response to the CXCR4 antagonist, AMD3100. In this study, we investigated whether MΦs were important in the retention and/or mobilisation of MSPCs. BM MΦs were depleted by administration of clodronate liposomes. Consistent with previous studies, MΦ depletion induced mobilisation of HSPCs into the blood and significantly enhanced their mobilisation by AMD3100. In contrast, pharmacological mobilisation of MSPCs, quantified by clonogenic (CFU-F) assays, was significantly reduced in mice pre-treated with clodronate liposomes. Similarly, mice pre-treated with Granulocyte-Colony Stimulating Factor (G-CSF), that also depletes BM MΦs, exhibited enhanced mobilisation of HSPCs and reduced mobilisation of MSPCs in response to pharmacological reagents. This data suggests that signals from MΦs are required for the mobilisation of MSPCs. Non-haematopoietic PDGFR-α+Sca-1+ (PαS) cells are a subpopulation of MSPCs in the BM. We detected a significant reduction in the ratio of cycling to quiescent PaS cells following depletion of BM MΦ with either clodronate liposomes or G-CSF; suggesting that MΦs are important in regulating the balance between the quiescent and cycling MSPC pool in the BM. These studies suggest that MΦs affect MSPC dynamics in the BM.

W-1091

DIFFERENTIAL EXPRESSION OF ALPHA4, ALPHA5 AND BETA1 INTEGRINS IN HEMATOPOIETIC STEM CELLS (HSCS) LACKING INFLAMMASOME ADAPTORS AND THEIR EFFECTS ON CHIMERISM FOLLOWING HSC TRANSPLANTATION

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Integrins serve important roles during the transendothelial migration (homing) and engraftment of allogeneic donor HSCs to the bone marrow (BM) niche prior to their commencement of hematopoiesis. Upon activation, pattern recognition receptors (PRRs) on donor HSCs may affect integrin expression which hinders engraftment and donor-recipient hematopoiesis (chimerism). Whether activation of specific PRRs on HSCs affects the expression of the integrins during homing and engraftment is not well understood. Our study assessed whether the presence or absence of the apoptosis-associated speck-like protein containing a CARD (ASC) and NOD-like receptor family, pyrin containing 3 (Nlrp3) inflammasome adaptors affected engraftment and peripheral blood cell (PBC) chimerism following an allogeneic HSC transplant. Additionally, we evaluated if there was a differential expression of engraftment associated α4, α5 and β1 integrin subunits in HSCs isolated from mice lacking the ASC or Nlrp3 inflammasome component relative to wild type (WT). Chimerism in three groups (n=6/grp) of recipient PBCs was assessed by FACs analysis one and two weeks following HSC transplant from allogeneic C57BL/6 WT, ASC-/-, or Nlrp3-/- donors. BALB/c recipient mice were preconditioned with sub-lethal TBI, anti-CD8 and anti-CD40L to receive donor HSCs. Expression of α4, α5 and β1 integrin subunits in three groups (n=2/grp) of WT, ASC-/- or Nlrp3-/- HSCs was assessed utilizing RT-qPCR. Relatively more chimeric BALB/c PBCs were observed at one and two weeks post-transplant from ASC-/- donors, but not Nlrp3-/-, relative to WT. Intriguingly, HSCs from both ASC-/- and Nlrp3-/- mice expressed significantly less α4 and β1, but not α5, than WT. Furthermore, we found that relatively less α4 and β1 was expressed in HSCs from ASC-/- than from Nlrp3-/- mice. Chimerism was enhanced when HSCs were transplanted from mice lacking ASC but not Nlrp3. We found lower expression of α4 and β1integrin subunits in HSCs from both ASC-/- and Nlrp3-/- mice, although significantly less α4 and β1 is present in those lacking ASC. Lower relative expression of α4 and β1 in ASC-/- HSCs and the greater percent of chimeric PBCs following HSC transplant from ASC-/- mice may provide insight as to the relationship between PRRs and their effect on overall engraftment to an allogeneic BM niche.

W-1092

THE ROLE OF RIBOSOMAL PROTEIN DEFICIENCY IN THERAPY-INDUCED MYELODYSPLASTIC SYNDROME PATHOGENESIS

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Therapy-related myelodysplastic syndrome (t-MDS) is a lethal complication of cytotoxic cancer treatment and a leading cause of non-relapse mortality among patients undergoing autologous hematopoietic cell transplantation (aHCT). We are studying a prospective, longitudinal cohort of patients undergoing aHCT for lymphoma at our center with serial sample collection and banking. We have identified impaired hematopoietic function and altered gene expression in CD34+ cells from pre-aHCT samples from patients who later developed t-MDS (cases) as compared to patients who did not develop t-MDS (controls). We hypothesized that genetic programs associated with t-MDS are perturbed long before disease onset. The alterations of genes related to ribosomes and translation highlight the potential contribution of altered ribosome function to disease development. Interestingly, inherited ribosomal disorders including Diamond-Blackfan Anemia (DBA) are characterized by cytopenias and a propensity for MDS development. We evaluated the expression of 79 ribosomal genes in cases and controls by multiplex qRTPCR. Expression of DBA associated genes RPL10, RPL11, and RPL27 was significantly reduced in cases compared to controls. Altered expression of RPL11 is hypothesized to contribute to hematopoietic dysfunction by modulating p53 levels. Therefore, we characterized the effect of reduced RPL11 expression on hematopoietic function and determined the role of p53 in mediating these effects. Knockdown of RPL11 by shRNA resulted in 5-fold increase in apoptosis and 2-fold inhibition of proliferation of MOLM13 hematopoietic cells that express wild type p53, but did not affect survival and proliferation of p53 mutated HL60 cells. This suggests that cellular responses to RPL11 deficiency may depend, in part, on intact p53 signaling. Furthermore, concomitant knockdown of p53 and RPL11 resulted in a 2-fold reduction of apoptosis compared to RPL11 knockdown alone. RPL11 knockdown in CD34+ cells resulted in 30% inhibition of proliferation, 90% decrease in colony forming potential, and a reduction in global protein translation. These results support a potential role of aberrant RPL11 expression in altered cell survival, proliferation, and differentiation in the early course of t-MDS development.

W-1093

SINGLE-CELL TRANSCRIPTOMIC RECONSTRUCTION OF CELL CYCLE AND LINEAGE SIGNATURE REVEALS THE CRITICAL ROLES OF BCL11A IN THE HEMATOPOIETIC STEM CELL COMPARTMENT

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Hematopoietic stem cells (HSCs) are a rare cell type with special ability of long-term self-renewal and multipotency to reconstitute all blood lineages. They can be purified from the bone marrow using cell surface markers. Recent studies have identified significant cellular heterogeneities in the HSC compartment with subsets of HSCs displaying lineage bias. We previously discovered that the transcription factor Bcl11a has critical functions in the lymphoid development of the HSC compartment. In this study, we employed single-cell transcriptomic analysis to dissect the molecular heterogeneities in HSCs. We profiled the transcriptomes of 123 highly purified wild type HSCs. Detailed analysis of the RNA-seq data identified cell cycle activity as the major source of transcriptomic variation, which allowed reconstruction of HSC cell cycle progression in silico. Single-cell RNA-seq profiling of Bcl11a-/- HSCs revealed the loss of quiescence and significant self-renewal defects, which were experimentally validated in the mouse. Remarkably, the Bcl11a-/- HSCs could be computationally segregated into two distinct myeloerythroid-restricted subpopulations with loss of lymphoid signature, which is consistent with the complete loss of lymphoid potential of these cells. Our study demonstrated the power of single-cell transcriptomics in dissecting cellular process and lineage heterogeneities in stem cell compartments, and further revealed the molecular and cellular defects in the Bcl11a-deficient HSC compartment.

W-1094

KIT RECEPTOR EXPRESSION DEFINES THE FUNCTIONAL HIERARCHY OF HSCS ACROSS SPECIES BARRIERS.

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Humanized mice are required for in-depth analysis of human hematopoietic stem cell (HSC) function and immunobiology. The currently available mouse models are problematic because stable engraftment of substantial numbers of human HSCs and the continuous generation of human myeloid cell types remain difficult to achieve. We generated three novel recipient mouse strains that combine immune deficiency with a functionally impaired endogenous HSC compartment mediated by a defective Kit receptor: BALB/c Rag2- Il2rg- KitWv/Wv (BRgWv), NOD/SCID Il2rg- (NSG) KitWv/Wv (NSGWv) and NSG KitW41/W41 (NSGW41). We find that the mutant Kit receptor opens up stem cell niches across species barriers and allows for robust and sustained engraftment of human HSCs after transfer into adult mice without the necessity for irradiation conditioning prior to transplantation. Following stable engraftment in mouse bone marrow niches, human HSCs give rise to lymphoid cells and to robust numbers of erythroid and myeloid lineage cells over extended periods of time in primary and secondary recipient mice. In the bone marrow, endogenous hematopoietic progenitor cells with a defective Kit receptor are largely replaced by human Kit-proficient hematopoietic progenitor cells because progenitor cell expansion requires normal signaling by Kit. Thus, human Kit-proficient donor cells have an advantage over endogenous murine Kit-mutant cells. We conclude that Kit-signaling regulates HSC engraftment across the human-mouse species barrier and that Kit deficient mice show great potential for the study of human HSC functions including self-renewal, differentiation and mechanisms of innate immunity.

W-1095

MARKER FREE CHARACTERIZATION OF HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS BY HIGH THROUGHPUT MECHANICAL PHENOTYPING

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Hematopoietic stem and progenitor cell (HSPC) transplantation has become a routine clinical procedure to treat hematological malignancies as well as acquired bone marrow failure syndromes. The mechanical properties of cells have been emerging as label-free, inherent marker of biological function and disease. Here, we present first results of the application of cell mechanical properties as a marker to study the stem cell potential of HSPCs. The characterization of HSPCs in the transplanted graft relies currently on molecular phenotyping based on certain surface proteins, e.g. CD34 and CD133, initially characterized by flow cytometry (FACS). In the current study, HSPCs were isolated from G-CSF mobilized peripheral blood and from bone marrow aspirates from hematological healthy donors using immunomagnetic CD34 and CD133 selection. In vitro differentiation and expansion of HSPCs was performed for bone marrow derived CD34+ cells. Mechanical characterization of HSPCs and differentiated cells was carried out using real-time deformability cytometry (RT-DC), a novel technique for the continuous mechanical screening of large cell populations (> 100,000 cells) with a throughput > 100 cells/s, approaching that of conventional flow cytometers. Using RT-DC we found differences in the deformability of CD34+ cells from mobilized peripheral blood as compared to bone marrow-derived CD34+ cells. Moreover, we could distinguish different populations within the CD34+ cell fraction taken from bone marrow. Interestingly, CD34+ and CD133+ cells displayed different mechanical properties - a distinction not detectable by FACS for CD34 and CD133 surface expression. We further demonstrated that monocytes, macrophages and granulocytes derived in vitro from CD34+ cells also display distinct mechanical fingerprints. In summary, RT-DC analysis of primary human HSPCs can distinguish mechanical phenotypes according to graft source in a reproducible manner. In addition, differential mechanical phenotypes can define stem cell subsets and progenitors/mature cells derived from these. These results suggest that mechanical phenotyping could become a standard flow cytometric approach.

W-1096

MIRNA-125A CONFERS MULTILINEAGE LONG-TERM REPOPULATING STEM CELL ACTIVITY TO MULTIPOTENT MURINE HEMATOPOIETIC PROGENITORS

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In the hematopoietic system all blood cells originate from a small population of stem cells (HSC) that can self-renew to maintain their pool, or differentiate into hematopoietic progenitors. These committed progenitors are able to unidirectionally differentiate into various blood cell types. Importantly, they do not have long-term self-renewal potential, are typically lineage-restricted, and fail to robustly reconstitute the hematopoietic system upon transplantation. Here we show that retroviral overexpression of miRNA-125a in multipotent progenitors (Lin-Sca-1+c-Kit+ depleted from CD150+48- cells), induces robust, long-term repopulation potential in myeloid and lymphoid lineages upon transplantation in primary, secondary and tertiary recipients while, as expected, control progenitors did not contribute to blood cell. Progenitor cell-derived chimerism levels uniformly reached values of ~50-90% already at 6 weeks post transplantation, increased up to 90% and remained stable in serial transplantations. The potential of miR-125a to confer stem cell activity to progenitor cells was strictly seed sequence dependent since a single point mutation within this region abolished the reconstituting activity completely. We did not observe any cases of hematological malignancies in primary recipients. However, 15 % mortality was observed in secondary recipients transplanted with progenitors, and 25% when miR-125a-overexpressing LT-HSCs were transplanted. In tertiary recipients, 35 and 50% of the recipients died, when transplanted with miR-125a overexpressing progenitors or LT-HSCs, respectively. Importantly, in surviving recipients we observed exhaustion of miR-125a overexpressing LT-HSC and progenitors. Apart from survival statistics, no functional differences were detected between progenitors and LT-HSCs overexpressing miR-125a. We performed quantitative proteomics with cells overexpressing miR-125a or its single nucleotide mutant. Stringent filtering identified 10 direct miR-125a targets. These included p38 kinase, 2 phosphatases involved in negative regulation of MAPK signalling, and proteins involved in vesicle transport or lysosomal degradation. Our findings offer new possibilities to use progenitors for transplantation purposes by modifying them with a single miRNA.

W-1097

ARYL HYDROCARBON RECEPTOR REGULATES HEMATOENDOTHELIAL DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

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The aryl hydrocarbon receptor (AHR) is an evolutionarily conserved transcription factor originally characterized for its role in mediating biological responses to carcinogenic environmental agents. Recent studies have elucidated the importance of AHR-mediated signaling for normal physiological function in the absence of environmental ligands, most notably the development of Th17 cells, regulatory T-cells, and natural killer (NK) cells. Additionally, AHR is highly expressed in hematopoietic stem/progenitor cells (HSPCs), and antagonism of AHR using small molecules results in a dramatic expansion of umbilical cord blood derived HSPCs suitable for transplantation. It remains unclear what role, if any, AHR plays during early human hematoendothelial development. We hypothesized modification of AHR signaling regulates early human hematopoietic cell development. To test this hypothesis, we differentiated human embryonic stem cells (hESCs) under defined hematopoietic-promoting media conditions for 15 days. qRT-PCR analysis demonstrated a significant increase in AHR transcript (7.16±1.02, $p<0.05$) at Day 11 of differentiation relative to undifferentiated hESCs, which correlated with an enriched CD31+CD34+ hematoendothelial population and the appearance of CD34+CD41+ and CD34+CD43+ hematopoietic progenitor cells. We modified the relative activity of AHR signaling by differentiating hESCs in the presence of 2,3,7,8-tetrachlorodibenzo-p¬-dioxin (TCDD), a prototypical AHR agonist, or StemReginin-1 (SR-1), an AHR antagonist, and assessed hematopoietic progenitor cell production at Day 12. Interestingly, TCDD treatment increased the number CD41a+, CD43+, and CD45+ hematopoietic cells relative to controls, while SR-1 treatment caused a reciprocal decrease in these populations. In assessing hESCs with an integrated RUNX1c-tdTomato reporter, which serves as a marker of definitive hematopoiesis from hemogenic endothelial cells, TCDD treatment further resulted in ~15% enrichment of tdTom+ cells from CD34+CD41- and CD34+CD43- subsets. Collectively, these results suggest AHR activation can promote hematoendothelial differentiation and may be used as a potential molecular target to enhance definitive hematopoietic development from human pluripotent stem cells.

W-1098

ENDOGLIN MODULATES BMP AND WNT SIGNALS TO SECURE MESODERM COMMITMENT TOWARDS THE HEMATOPOIETIC LINEAGE

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In mice, the absence of the TGF-β co-receptor endoglin (Eng) results in embryonic lethality. We showed that induction of Eng (iEng) results in increased hematopoiesis, which happens at the expense of the cardiac lineage. This is also the case in E8.5 explants. Since Wnt signaling is critical for cardiogenesis, we hypothesized that Wnt activation may be involved in Eng-mediated cardiac repression. Consistently, inhibition of the canonical Wnt signaling pathway abolished effects resulting from Eng overexpression. To determine whether Eng regulates Wnt activation, we introduced a β-catenin-dependent reporter into iEng ES cells. We observed distinct up-regulation of reporter activity as well as higher amounts of active β-catenin in Eng-induced EBs showing that Wnt activation is directly regulated byEng. We have previously demonstrated that the positive effect of Eng on hematopoiesis occurs through BMP signaling, suggesting a potential common downstream effector that supports Eng-mediated regulation of BMP and Wnt signals to

enhance hematopoiesis. SMAD1, a downstream component of the BMP signaling pathway, has been shown to integrate BMP and Wnt/GSK3 signaling at the phosphorylation level during embryogenesis. To test this, we generated an inducible SMAD1 ES cell line that carries mutated GSK3 conserved sites. Our findings show that these cultures display enhanced hematopoiesis, similarly to iEng, confirming the importance of BMP and Wnt modulation in SMAD1 activation during this process. To further elucidate how Eng specifies early mesodermal precursors into either cardiac or hematopoietic cells, we utilized the mesodermal reporter Bry-GFP ES cell line, which revealed that Eng identifies cells with both hematopoietic and cardiac potential, and that Eng controls lineage specification by modulating BMP and Wnt signals. Whole transcriptome analysis from Eng+ sub-fractions defined the detailed gene expression profiles of early hematopoietic and cardiac precursors as well as the potential gene expression signatures governed by BMP and Wnt signals. Our study uncovers a novel role for endoglin as a mediator between BMP and Wnt signaling during mesoderm specification, and provides mechanistic insight on the understanding of how hematopoietic and cardiac progenitors are established during embryogenesis.

W-1099

SINGLE CELL DE-CONVOLUTION OF TRANSCRIPTIONAL REGULATORS IN HUMAN HEMATOPOIETIC IMMUNE PROGENITORS

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Mature blood cells are generated from a complex stepwise process in which hematopoietic stem cells (HSC) give rise to lineage restricted hematopoietic progenitors and their progenies. While the lineage progression of adult hematopoiesis has been extensively studied, little is known about molecular mechanisms involving the coordination of transcription factors (TFs) and regulatory signaling pathways driving the behavior of hematopoietic stem/progenitor cells. Further complicating this is the variety of hematopoietic tissues studied, the rarity of progenitor cells, and inconsistent reports between human and mouse. Recently, we demonstrated the ability of single cell mass cytometry (CyTOF) to organize developing immune cells in human bone marrow (BM) – from HSC to mature B cells. Now, taking advantage of this suite of techniques we have expanded this more broadly to investigate molecular networks orchestrating normal human hematopoiesis towards a host of cell progenitors (i.e. multi-potent, T, NK, Monocyte). To get at the single cell molecular networks driving this progression we have optimized the simultaneous analysis of 40+ features including expression of development TFs (GFI1, GATA2, BMI1, C/EBPα, RUNX1, GATA3) and regulatory behaviors (i.e., cell proliferation, apoptosis, senescence) all in combination with markers of cell identity used to define hematopoietic lineage fate. In order to unify our observations we analyzed primary tissues such as BM, cord blood, thymus, and mobilized peripheral blood. Analyses of this data have revealed a new organization of lineage-restricted precursors based on differential transcriptional factor usage across all human hematopoietic tissues. In particular, we identified a new, rare T cell progenitor in the BM that appears to migrate and expand in the thymus. More broadly, the simultaneous analysis of TFs, cell cycle, and comprehensive cell identity allowed us to model cellular behavior and to establish hierarchies of primary human hematopoiesis system-wide with an unprecedented level of granularity. Hence, these results provide new insight into the complex process of normal human immune development, and also a framework to understand general stem cell specification or dysfunctional processes, like emergence of leukemia, in the future.

W-1100

MITIGATION OF HEMATOPOIETIC TOXICITY IN SUBLETHALLY IRRADIATED MICE BY SHORT-TERM SONIC-HEDGEHOG TRANSIENT GENE THERAPY

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The hematopoietic syndrome represents the first therapeutic challenge following exposure to high doses of ionizing radiation. The protection of hematopoietic stem and progenitor cells (HSPCs) continuum and environment is required for recovery from radiation-induced myelosuppression. To favour the regeneration of residual bone marrow after radiative stress, we propose the concept of a global niche therapy. This strategy is based on the transient local production/secretion of Sonic hedgehog (Shh) morphogene at the niche level following transduced adipocyte derived stem cells (ASC) injection. ASCs were nucleofected using pIRES2 plasmids and Amaxa technology. Highly irradiated monkeys (8-Gy gamma total body irradiation TBI) were given Shh-ASCs 2 days (D) after TBI via intra-osseous injection and subsequently exhibited an accelerated multilineage hematopoietic recovery. The aim of the present study was to identify the Shh target(s) at the niche level. B6D2F1 mice were sublethally irradiated (7 Gy 60Co gamma TBI) and then received (retro-orbital route) phosphate-buffered saline, mock- (each mouse 0.85x106) or Shh-ASCs (each mouse 1x106 total among which 0.33 x106 Shh-ASCs) on D2 (each group n=10). Mice were euthanized on D7 (n=5) and D14 (n=5) and bone marrow hematopoiesis and microenvironment structures were semi-quantitatively evaluated at the humerus and femur level following hematoxylin-eosin staining. Radiation induced a severe depletion of hematopoietic cells 7 days after TBI especially at the epiphysis level and the animals partially recovered on D14. On D7 epiphyseses were partially protected in Shh-ASC animals in which +/- large hematopoietic islands were observed close to epiphyseal-plate or cortical bone (hematopietic cells score: 1.4 +/- 0.7 versus 0.2 +/-0.22 and 0.3 +/- 0.1; baseline = 4, Shh-ASC versus other groups $p<0.05$). Sinusoids were severely damaged in all groups except at the level of Shh-ASC preserved hematopoietic islands. Osteoblastic stimulation following irradiation was huge in the 3 groups on D7 and decreased on D14 especially in Shh-ASC animals. Work is going on to better understand the mechanisms involved in Shh induced hematopietic protection/stimulation.

CARDIAC CELLS

W-1102

CARDIAC EXTRACELLULAR MATRIX INFLUENCES HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTE MATURATION

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Pluripotent stem cell-derived cardiomyocytes (CM) hold great promise for the treatment of heart disease because they can be used for cellular therapies as well as preclinical pharmacologic drugs studies. However, it is well established that pluripotent stem cell-derived CM are immature, with characteristics of fetal CM rather than adult CM. These characteristics include disorganized sarcomere structures, automaticity, improper calcium handling/electrophysiological signaling and different responses to pharmaceutical drugs compared to mature CMs. It is necessary therefore to understand what influences CM maturation, and to find strategies to mature the pluripotent stem cell-derived CMs. We are exploring the possibility that the heart's native ECM will drive CM maturation and behavior similarly to the way ECM affects differentiation of many cell types such as neural progenitors and chondrocytes. For this reason, we have generated cardiac ECM from fetal and adult bovine heart tissue through SDS-mediated decellularization, and find that in contrast to fetal cardiac ECM, the adult ECM promotes induced pluripotent stem cell-derived CM maturation, as assessed by enhanced expression of mature CM markers. Even more strikingly, we have seen further increased levels of maturation when iPSC-derived CM are seeded into a three-dimensional cardiac ECM scaffold compared to growth on 2D surfaces. In summary, this study demonstrates the use of native cardiac ECM as scaffold that promotes CM maturation. These findings have important implications for studies using iPSC-derived CM to screen for novel drugs, and as therapies for heart disease.

W-1103

ISOLATION AND CHARACTERIZATION OF WT-1 POSITIVE PROGENITOR CELLS FROM THE RAT AND HUMAN HEART

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Epicardium-derived cells (EPDC) play a fundamental role in embryonic heart development, contributing to coronary vascular precursors, fibroblasts and cardiomyocytes. In the adult heart, EPDC (key marker gene: WT-1) can be reactivated in response to tissue injury like myocardial infarction (MI). EPDC are therefore considered as endogenous cell source with the potential to induce cardiac regeneration after MI. In this study, we elaborated and validated techniques for the isolation and cultivation of rat and human WT-1 positive cells. For the induction of EPDC reactivation in the rat, MI was induced by ligation of the LAD for 60min and subsequent reperfusion. EPDC were isolated 5d after MI by a protocol using selective enzymatic digestion of the surface of infarcted hearts. We found that EPDC can be well cultivated up to 20 passages with a doubling time of about 35 hrs. They display a MSC-like morphology and retain the expression of WT-1 (>90%), throughout in vitro expansion. Flow cytometric analysis revealed that these cells express stem/progenitor cell markers such as CD73 (>80%) and CD90 (>75%). Distinct cardiac marker genes such as Gata4, Tbx5 and troponin T, the EMT marker alpha-smooth muscle actin and the embryonic gene Tbx18 were also expressed as assessed by quantitative real time PCR and immunofluorescence. EPDC display remarkable endocytic properties and avidly take up nanoparticles (130 nm). In a translational attempt, we have established a protocol for the isolation of WT-1 positive progenitor cells from human atrial biopsies (bypass surgery after MI). To this end, the tissue was chopped and enzymatically digested. Following a preplating step, the nonadhering cells were transferred to a secondary culture plate. These cells can also be well cultivated up to 10 passages and are positive for WT-1 (>90%), CD73 (>85%), and CD90 (>70%). They display a similar phenotype, expression profile and similar endocytic properties as their murine counterparts. In conclusion, we report techniques for the successful culture of WT-1 positive progenitor cells from rat and men and demonstrate that these cells express stem/progenitor cell as well as cardiac marker genes. Their ability to endocytose may represent a mechanism by which instructive signals are delivered that promote their differentiation.

W-1104

DOXORUBICIN INDUCES ACUTE UPREGULATION OF THE SDF-1 RECEPTOR CXCR4 BY MODULATING THE MIR-200C/ZEB1 AXIS IN HUMAN CARDIAC STROMAL AND C-KIT+ PROGENITOR CELLS.

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Doxorubicin (Dox) is higly effective against cancer, however cardiotoxicity is a severe side effect. Despite this, molecular mechanisms of Dox-induced cardiotoxicity are still unclear, Dox is known to affect the viability of cardiac progenitor cells both in vitro and in vivo. In this study, we show that Dox upregulates CXCR4 modulating the miR-200c / Zeb1 pathway both in human cardiac stromal cells (CStCs) and c-Kit+ cardiac progenitor cells (CPCs). Human CStCs treated with Dox (1 µM) showed a time dependent induction of miR-200c as assessed by qRT-PCR. In parallel, downregulation of miR-200c target gene Zeb1 was confirmed by qRT-PCR and WB and was significant versus control at 24h. ChIP analysis followed by qRT-PCR on CStCs treated with Dox for 24h vs untreated cells, revealed the presence of Zeb1 binding sites on the Cxcr4 promoter. In agreement, Dox significantly induced CXCR4 expression in CStCs at 24h as revealed by WB and FACS analysis. Similar results were obtained in human CPCs. Moreover, Zeb1 over-expression decreased CXCR4 basal levels in CStCs and impaired its induction upon Dox treatment. Zeb1 silencing had opposite

effects. Treatement with SDF-1 (100 ng/ml) resulted in a significant impairment of Dox-induced apoptosis at 48h in CStCs. SDF-1 protective effect was abrogated in the presence of AMD3100 (25μg/ml). Our study shows for the first time that CXCR4 is regulated by miR-200c/Zeb1 axis in both human CStCs and CPCs. Specifically, Dox induces Zeb1 donwregulation and consequent reversal of Cxcr4 promoter repression, thus resulting in CXCR4 upregulation. Our results suggest that CXCR4 upregulation could have protective effects against Dox induced apoptotic pathways in CStCs.

W-1105

MESENCHYMAL STEM CELLS POTENTIATES CARDIAC PROGENITOR CELLS SURVIVAL AND ENGRAFTMENT ATFER TRANSPLANTATION IN MYOCARDIAL INFARCTION

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Pluripotent stem cell-derived cardiac progenitor cells (CPCs) can differentiate into cardiomyocytes, endothelial cells and vascular smooth muscle cells in vitro and in vivo without teratoma formation. This potential makes CPC as a prospective cell source to cardimyocyte regeneration. However, the poor cell survival and engraftment after transplanting into an ischemic hostile environment that limit the application potential of CPC for heart repair. We found that mesenchymal stem cells (MSCs) can promote the proliferation and myocardial differentiation of CPCs. Nevertheless, there is little information about mechanisms of these two cellular communication and not clear effects in vivo hybrid application of two types of cell for heart repairs. Since the niche is very important to influence the fates of CPC, we proposal the hypothesis that components of MSC secreted extra cellular matrix play critical role to support Nkx2.5+ CPC survival and proliferation. Fibronectin, a key component of ECM highly enriched in MSC will be focus on this study. In our research process, the ESC-derived GFP+ CPCs are sorted out, labeled, mixed with mouse MSCs or fibronection and injected into infarcted area of the mouse heart. The groups were classified as: A). CPCs group, MSCs group, CPCs + MSCs group; B). CPCs + PBS group, CPCs + fibronection group, fibronection group. After 4 weeks, heart function and the labeled cells' differentiation of the animal model will be identified. The survival rate of CPCs will also be tested and compared between different groups. By statistical analysis, the heart function, cell survival and cardiac differentiation of injected CPCs will be tested. Our study aims to find a more optimized and effective cell therapy after heart infarction.

W-1106

TRANSCRIPTIONAL REGULATORY NETWORKS IN HUMAN EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES EXPOSED TO DOXORUBICIN

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Doxorubicin is one of the most successful chemotherapeutic agents used for a variety of cancers, including leukemia, lymphomas, and many solid tumors. However, the severe cardiotoxicity associated with the doxorubicin treatment limits its clinical use. The exact mechanisms involved in doxorubicin-induced cardiomyopathy are not known, but the fact that the cardiotoxicity is dose-dependent and that there is a variation in time-to-onset of toxicity, gender- and age differences suggest that several mechanisms may be involved. In this study, transcriptional regulatory networks activated upon doxorubicin exposure have been investigated in cardiomyocytes derived from human embryonic stem cells (hESC). The cells were exposed to a low- (50nM), medium- (150nM), and high (450nM) dose of doxorubicin for 48h, followed by a 12 days wash-out period. Total RNA was extracted after 24h and 48h of doxorubicin exposure as well as after 5 and 12 days recovery, post exposure. The global transcriptional profiles in the cells were analyzed using the WT Gene 2.0 ST arrays from Affymetrix Inc. In a previous study, we analyzed this dataset, in total 51 microarrays, and revealed clusters of genes that were differentially expressed upon doxorubicin exposure, some of which remained even after the wash-out period. In the present study, the promoter sequences in selected genes were further studied in more detail. Identified overrepresented motifs in these promoter sequences were mapped and compared to known human transcription factors. Furthermore, the mRNA levels of known human transcription factors were investigated and based on their expression pattern in combination with the expression of their putative target genes we propose regulatory networks that may be of importance for understanding doxorubicin-induced toxicity in cardiomyocytes. Taken together, the results presented in this study identify several transcriptional factors that regulate critical genes that may help to explain the cellular mechanisms behind the late onset apoptosis, associated with doxorubicin-induced cardiomyopathy.

W-1107

REVERSE-ENGINEER TO INCREASE THE PHYSICAL SIZE OF HUMAN PLURIPOTENT STEM CELL-DERIVED VENTRICULAR CARDIOMYOCYTES VIA CELL FUSION

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Human pluripotent stem cells (hPSCs) including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can self-renew while maintaining their pluripotency to differentiate into cardiomyocytes (CMs), providing a potential unlimited source of donor cells for replacement therapies and other applications. However, hESC-CMs are structurally and functionally immature. For instance, hESC-CMs are >10x physically smaller than the adult counterparts. Furthermore, the majority are mono- rather than bi- or multi-nucleated which is an evolutionary adaptive response in metabolically active cells (e.g., by doubling the RNA content for promoting protein synthesis). Developmentally, bi-nucleated CMs arise from acytokinetic mitosis during the final but incomplete round of cell division, followed by physiological hypertrophy. Here, we attempted to reverse-engineer the physiological hypertrophy of hESC-derived ventricular cardiomyocytes (VCMs) by increasing their physical size and nucleation status via chemical-induced cell fusion. To induce fusion, polyethylene glycol (PEG) was added to a mix of GFP- and -tdTomato-labeled MLC2v+-cTNT+-hESC-VCMs. To avoid ambiguity, successfully fused heterokaryons were identified as doubly

GFP+/tdTomato+ cells, followed by patch-clamp analysis of the cell capacitance. Indeed, heterokaryons almost doubled in size (control: 91±12 pF, n=17 vs. heterokaryon: 194±18 pF, n=9). Consistently, the proportion of multi-nucleated cells also increased from 20% in control to ~90% after fusion. However, the electrophysiological properties, Ca2+-handling and mitochondrial functions of the fused syncytia were not different from those of control hESC-VCMs. To Sum, although acute functional changes were not observed upon fusion, we conjecture that gradual structural, functional and genomic remodeling exist but take time, and therefore we are currently probing for long-term responses after fusion.

W-1108

VIDEO-BASED ANALYSIS OF HUMAN IPS CELL DERIVED CARDIOMYOCYTE BEATING CHARACTERISTICS

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Since the initial discovery, the human induced pluripotent stem cell (hiPSC) derived cardiomyocytes have been harnessed to serve the research of cardiac arrhythmias. It has been widely demonstrated that the disease phenotype, including the arrhythmias, is present also in iPS derived cardiomyocytes. However, the traditional tools for analyzing these cells have been mainly limited to studying their electrophysiological properties. We have previously described a method for analyzing the mechanical beating behavior of single cardiomyocytes. Here we describe in more detail the capabilities and performance of this analysis tool. In this study, we demonstrate that our analysis tool can be used to detect the common changes in the beating behavior of human cardiomyocytes. The beating movement of a single cardiomyocyte is recorded on a high quality video, which is then processed and analyzed by the software to obtain detailed data on the beating characteristics. Changes in beating frequency and duration of mechanical activity, as well as durations of the different phases of the beat can all be detected by using our analysis tool. We also show that it can be used to reliably analyze commercially available human cardiomyocytes. All this together provides a fast and reliable tool for screening cardiomyocyte beating characteristics, which is extremely useful for example in disease modeling or drug screening. The existing methods for studying cardiomyocyte functionality mainly focus on the electrophysiology of the cells. Our analysis tool is a good addition to the current pattern of characterization methods as it brings information of the mechanical properties of the cell. Although it does not reveal the electrophysiological properties of the cell, it still has great advantages over the existing methods. It is fast and easy to perform (compared to patch clamp), does not rely on analysis of single ion channel proteins (as calcium imaging), but still gives the resolution of one single cell (compared to multielectrode arrays). In conclusion, we provide an accurate, non-invasive and label-free method to analyze the functionality of hiPSC derived cardiomyocytes without extensive technical skills and instrumentation.

W-1109

DEFINED MICRORNAS PROMOTE THE MATURATION OF EMBRYONIC STEM-CELLS DERIVED CARDIOMYOCYTES

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Advances in our ability to differentiate pluripotent stem cells into somatic cells of many lineages provide us with a platform for drug screening, disease modeling and regenerative medicine. Due to the high prevalence of cardiovascular disease, cardiac cells are of particular interest. Several outstanding groups have developed reproducible and efficient systems to differentiate pluripotent cells into cardiac cells. However, these pluripotent stem cell-derived cardiomyocytes often display the structural and functional attributes of fetal cardiomyocytes rather than adult cardiomyocytes. This significantly limits the use of these cells for applications such as drug screening or regenerative medicine. Thus, a method for increasing the maturity of pluripotent stem cell-derived cardiomyocytes is highly desirable. Studies of anatomy and developmental biology suggest that the function of heart is considerably dependent on endothelial cells. We have shown that endothelial cells play a role in enhancing the maturation of murine embryonic stem cells-derived cardiomyocytes (ES-CMs). We have identified a cluster of miRNAs that are upregulated in cardiomyocytes upon their coculture with endothelial cells. Exogenous addition of this combination of miRNAs was able to significantly increase the molecular, structural and functional maturation of ES-CMs. Bioinformatics revealed ErbB4 as the target of these four miRNAs, and a luciferase reporter assay confirmed that they together targeted ErbB4. This finding was further confirmed by siRNA-induced downregulation of ErbB4, resulting in the enhanced maturation of ES-CMs. To apply these findings to human cardiomyocyte maturation, we tested the same miR combination in human ES-CMs. The enforced expression of this miR-combo was able to increase maturation of ES-derived cardiomyocytes into a more adult-like state, evidenced by an increased binucleation ratio, lower ANF expression, improved respiratory capacity, more negative resting membrane potential and polarized Connexin-43. In conclusion, we present a novel approach for improving the maturity of cardiomyocytes differentiated from pluripotent stem cells. This is a significant step towards realizing the full potential of iPS and ES-CMs as platforms for drug screening and disease modeling.

W-1110

UNIVERSAL PROTOCOL FOR EFFICIENT 2D AND 3D DIFFERENTIATION IN HUMAN PLURIPOTENT STEM CELLS TO CARDIOMYOCYTES

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Consistent supply of cardiomyocytes is key to maximizing the potential of human pluripotent stem cells (hPSCs) in disease modelling, drug toxicity screening and cardiac regeneration therapy. However, current cardiac differentiation methods are restricted

to 2D monolayer or 3D embryoid bodies (EB) approaches that are hampered by lack of reproducibility and transferability across different hPSC lines. We provide a universal method for 2D and 3D cardiac differentiation that efficiently generates cardiomyocytes consistently across multiple hPSC lines. Gene expression arrays and flow cytometry were utilized to map the temporal prolife of differentiation to characterize cardiac subtype derivation during directed differentiation. Our serum-free monolayer and EB-based protocol was verified on hESC, normal and disease-specific hiPSC lines (total of 5 lines). All cell lines displayed a high degree of contraction (85-90%) by day 12-14 under monolayer and EB-based differentiation methods. Kinetic quantitative gene expression profiling (day 0, 2, 4, 6, 8 and 14) of 30 developmental cardiac markers expressed at different stages were evaluated. Gene expression profiling showed sequential upregulation of early mesodermal (brachyury) and cardiac mesodermal (Isl1) markers, cardiac committed markers (GATA4, NKX2.5) and followed by cardiac specific markers (MLC2a, MLC2v, cTnI, MYH7). A significant upregulation of MLC2a was observed as early as day 4 of differentiation. However, ventricular marker, MLC2v was seen only by day 14 onwards along with cardiac troponin expression. Flow cytomertic analysis revealed 70-90% expressed cardiac troponin I (cTnI) on day 14. We describe a simple off-the-shelf protocol that is universally applicable to 2D and 3D differentiation to produce large quantities of cardiomyocytes, in a cell-line insensitive manner, which is crucial in translating regenerative potential of hPSCs.

W-1111

INVESTIGATING THE PATHOPHYSIOLOGY OF CARDIAC ARRHYTHMIAS USING INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES FROM PATIENTS AND CONTROL INDIVIDUALS

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Congenital heart arrhythmia conditions such as Brugada syndrome (BrS) can cause ventricular fibrillation and lead to cardiac arrest. Some limited screening and risk assessment is possible based on idiopathic ventricular presentations and/or family history, and management is currently only achievable via implantable cardioverter defibrillators. Furthermore, mutations in a variety of genes have been linked to BrS and genetic screens indicate that in some cases it may be oligogenic, meaning animal models are not feasible and the exact disease mechanism(s) remain to be determined. In order to investigate the cardiomyocyte (CM) pathophysiology of heart arrhythmias, we are generating induced pluripotent stem cell (iPSC) lines from a consenting cohort of predominantly BrS patients. Dermal fibroblasts derived from skin punch biopsies are reprogrammed using both non-integrating episomal and lentiviral vectors. Multiple clonal lines from each patient are expanded and characterised for features of pluripotency, including self-renewal over multiple passages, expression of pluripotency-associated cell surface markers and transcription factors, and in vitro differentiation into derivatives of all three germ layers. Using patient and control iPSCs we have refined a targeted cardiac differentiation protocol employing embryoid body (EB) formation, subjecting cells to stepwise treatment of various growth factors and small molecule inhibitors. After 14 days, >85% of cardiac EBs (CEBs) from multiple iPSC lines spontaneously beat. Temporal induction of mesodermal and cardiac differentiation markers was observed, with >50% of cells expressing the cardiomycocyte marker TNNT2 after 3 weeks. Patch clamp analysis of iPSC-CMs dissociated from CEBs reveals action potentials with ventricular, atrial or pacemaker-like morphology. Analysis of CEBs between days 40-50 by multi-electrode array has indicated effects on field potential, including field potential duration, with increasing doses of the drug Ajmaline. Experiments are being expanded to incorporate further patient vs control analyses to identify whether differences in CM features such as gap junctions are present, and whether iPSC-CMs recapitulate known features of BrS pathophysiology, e.g. response to challenge such as ion channel inhibition.

W-1112

SINGLE CELL EXPRESSION ANALYSIS REVEALED A NOVEL REGULATORY MECHANISM DURING DIFFERENTIATION INTO CARDIOMYOCYTE FROM INDUCED PLURIPOTENT STEM CELLS

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Previous embryonic developmental studies have revealed key factors which drive cardiac differentiation, and accordingly an efficient in vitro directed differentiation methods into cardiomyocytes from pluripotent stem cells have been developed. During the differentiation process, the differentiating cells undergo a drastic change including gene expression, cellular morphology, and metabolic states. However, there are many unclear points regarding regulatory mechanisms during differentiation process, and heterogeneity in the population of the differentiating cells hindered the precise analysis and evaluation of gene expression. Here we show, using single cell RNA sequencing that several key factors of cardiac differentiation were expressed heterogeneously during the cardiac differentiation from induced pluripotent stem cells. We focused on TBX5, a key transcriptional factor of cardiac differentiation. We found that TBX5 was expressed heterogeneously and the expression of several genes (TBX5-correlated genes; TCG) was correlated with TBX5 expression especially in the cells on day 5. We focused on TCG1, which is one of the genes, whose expression is most highly correlated with TBX5 expression and found that knockdown and overexpression of TCG1 varied expression of mitochondrial genes, indicating that TCG1 was a key factor of metabolic change during the process of early cardiac differentiation. We think these findings provide insights into regulatory network at mesodermal and cardiac progenitor stages during cardiomyocyte differentiation.

W-1113

TECHNIQUES FOR HIGH THROUGHPUT CARDIOTOXICITY STUDIES USING HUMAN STEM CELL-DERIVED CARDIOMYOCYTES

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Cardiotoxicity is a leading cause for the recall of commercial pharmaceutical drugs and the failure of compounds during drug development. Traditional models for studying cardiotoxicity in vitro use non-cardiac cells, such as CHO or HEK cells, transduced with cardiac-muscle related ion channels. These models lack the complexity of in vivo cardiomyocytes, thus limiting their predictive power during drug screening. Use of animal models for drug screening is confounded by species differences in ion channels involved in the cardiac action potential. Pluripotent stem cell-derived functional cardiomyocytes provide a simple and renewable alternative model for in vitro drug toxicity studies as well as for cardiac disease modeling and the development of clinical therapies. We have previously shown that the StemXVivo™ Cardiomyocyte Differentiation Kit efficiently differentiates human pluripotent stem cells into functional cardiomyocytes. In this study, we provide further evidence for the functional validity of these cells using multiple techniques that include cell survival assays and calcium channel visualization. We expose differentiated cardiomyocytes to small molecules known to modulate cardiac function, including beta-adrenergic agonists and antagonists, hERG inhibitors, kinase inhibitors, and DNA damaging molecules. We observe that cardiomyocytes respond as expected with changes in cardiac physiology respective to each small molecule. Last, we show that small molecule and drug testing using cardiomyocytes derived from human pluripotent stem cells using the StemXVivo Cardiomyocyte Differentiation Kit are amenable to high throughput screening assays.

W-1114

ROLE OF INTEGRINS DURING CARDIAC DEVELOPMENT IN HUMAN PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

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Integrins mediate cell-extracellular matrix interactions that govern numerous physiological processes including cell shape and cell cycle. Their role during transition of fetal cardiomyocytes into adult mature cardiomyocytes during cardiac development remains elusive. In this study, using human pluripotent stem cells (hPSCs) as an in vitro humanized model for cardiac development, we aim to understand integrin regulation during various stages of cardiogenesis ultimately leading to cardiomyocyte maturity. Human PSCs were differentiated into cardiomyocytes using a directed differentiation protocol which emulates mammalian cardiac development. Temporal quantitative gene expression and Western blot analysis were utilized to characterize integrin expression during various stages of cardiogenesis. Quantitative gene expression analysis during cardiogenesis revealed hPSCs transitioned through cardiomesoderm (T, Mesp1), cardiovascular progenitors (Kdr, Isl1), cardiac committed precursors (NKx2.5, Gata4) and finally contracting cardiomyocytes (Tnnt2, Myl2) thereby emulating cardiac development in vitro. Corresponding with these various stages of development, distinct integrin expression profiles were revealed by Western blot analysis. While integrin α4 and α5 was significantly up-regulated during early stages of cardiogenesis, integrin β1D was only expressed in cardiomyocytes, suggesting stage-specific temporal regulation of integrins. Interestingly, integrin β5 demonstrated a gradual increase throughout differentiation with maximal expression in cardiac committed precursors and cardiomyocytes. Increased integrin signaling resulted in up-regulation of Ca2+ ion channels and organized inter-sarcomeric distance between Z-bands of cardiomyocytes that coincided with cellular maturity. In conclusion, integrins play versatile roles during various stages of cardiogenesis and activation of specific isoforms may regulate maturation of fetal hPSC-derived cardiomyocytes, thus providing a valuable model to study and to understand adult-onset cardiac disorders in vitro.

W-1115

CYCLIN A2 'REPROGRAMS' ADULT MAMMALIAN CARDIOMYOCYTES TO A PROGENITOR STATE

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The dedifferentiation of specialized cells and cycling of resultant progenitor/stem cells is thought to be important for regeneration, particularly in adult organs with low abundance of active cycling cells. In mammalian heart, 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. To investigate the molecular mechanisms of cell division in adult mammalian cardiomyocytes we have optimized culture methods for adult human and mouse cardiomyocytes. The isolated cardiomyocytes start to adhere and spread after 1 week of culture. We induced expression of CCNA2 using adenovirus encoding 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 α-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 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.

W-1116

A NOVEL HUMAN PLURIPOTENT STEM CELL-BASED PLATFORM FOR VISUALIZING AND PREDICTING CARDIAC ARRHYTHMIAS

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Drug-induced ventricular arrhythmias in the form of torsades de pointes (TdP) can be life-threatening and hence a major concern in drug safety. Indeed, TdP is a major cause for drug attrition and market withdrawal, owing to the lack of effective risk prediction platforms prior to human trials. For instance, in the Cardiac Arrhythmia Suppression Trial (C.A.S.T.) conducted during 1986-98, several 'anti-arrhythmics' such as flecainide were found to ironically increase mortality by causing lethal arrhythmias after testing in over 1700 patients. Conventional screening of TdP risk performed in non-cardiac or non-human models are sub-optimal in reflecting the actual cardiac responses to drug treatment in human. Thus the use of human pluripotent stem cell (hPSC)-derived ventricular cardiomyocytes (VCM) for disease modeling and arrhythmogenicity screening has been pursued. However, earlier studies were primarily conducted with single cells or disorganized clusters; while single-cell traits such as action potential prolongation, early and delayed after-depolarization provide convenient estimates, they are merely surrogates or triggers of but not arrhythmias per se, which are by definition multi-cellular reentrant events. Aligned hPSC-VCM sheets (a.k.a. human ventricular cardiac monolayers or hvCML) generated by a micro-groove technology were used in this study to recreate the structural and functional anisotropy seen in native heart. Drugs with indicated TdP risk as listed on CredibleMeds® were tested to evaluate the predictability of arrhythmia. Wave propagation upon drug treatment was measured using optical mapping while action potential duration and conduction velocity could also be computed. Sustained and non-sustained arrhythmia could readily be visualized as spiral waves. In addition to AP defects, flecainide was found to induce reentrant arrhythmias in ~20% of hvCML (n=11) comparing to 0% of control drug-free conditions. Thus the pro-arrhythmic properties of this failed CAST drug could readily be detected. Based on these data, a novel scoring algorithm for assessing arrhythmogenicity is proposed. We conclude that our current model would better predict the clinical risk of drug-induced arrhythmia with improved sensitivity, thereby facilitating the development of new pharmaceutical regimes.

W-1117

SINGLE CELL TRANSCRIPTOME ANALYSIS DISSECTS CELL FATE DETERMINATION FROM IPS CELLS TO CARDIOMYOCYTE

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During human development, a single-cell fertilized egg generates hundreds of different types of the cell. This cell fate specification is finely regulated by epigenetic mechanism. However, there is little understanding of the mechanisms by which a particular gene network at a branch of the cell differentiation defines direction to specific cell type. For the clinical application, it is also important to generate a specific cell type with a high efficiency, by precise control of differentiation. Continuous supply of cardiomyocyte is required for cell-based therapy and drug screening to evaluate cardiotoxicity. We aim to understand the detailed dynamics of transcription during differentiation from induced pluripotent stem (iPS) cells to cardiomyocyte and increase the efficiency of cardiac differentiation. We performed single cell RNA-seq using iPS and cardiomyocyte-directed cells. We differentiated human iPS cells into cardiomyocyte by changing the growth factors at each time point, and harvested the cells at day 1, 3, 5, 7, 9, 21 and 30 after induction of directed differentiation. We performed RNA-seq for twenty-four of the singlet cells harvested at each time point. Principle component analysis showed heterogeneity of gene expression in each day point, and enabled data to sort samples into differentiation status. Our newly developed method modified from Weighted Correlation Network Analysis (WGCNA) identified core gene expression modules of differentiated and iPS cells. In addition, we could detect several groups of genes whose expression were dynamically changed during the differentiation process. We propose time-information-free analysis as a powerful approach for unveiling the dynamics of transcriptome in reprograming and differentiation.

W-1118

EMPLOYING AN IN VITRO NICHE FOR MURINE CARDIAC STEM CELLS ALLOWS TO DELINEATE A TRANSCRIPTIONAL NETWORK AT THE BEGINNING OF CARDIOMYOGENESIS

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Cardiac stem cells depend on a tissue specific stem cell niche to survive, self-renew and differentiate. Reconstruction of basic niche conditions should permit to isolate and maintain cardiac stem cells in vitro. Temporal co-culture of whole heart cell populations with fibroblasts, secreting leukemia inhibitory factor, and embryonic stem cells under a tight selective regime resulted in the reproducible isolation of clonal murine cardiac stem cell lines. These cardiac stem cell lines remain genotypic and phenotypic stable over more than 149 passages when cultured in the presence of leukemia inhibitory factor, serum, and factors secreted by murine fibroblasts. In the absence of these factors cardiac stem cells exclusively differentiate to cardiomyocytes, vascular endothelial cells and smooth muscle cells. Here we demonstrate the interaction of the transcription factors nanog, brachyury, mesp1 and nkx2.5 with the nanog, brachyury, mesp1, nkx2.5, and desmin genes in cardiac stem cells by chromatin immunoprecipitation and provide evidence that these physical interactions change in the course of cardiomyogenesis. These data suggests that stemness factors such as nanog interact with early mesodermal and myocardial transcription factors, and vice versa, in a basic dynamic network regulating the transition from self-renewal to differentiation. Additionally, these results demonstrate feasibility of in vitro maintenance of cardiac stem cells and provide the foundation for studying the molecular regulation of early cardiomyogenesis and stem cell based myocardial regeneration in vitro.

W-1119

GMP MANUFACTURING AND PRECLINICAL TESTING OF AN ADVANCED THERAPY MEDICINAL PRODUCT FOR ACUTE MYOCARDIAL INFARCTION TREATMENT

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The ischemia produced after an Acute Myocardial Infarction (AMI) causes the death of millions of cells inducing an inflammatory process and triggering a tissue healing response. This process finishes with the formation of a fibrotic scar without contractile capability, usually followed by cardiac remodeling and chronic heart failure (CHF) development. The high prevalence of ischemic heart disease and the absence of effective treatments able to prevent the development of chronic heart failure after the ischemic insult have encouraged search for feasible alternatives to treat this pathology, among them cell therapy has arose high expectations. A total of 7 GMP-accredited cell productions were obtained and characterized by genome wide array expression analysis, confirming the robustness of the manufacturing process and the bio-equivalence among different batches. Preclinical studies in small and large animal models showed the safety and the efficacy of the cellular products. Dosing, timing and delivery methods were optimized to promote a strong myocardium regeneration and functional recovery. Intracoronary CSCs administration showed a reduction of scar size and an improvement of cardiac function in an infarcted pig model when compared with placebo treated animals. In-vivo studies in immunodeficient mice and in large animal models demonstrated that CSCs are safe and no-observed-adverse-effect-level (NOAEL) assays showed no toxicity when 100 million CSCs were intracoronary administered. Finally, biodistribution studies after intracoronary or intramyocardial administration revealed a high cardiac tropism of manufactured CSCs. The results from non-clinical studies showed that the approved Advanced Therapy Medicinal Product (ATMP) is effective and safe, and offer an affordable and off-the-shelf cellular product for the treatment of the Ischemic Heart Disease. Coretherapix has initiated a clinical trial for testing a cell therapy product based on the intracoronary administration of allogeneic Cardiac Stem Cells (CSCs) for promoting myocardial regeneration.

W-1120

IDENTIFICATION AND ISOLATION OF CHAMBER-SPECIFIC PROGENITORS DERIVED FROM PLURIPOTENT STEM CELLS

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Cardiomyocytes derived from pluripotent stem cells (PSCs) represent a promising platform for in vitro disease models, drug discovery, and cell based heart therapeutics. Protocols that allow a yield of >90% cardiomyoctes from PSC cultures are now readily available. However, PSC-derived cardiomyocytes remain electrophysiologically and functionally heterogeneous. The heart develops from two developmentally distinct populations of cells called 'cardiac progenitor cells', referred to as the first- and the second heart field, which serves as 'building blocks' of the left- and right ventricular heart chamber, respectively. Yet, it is still unclear whether cardiomyocytes from these distinct CPC populations are functionally and molecular distinct. By a candidate approach based on CPC expression patterns in mice combined with lineage trace analyses, we identified differential expression of transcription factors and surface molecules that marks the two distinct CPC populations. Using these surface candidates we were able to isolate distinct CPCs from PSC cultures using the Sony SH800 cell sorter. Gene expression patterns of these two PSC-derived CPC populations correlated well with in-vivo expression patterns of the developing right and left ventricle in mice. Further gene expression analyses of the two populations revealed functional characteristics and differential expression of previously reported candidates (Tbx5, HCN4 and Tbx1, FGF10, respectively) of the first- and the second heart field. Our results demonstrate that distinct chamber-specific CPCs that give rise to the right- and left ventricle can be identified and isolated directly from differentiating PSC cultures. This offers a novel method to study left and right ventricular cardiomyocytes from PSCs and provides a platform to study early human cardiogenesis and how distinct CPCs develop and contribute to congenital heart disease.

MUSCLE CELLS

W-1122

THE EFFECT OF LOW ENERGY LASER IRRADIATION IN MODEL OF DENERVATION AND DISUED ATROPHY

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Denervation and disuse induced muscle atrophy, a common clinical neuromuscular pathology, are important issues in neurology. Our previous study, using denervation and disuse animal model had found that accompanying with the muscle tissue atrophy are an increase in density of stem cells expressing Pax3 and/or Pax7 and an increase in their apoptosis. It might be that the death of stem cell is the one of the main cause leading to the final atrophy of muscle. Low energy laser radiation (LLL) had been use in clinical for decade in treating pain or other disorders. Previous report had indicated that topical application of LLL could increase the proliferation of muscle stem cells in cell culture, and reduce the fibrosis of muscle in animal models. Here, we postulate that LLL could rescue the atrophy of muscle induced by denervation or disuse by increasing the proliferation of stem cells and reducing the apoptosis of stem cell. We developed denervation and disuse animal models using male Sprague-Dawley rats. The dysfunction of denervated or disused leg was examined by SFI and gait appearance scoring. The muscle strength was determined by measuring action potential using needle EMG in vivo. The histology change of muscle atrophy was examined by HE staining of muscle tissues. The density of muscle stem cells and apoptosis were quantified by Pax3/Pax7/Tunel immunofloresnce histology in slide-mounted muscle tissue. LLL was applied to the leg right after been denervated or disused once every day. The difference among groups was analyzed by one way-ANOVA.
The results showed that both denervation and disuse treatment produced profound dysfunction of leg, abolished or reduced the tibia muscle strength, and increased the proliferation and apoptosis of stem cell. Application of LLL did not significantly improve the function of treated-legs, but did improve transiently the strength of muscle

in disused animals. Furthermore, LLL could potentiate the increase of proliferation of stem cell and reduce the apoptosis of cells. In conclusion, the finding that both denervation and disuse could induce proliferation and apoptosis of muscle stem cells and application of LLL could counteracts the apopotosis effect indicates the clinical potential of the use of LLL in treating patients.

W-1123

FOUR TRANSCRIPTION FACTORS MAKE MEF HAVE HIGH CAPACITY OF MYOGENIC DIFFERENTIATION AND PROLIFERATION

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The supply of myogenic stem cells is still challenging. To establish cells with myogenic differentiation ability and consistent proliferation capacity, we transduced four transcription factors to mouse embryonic fibroblasts. After transduction of four transcription factors, the cells show higher expression level of myf5, myoD, myogenin, which are well-known skeletal muscle markers, when compared to mouse embryonic fibroblasts. And the transduced cells differentiate to muscle and form myotubes which have multi-nucleus in skeletal muscle differentiation media. Also the transduced cells can keep passaging till 50 generation with consistent proliferation rate. For getting single cell clone, we performed single cell clonal selection with CD90.2 markers. The cells with CD90.2 (-) show high expression of pax7, myf5, myoD and myogenin. On the other hand, CD90.2 (+) cells have low or no expression of pax7, myf5, myoD and myogenin. After single cells selection with CD90.2 (-), the cells showed much higher level of myogenic markers such as myf5, myoD and myogenin. The sorted single cells are negative for PDGF-alpha receptor, CD90.2 and CD106 whereas the unsorted transduced cells are positive for PDGF-alpha receptor, CD90.2 and CD106. Interestingly, the sorted single cells are positive for integrin alpha 7 and CD73, which are myogenic precursor cell markers, while unsorted transduced cells show negative or low level of integrin alpha 7 and CD73. In conclusion, we reveal that the four transcription factors make mouse embryonic fibroblasts have high capacity of skeletal muscle differentiation and proliferation. And sorting with CD90.2 (-), helps cells have high capacity of myogenic differentiation.

W-1124

A SYSTEMS APPROACH TO IDENTIFY MECHANISMS OF MUSCLE STEM CELL SELF-RENEWAL

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Satellite cells are a distinct and small population of skeletal muscle resident stem cells responsible for regeneration and maintenance of mature skeletal muscle. Transcription factors from the Pax gene family are critical for satellite cell biogenesis, survival and self-renewal. Pax7 expression persists in activated, proliferating satellite cells but is rapidly down-regulated in cells that commit to terminal myogenic differentiation. These facts suggest that an active mechanism exists in satellite cells to sustain Pax7 expression for stem cell maintenance or to mediate its degradation for entry into myogenic differentiation. We aim to find out if such a pathway exists and, if so, to identify the molecular determinants that govern Pax7 protein stability. Our data indicate that Pax7 is degraded via the ubiquitin-proteasome system. To isolate the molecular machinery that mediates Pax7 ubiquitylation we will perform a multi-parametric high-throughput, loss-of-function lentiviral RNAi screen against all known ubiquitin related genes. The ultimate goal of this project is to identify the mechanism and the putative factors underlying Pax7 stability and the therapeutic potential of targeting these for muscle development and regeneration related disease.

W-1125

HEPATOCYTE GROWTH FACTOR IMPROVES STEM CELL DYSFUNCTION AND REDUCES FIBROSIS IN MUSCULAR DYSTROPHY

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The NF-κB pathway has been implicated in a number of conditions including chronic inflammation and diseases such as Duchenne muscular dystrophy (DMD). Using mdx mice, a model of DMD, our group previously found that inhibiting the NF-κB pathway through global p65 suppression (p65+/-) decreased inflammation, necrosis, and fibrosis in vivo. In addition, muscle-derived stem cells (MDSCs) from p65+/- mice showed increased proliferation, myogenic differentiation, and muscle regeneration. Interestingly, hepatocyte growth factor (HGF) expression was significantly increased in whole muscle tissue of p65+/- mice. HGF activates satellite cells and has anti-inflammatory properties; however, the short half-life of HGF in vivo has hindered its use therapeutically. New controlled release technologies such as coacervate, composed of heparin and poly(ethylene argininylaspartate diglyceride) (PEAD), allow for the sustained release of HGF. In the current study, we examined the role that HGF plays in the function of primary cells from p65+/- mice. HGF mRNA expression was increased in muscle cells, but not infiltrating macrophages, in acutely injured muscles of p65+/- mice compared to wild-type littermates. Although the mechanism in skeletal muscle is still unclear, our results indicate that p65+/- macrophages expressed higher IL-10 mRNA levels and had increased surface expression of M2 activation markers (RELM-α and CD163), suggesting a potential anti-inflammatory role. Therefore we hypothesized that delivery of exogenous HGF could have a beneficial effect on dystrophic muscle, as observed with NF-kB inhibition. Although our results are preliminary, we found that HGF delivered to severely dystrophic muscle via heparin-coacervate significantly decreased muscle fibrosis and necrosis. We also observed that HGF with coacervate modulated MDSCs in vitro by increasing cell survival under oxidative stress (n=3). We are currently performing a full-scale evaluation of the efficacy of HGF coacervate to improve skeletal muscle regeneration. Controlled HGF delivery, likely more specific than ubiquitous anti-NFkB treatment, might be beneficial for muscle repair by creating an environment more conducive to endogenous and/or transplanted cell-mediated muscle regeneration, offering a new therapeutic approach for DMD.

W-1126

IDENTIFYING SIGNALS FROM THE SKELETAL MUSCLE NICHE THAT REGULATE SATELLITE CELL FUNCTION

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Recent studies suggest that muscle-resident fibro-adipogenic precursors (FAPs) are a critical component of the satellite cell niche. FAPs help to orchestrate efficient muscle regeneration and potentiate satellite cell differentiation. This activity appears to derive in part from soluble factors produced by FAPs, as FAP conditioned medium also stimulates satellite cell differentiation. We found that FAP-like cells are also present in tissues other than skeletal muscle. Surprisingly, FAPs isolated from mouse lungs were able to stimulate satellite cell differentiation in vitro similar to FAPs from skeletal muscle. These data suggest that tissue-resident stromal cells in diverse tissues may utilize common signaling mechanisms to control the fates of their respective tissue-resident stem cells. In addition, this observation presents a unique opportunity to clarify the molecular mechanisms by which muscle FAPs modulate satellite cell activity. Taking advantage of the shared activity of muscle and lung FAPs in regulating muscle satellite cells, and the lack of this activity in mouse embryo fibroblasts (MEFs), we performed comparative RNA sequencing analysis to identify transcripts commonly enriched in lung and muscle FAPs from mice, but depleted in MEFs. This gene set was further prioritized by selecting for secreted molecules that were enriched in human FAP-like cells as compared to human satellite cells, yielding a list of factors that might act as conserved signals in the intercellular communication between FAPs and satellite cells in mice and humans. This analysis identified Hepatocyte Growth Factor (HGF) as a potential candidate for validation of our approach. HGF has been well studied as a satellite cell regulator, and our analysis indicates that this molecule is expressed by FAPs but not satellite cells. Confirming prior studies, we found that HGF stimulated differentiation and clonal expansion of satellite cells in vitro. Taken together, this study reports a new strategy for identifying paracrine factors produced locally in the skeletal muscle niche that may regulate satellite cell function. HGF is likely to represent one such factor that participates in the interactions between FAPs and satellite cells and thereby influences muscle regeneration.

W-1127

SKELETAL MUSCLE DERIVED MYOBLAST DIFFERENTIATION INHIBITORY MECHANISME IN SIMULATED MICROGRAVITY ENVIRONMEN

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Patients with muscle intractable disease such as muscular dystrophy have a high hope for regeneration medicine. However, unfortunately, it does not become an established therapy for muscle. Data from astronaut studies and model microgravity experiments show that gravity regulates cell proliferation, differentiation, and function. Muscle is one of the sensitive tissues for gravity. Microgravity and its simulated conditions preferentially disturb differentiation of skeletal muscle cells. We consider the mechanisms of muscle differentiation by gravity give us new aspects of stem cell therapy for muscle intractable disease. A clinostat is one of the devices for providing the simulated microgravity environment to examine the weightless and space-flight research. In this study, rat skeletal muscle derived myoblasts (L6) were induced muscle differentiation in normal 1G environment and 10^-3 G environment. Simulated microgravity environment was provided using newly developed three-dimensional-clinostat, the GRAVITE (Space Bio-Laboratories Co, Ltd. and KITAGAWA IRON WORKS CO., Ltd., Japan), controlled by rotation of two axes, resulting 10^-3 G average over time. And then, we investigated mRNA expression control systems, such as synthesis, degradation, and DNA demethylation. In microgravity, muscle differentiation was inhibited and MyoD mRNA expression was significantly decreased. This result indicated MyoD was the most sensitive and important gene to gravity in MyoD family of muscle differentiation specific genes. Additionally, the MyoD expression was controlled DNA demethylation. In summary, simulated microgravity increased MyoD DNA methylation resulted in inhibition of muscle differentiation. We already reported that microgravity had proliferative effect and differentiation inhibitory effect on stem cells. And we showed that hyper-gravity environment enhanced muscle differentiation. This new insight related to muscle differentiation control mechanism by microgravity helps us to establish the attractive stem cell culture and muscle differentiation methods using gravity.

KIDNEY CELLS

W-1129

ESTABLISHMENT OF NOVEL STEM CELL-DERIVED KIDNEY MODELS FOR DRUG DISCOVERY AND SAFETY

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Animal models used in preclinical testing are not sufficiently predictive and offer limited throughput for screening purposes. Pluripotent stem cell (PSC)-derived models offer new opportunities to improve drug discovery processes and bring safe medicines to the market. Despite the widespread applications of PSC-derived liver and heart models, kidney remains under-represented in drug discovery and safety screens, calling for innovative strategies to recreate the complex renal function in vitro. Here, we combined genetic and pharmacological approaches to rapidly and efficiently differentiate human induced PSC into metanephric mesenchyme (MM) capable of undergoing nephron-like epithelialization. In our attempt to control the differentiation and maturation of MM cells into functional renal tubule epithelial cells specifically, we elaborated a high content imaging-based screening strategy to identify novel compounds and pathways that induce an AQP1+ phenotype. Ultimately, our goal is to characterize multi-species PSC-derived

kidney models to enable cross-species and in vitro to in vivo translatability studies.

W-1130

EFFECTS OF EXOSSOMES (EXOS) DERIVED FROM RENAL PROGENITOR CELLS (RPCS) ON THE LPS TOXICITY IN IMMORTALITY HUMAN MESANGIAL CELLS (IHMCS) AND RENAL EPITHELIAL CELLS (LLCP-K1)

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Aim: In this study we investigate the effects of EXOs derived by rPCs on the iHMCs or LLCP-K1 treated with LPS. Methods: rPCs were characterized by (CD24, PAX2 and CD133) and negative (CD45, Thy-1 and pancytokeratin). rPCs were previously treated with LPS (100µg/ml), gentamicin (G; 2mM) or cisplatin (Cis; 6µM) (preconditioned - PC) or not (nPC) for 24 hours. The EXOs were characterized by CD81 or CD63. The iHMCs or LLCP-K1were treated with LPS (100µg/ml) or PBS (vehicle) for 24 or 72 hours or LPS+EXOs nPC (35µg/ml) or PC-EXOs (35µg/ml) for 72h. Cellular viability, caspase 3, IL2, IL6, IL10 and NO were evaluated. Results: rPCs were positive by CD24, PAX2, CD133, negative for CD45, Thy-1, pancytokeratin. EXOs were positive to CD81, CD63. LPS Treatment decreased cell viability versus PBS 24h (408±18.8vs.150±7.9; %; $p<0.05$) or 72h (385±12.6vs.67.5±7.4;%; $p<0.05$). When these cells were treated with EXOs-nPC, cell viability increased for 24h (312±7.4 vs. 150±7.9;%; $p<0.05$) or 72h (266±14.6 vs. 76.6±5.7;%; $p<0.05$). The LPS 72h increased expression of caspase 3, IL2, IL6 and decreased IL10 vs. CTL. These cytokines and caspase 3 decreased when iHMCs received nPC-EXOs were intensifying in PC-EXOs and increased IL10. NO (nmoles/mg) increased in LPS vs. CTL 72h in iHMCs (82.8±9.5 vs. 171.2±27.8; $p<0.05$), decreased in nPC-EXOs (145.4±4.6 vs. 171.2±27.8; $p<0.05$) and PC-EXOs with LPS, G or Cis for 72h, respectively (109.5±11.2; 115.8±5.8; 103.2±9.3; $p<0.05$). Similar results were obtained with LLCP-K1. NO increased in LPS vs. CTL (24.5±1.8vs.129.8±16.4;$p<0.05$) and decrease in nPC-EXOs (129.8±16.4 vs.72.6±6.6; $p<0.05$) and PC-EXOs with LPS, G or Cis for 24h, respectively (76.3±5.9; 41.7±5.1; 33.8±1.3; $p<0.05$). Conclusion: These results suggested that EXOs derived from rPCs have the ability to protect the toxicity in iHMCs and LLCP-K1 induced by LPS observed by apoptosis, cytokines pro-inflammatory and NO modulation. These effects were mediated by EXOs and intensified in PC-EXOs.

PANCREATIC, LIVER, LUNG OR INTESTINAL/GUT CELLS

W-1131

SNAI1 IS REQUIRED FOR STEM CELL MAINTENANCE IN THE MOUSE INTESTINAL EPITHELIUM

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The Snail family of transcription factors are well known for mediating epithelial to mesenchymal transitions and cell motility during both embryonic development and tumour invasion. Although they are generally regarded as markers of mesenchymal cells, Snail proteins have recently been implicated in regulating stem cell populations in several organs. Snai1 is normally expressed in the intestinal epithelium within the crypt base columnar (CBC) stem cells and transit amplifying cell populations. Our studies have shown that both the expression and cellular localisation of Snai1 is dependent on canonical Wnt signalling, a key regulatory pathway of intestinal stem cells. Snai1 is up-regulated in polyps from Apcmin/+ mice indicating that Snai1 may also play a part in the early stages of tumorigenesis in addition to promoting invasion of intestinal tumours. We investigated Snai1 function in the mouse intestinal epithelium using an inducible conditional knockout approach and found that knockout of Snai1 results in apoptotic loss of CBC stem cells and a bias towards differentiation of secretory lineages. Further analysis of the effects of Snai1 loss on the CBC stem cell population using a combination of Fluorescent Activated Cell Sorting (FACS), lineage tracing and in vitro organoid culture showed that loss of Snai1 results in a decrease in cell proliferation and apoptotic loss of stem cells. In contrast, ectopic expression of Snai1 using a conditional transgenic approach results in an increase in cell proliferation at the crypt base which correlates with elevated levels of expression of CBC stem cell markers. In addition, a decrease in secretory Paneth and enteroendocrine cells is observed. Our functional studies show that intestinal epithelium where Snai1 is depleted fails to produce a proliferative response following radiation induced damage. These studies demonstrate a critical role for Snai1 in survival of CBC cells and regeneration of the epithelial cell layer following damage. In conclusion, this suggests that Snai1 has a key role in stem cell maintenance and control of cellular differentiation.

W-1132

BETA-CELLS GENERATED FROM HUMAN PLURIPOTENT STEM CELLS TO BE USED IN IN VIVO AND IN VITRO APPLICATIONS

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The great possibilities of using human pluripotent stem cells (hPSC) in both clinical therapies as well as for in vitro applications are promising and have created a growing interest in the pharmaceutical and biotech industry. Novo Nordisk; a leader company in diabetes care, Takara Bio Europe AB (former Cellectis/Cellartis); a pioneer in stem cell biotechnology, and Lunds University started in 2008 a joint program in diabetes regenerative medicine. The purpose of this program is to generate insulin-producing beta-cells from human Embryonic Stem Cells (hESC) in order to cure Type 1 Diabetes (T1D) by transplanting these cells into patients. T1D is an autoimmune disease and patients are normally treated with a continuous administration of insulin-related drugs, since their immune system attacks and destroy the beta-cells in the pancreas, resulting in an insufficient production of insulin. Presently, the project has developed a four-step differentiation protocol, generating functional beta-cells from human pluripotent stem cells in vitro. These cells are presently being transplanted into animal models. Along with the protocol development a clinical grade media system has been established to culture cells complaint with GMP conditions. Beside clinical use of these generated beta-cells, a system using the same cells for in vitro applications are being set-up. An in vitro-based beta-cell product would offer the possibility to screen compounds for regulating insulin secretion, but also provide the opportunity to study beta-cell function, GSIS (Glucose Stimulated Insulin Secretion), calcium influx signaling, mechanism of insulin secretion, beta-cell transdifferentiation, etc. This novel system, directed towards in vitro applications, is presently not available in the market and could replace the use of human primary pancreatic islets from deceased donors. Such system will also provide a better access of beta-cells and reduced batch variability compared to primary islets.

W-1133

FACILITATE AND STANDARDIZE HESC - AND HIPSC DERIVED HEPATOCYTE EXPERIMENTS IN 2D- AND 3D CULTURES

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Human hepatocytes display substantial functional inter-individual variation regarding drug metabolizing functions. This diversity is mirrored in hepatocytes derived from different human pluripotent stem cell (hPSC) lines, shown by evaluation of twenty-five hPSC lines originating from twenty-four different donors (A .Asplund et. al, for submission). With regard to functional aspects, such as Cytochrome P450 activities, we observed that hepatocytes derived from different hPSC lines reflected the inter-individual variation characteristic for primary hepatocytes obtained from different donors, while these activities were highly reproducible between repeated experiments using the same line. We also found that our standardized hepatic differentiation protocol did generate a homogenous hepatocyte culture from all hPSC lines tested, which do facilitate the emerging possibility to compile panels of hPSC-derived hepatocytes of particular phenotypes/genotypes relevant for drug metabolism and toxicity studies. To further harmonize hepatocyte experiments using multiple donors, we have continued to develop several aspects of the differentiation procedure. The use of frozen batch controlled media for Definitive Endoderm (DE) differentiation and differentiation towards hepatocytes do minimize potential variations caused by batch-to-batch variations in medium components. Moreover, the use of frozen banks of DE cells from different donors does further facilitate the synchronization of more complex studies, where data from several donors are of interest. The concept to differentiate from frozen DE cells followed by further differentiation towards hepatocytes in more complex 3D scaffold systems has successfully been tested in the EU project NanoBio4Trans and will be presented.

W-1134

INSULIN MUTATION CAUSING NEONATAL DIABETES CORRECTED WITH CRISPR IN PATIENT-DERIVED IPSC

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Permanent neonatal diabetes is commonly caused by mutations affecting genes encoding proteins that are important for beta-cell function, such as ATP-sensitive potassium channels or insulin. Insulin gene mutations lead to the accumulation of misfolded insulin protein, causing endoplasmic reticulum (ER) stress that eventually triggers beta-cell apoptosis and subsequent diabetes. ER stress is also an important pathogenetic mechanism in both T1 and T2 diabetes. In order to establish a human disease model of ER stress-caused diabetes, we derived iPSC lines from two Finnish families carrying heterozygous mutations in the insulin gene. These mutations are dominant and the carriers develop diabetes at 3-4 months of age. The patient-specific iPSC lines were differentiated in vitro successfully to pancreatic endocrine progenitors using an optimized protocol and, further, into functionally immature beta cells. To overcome the variability in the differentiation between the control and patient-specific iPSC, we generated isogenic cell lines by correcting the insulin mutation using CRISPR technology. A combination of guide RNAs targeting next to the mutation site, together with 70 bases single stranded DNA repair template and Cas9 resulted in efficient correction of the insulin mutation by homologous recombination. After electroporation of these components to the patient iPSCs, 22.5% of isolated clones had integrated the correction repair template. Corrected cells were differentiated in parallel with mutant cells to the beta cell lineage and differentiated endocrine progenitors were transplanted under the kidney capsule of immunodeficient mice to obtain beta cell maturation in vivo. Electron microscopy and qPCR was used to examine structural and transcriptional changes caused by ER stress in differentiated beta cells. This model provides insight into the pathogenetic mechanisms of beta-cell failure not only in these families, but also in more common forms of diabetes. It could also serve as a drug-screening platform for ER stress inhibitors.

W-1135

SELF-ORGANISATION AND DIFFERENTIATION OF HESC DERIVED HEPATOCYTES UNDER DEFINED CONDITIONS

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In this study we aimed to improve cell function and maturation of pluripotent stem cell (PSC) derived hepatocytes under defined conditions. To develop accurate models of human biology "in a dish", or pioneering cell based therapies, it is important consider the starting material and process definition. Our focus is on the major metabolic cell type of the liver, the hepatocyte. While a number of hepatocyte models exist, their drawbacks outweigh their advantages, currently limiting widespread deployment of the cell based technology. Therefore, other sources of human hepatocytes have been explored. One of the most promising sources of human cells, are pluripotent stem cells (PSCs). PSCs represent an exciting cell resource as they are amenable to scale up and directed differentiation, promising an unlimited supply of somatic cell(s) for application, if the appropriate differentiation procedures exist. In the past, we have used biological matrices, prepared from human or animal tissue, to support our differentiation process. While enabling to an extent, those substrata display lot to lot variation, serving as a significant barrier to scale-up and therefore application. In these studies, we have tackled this issue, opting to use biologically relevant and full length liver matrices, which have been produced using recombinant expression systems. The focus of our studies was the supportive properties of human recombinant laminins in the context of PSC derived hepatocyte self-organisation and function. Human PSCs were cultured on laminin 521, 111 and matrigel, prior to the onset of differentiation as described in Szkolnicka et al., 2014. At key stages during differentiation, stem cell derived definitive and hepatic endoderm gene expression and function were characterized. Hepatocytes were generated from PSC cultures on all three substrate. When cultured on laminins the hepatocytes demonstrated significantly higher levels of cytochrome p450 activity compared to cells on matrigel. Furthermore, we observed overt differences in cell size, assembly and organisation on laminin coated surfaces. We believe that these studies represent an important advance, which permit large manufacture of stem cell derived hepatocyte populations for laboratory and clinical application.

W-1136

A COMPREHENSIVE TEMPORAL ANALYSIS OF DIFFERENTIATING PANCREATIC β-ISLET CELLS FROM HUMAN EMBRYONIC STEM CELLS PROVIDES GLOBAL INSIGHTS INTO MATURATION

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While the differentiation of stem cells into pancreatic beta cells offers tremendous potential as a cure for diabetes, many challenges exist toward using these cells in therapy. To better understand the maturation process, we performed a global temporal analysis of human stem cells as they differentiate into pancreatic β-islet cells using quantitative proteomics and transcriptomics. This temporal developmental profiling approach allowed us to determine changes unique to specific differentiation states and allowed us to identify a number of candidate key factors involved in the differentiation and maturation of hESC into pancreatic β-islet cells as well as specific markers of pancreatic beta cell progenitors. Human pancreatic cells from postmortem tissue was used to compare and characterize the maturity of the hESC derived β-Islet cells.

W-1137

TARGETED CORRECTION AND RESTORED FUNCTION OF CFTR GENE IN CYSTIC FIBROSIS INDUCED PLURIPOTENT STEM CELLS

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Recently developed reprogramming and genome editing technologies make possible the derivation of corrected patient-specific pluripotent stem cell sources - potentially useful for the development of new therapeutic approaches. The primary defect in Cystic Fibrosis (CF), an autosomal recessive disorder, is the regulation of epithelial chloride transport by a chloride channel protein encoded by the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Recurrent pulmonary infections are responsible for 80 to 90% of the deaths in CF patients. Starting with skin fibroblasts from patients diagnosed with CF, we have derived and characterized induced pluripotent stem cell (iPSC) lines. We then utilized zinc finger nucleases (ZFNs), designed to target the endogenous CFTR gene, to mediate correction of the inherited genetic mutation in these patient-derived lines via homology directed repair (HDR). We observed an exquisitely sensitive, homology-dependent preference for targeting one CFTR allele vs. the other. Differentiation for a total of 19 days in a protocol designed to derive anterior foregut endoderm, subsequently up-regulated expression of NKX2-1, SOX9, TP63, FOXP2, FOXA2, and CFTR, suggesting commitment of at least a sub-population of cells within the endodermal culture to a lung epithelial cell fate. Once differentiated, mutant CF iPSCs yielded neither mature CFTR protein nor CFTR-specific chloride channel activity (as assayed in Ussing chamber experiments) - whereas corrected CF iPSCs and the normal control WA09 hESCs yielded mature CFTR protein and CFTR¬ specific chloride channel activity. In vitro differentiation of the mutant CF iPSCs into lung epithelial cells and tissue, controlled for by the parallel differentiation of the otherwise isogenic corrected CF iPSCs, may provide a valuable tool for drug screening and examining the functional consequence of mutant CFTR expression. Furthermore, corrected CF iPSCs present a potential source of patient-specific cells capable, in vitro, of differentiation into various lung stem/progenitor cells --either for transplantation of autologous lung cells or for seeding de-vitalized lung scaffolds ex vivo to generate autologous lungs.

W-1138

A NOVEL KIT THAT EFFICIENTLY GENERATES STEM CELL-DERIVED PANCREATIC PROGENITOR CELLS

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It is estimated that 347 million adults and children worldwide are currently living with diabetes. Donor human islet cell transplantation has been shown to be a more effective therapy for glycemic control than the traditional direct administration of insulin. Unfortunately, the lack of donor organs renders this option unworkable for millions of patients. Alternative therapies using pancreatic progenitor or insulin secreting cells generated from pluripotent stem cells have been shown to correct the diabetic phenotype in mice. Here, we introduce the StemXVivo™ Pancreatic Progenitor Cell Differentiation Kit, which efficiently directs pluripotent stem cells into pancreatic progenitors. Using quantitative PCR and immunocytochemistry we show that this kit differentiates both embryonic stem (ES) and induced pluripotent stem (iPS) cells, in a step-wise manner, through key stages of pancreatic development. Pluripotent stem cells are first directed toward definitive endoderm, as demonstrated by an increased expression of SOX17 and a decrease in expression of the pluripotency marker, Oct-4. Endoderm is then directed to differentiate into HNF-1 β+ primitive gut tube cells. Lastly, cells differentiate into PDX-1+ pancreatic progenitors and begin to express the key endocrine markers, NKX6.1 and SOX9. Using flow cytometry, we demonstrate that this kit is capable of generating > 70% PDX-1+ cells. In summary, the StemXVivo Pancreatic Progenitor Cell Differentiation Kit efficiently and reproducibly drives both ES and iPS cells into pancreatic progenitors. Cells derived using this kit can provide a renewable cell source to aid in drug candidate screening and to advance cell-based therapy for the treatment of diabetes.

W-1139

HUMAN LIVER ORGANOIDS - A LONG-TERM ADULT STEM CELL CULTURE SYSTEM

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Due to the rising incidence of liver disease, the demand for transplantable liver cells is steadily increasing. Despite the enormous replication potential of the human liver, there are currently no culture systems available that sustain hepatocyte replication and/or function in vitro. Recently, we established conditions that allow long-term expansion of single adult human liver stem cells isolated from small liver biopsies. These stem cells are of ductal origin and can be readily converted into functional hepatocytes. Liver cells grow as spherical organoids in a 3D matrix with defined culture medium. The high growth rate of human liver organoids enables us to generate billions of liver cells within 3-4 months from a single isolated stem cell. The expanded cells are highly stable at the chromosome and structural level, while single base changes occur at a very low rate similar to that of the germ-line. Differentiated organoids secrete essential serum proteins, produce bile acids and metabolize drugs. In vivo, human liver organoids can engraft acutely damaged mouse livers and contribute to their function. In vitro, the cells serve as model systems for genetic liver diseases such as α1-antitrypsin deficiency or Alagille syndrome. Furthermore, the genome of human liver stem cells can be precisely edited to generate knock-outs, knock-ins or introduce mutations. Clonal long-term expansion of primary adult liver stem cells opens up new avenues for basic research, regenerative medicine and gene therapy and is thus a promising tool for understanding and countering liver disease.

W-1140

MUCUS PRODUCED BY STEM CELL DERIVED HUMAN COLONOIDS IS AN EARLY TARGET IN ENTEROHEMORRHAGIC E. COLI INFECTION

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Enterohemorrhagic E. coli (EHEC) is the major disease-causing foodborne E. coli in the USA. In humans, EHEC colonizes the proximal colon and causes watery diarrhea that can progress into hemorrhagic colitis and life-threatening hemolytic uremic syndrome. There are no treatments against EHEC infections and currently no disease model exists that fully recapitulates EHEC infection in humans. Primary ex-vivo cultures of human colonic epithelium derived from LGR5+ stem cells potentially allows for the study of early stage EHEC infection. We established methods to grow LGR5+stem cell-derived human proximal colonoids as 2D monolayer cultures to study the interaction between the apical surface of the epithelium and EHEC. Colonoid monolayers achieve confluency in 2 weeks, as determined by transepithelial electrical resistance (TER). Cultures were then differentiated for 5 days into surface-like colonocytes and goblet cells, and then were apically infected with EHEC O157:H7. Using immunoblotting, immunofluorescence, scanning and transmission electron microscopy, we characterized effects on the colonic mucus layer. Differentiated colonoid monolayers showed a significant increase in TER (>3-fold), well-developed brush borders, and a significant decrease in the stem cell marker, LGR5, compared to undifferentiated monolayers. The appearance of goblet cells following differentiation resulted in mucin2 (MUC2) expression and formation of a thick (>25μm) apical mucus layer. EHEC infection eliminated the MUC2-positive mucus layer and led to bacterial attachment to and effacement of the brush border, characteristics of EHEC infection. The EHEC-secreted metalloprotease, StcE, has been previously suggested to cleave mucins. However, an EHEC stcE deletion mutant (EHECΔstcE) failed to protect mucus integrity, indicating that bacterial factors other than StcE are involved in mucus degradation. Finally, there were significantly less EHEC attached to undifferentiated colonoids, which lack a mucus layer, suggesting that mucus promotes EHEC adherence to epithelium. Thus, we have developed a novel human colonoid monolayer ex vivo model derived from adult stem cells

that resembles the proximal colon and serves as a physiologically relevant human model to study EHEC infections of the human colonic epithelium.

W-1141

NANOTOPOGRAPHY DIRECTS DIFFERENTIATION OF HUMAN PLURIPOTENT CELLS INTO PANCREATIC LINEAGE

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Human pluripotent stem cells (hPSCs) have been proposed as a promising candidate to treat diabetes mellitus. We previously reported a five-stage protocol to generate three-dimensional pancreatic islet-like spheroids from hPSCs. However, our and other protocols reported so far mostly do not reflect nanotopological structures created by extracellular matrices that surround the developing pancreas. In the present study, we investigated how topographical cues created by nanopore structures affect the pancreatic differentiation of human ES and iPS cells using nanopore-patterned chips with different pore diameters (100 ~ 230 nm). Quantitative PCR analysis showed that a nanopore pattern (NP) of 200 nm (in diameter) induced the highest level of PDX1 expression at the progenitor stage in the pancreatic differentiation of hPSCs. Immunostaining and fluorescence-activated cell sorting (FACS) analyses showed that the 200 nm NP significantly increased the percentage of pancreatic progenitors (PDX1+ and NKX6.1+), compared to the conventional flat culture dish (FL) (9.7% in the FL vs. 50.8% in the 200 nm NP, respectively). After further differentiation, these pancreatic progenitors produced a large number of three dimensional clusters that expressed insulin and c-peptide. We also investigated the underlying mechanism of the enhanced pancreatic differentiation on the nanopore pattern and found that downregulation of TAZ was directly involved in the upregulation of PDX1 and neurogenin3. Our study demonstrated that the pancreatic differentiation can be enhanced by nanotopographical environments in the differentiation of hPSCs.

W-1142

LUNG STEM CELLS AND THEIR POTENTIAL IN 3D-CULTURE SYSTEM

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Lung disease is the third most common cause of death in the United States and Europe. In many cases, lung transplantation is the only definitive treatment; yet only 2700 lung transplants are performed each year, limited primarily by a lack of donor organs. The development of engineered lung tissue, created using a patient's own cells, could have a significant impact on the treatment of end-stage lung disease. We therefore set out to develop robust 3D culture systems for normal and diseased human lung using rotating bioreactors. To generate the human lung in 3D, we use cellular scaffolds from human or mouse lungs and populated them with defined stem and progenitor cell populations. The adult human lung stem cell (HLSC) population we focus on is characterised by high expression of Lgr6 and E-cadherin. Lgr6+/E-cadherinhigh lung cells can be cultured indefinitely, successfully re- cellularise the human scaffold and differentiate into bronchio-alveolar cell types. The re-cellularised lungs grow in slice cultures and in rotating bioreactors and we observe increased cellular survival and improved lung morphology when the cells are grown in this way. Thus, we demonstrate progress towards the development of functional 3D engineered lung tissue in a rodent/human decellularised model. Our novel 3D technique, to create accurate in vitro models for normal and diseased human lung, can be applied to a wide area of research fields: ranging from the basic understanding of human lung biology to the evaluation of cellular responses to pharmaceutical compounds in drug discovery programs.

W-1143

LAMININ 521 AND 221 CONTAINING MATRICES ALLOW DIFFERENTIATION OF HEPATOCYTE-LIKE CELLS UNDER DEFINED CONDITIONS

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Despite the enormous success of human liver transplantation to treat end-stage liver failure, donor deficiency highlights outmost necessity for generating new therapeutic modalities. In vitro expansion and maintenance of isolated hepatocytes as well as liver stem cells is problematic due to their propensity for apoptosis and de-differentiation. Recent studies have implied significant potential for the use of human embryonic stem (hES) cells in tissue regeneration. However, reproducibility and efficiency of existing differentiation protocols as well as maturation status of generated hepatocytes frequently pose considerable limitations. Laminins represent 16 different, cell type-specific matrix proteins that have been shown to regulate cell differentiation, phenotype stability and resistance to apoptosis of many different cell types, including hES cells. Our mRNA expression analysis revealed prominent expression of laminin-521 (LN-521) and laminin-221 (LN-221) in normal human liver. Here, a combination of biologically relevant recombinant LN-521 and LN-221 was used as cell culture matrix for hES cell differentiation into hepatocytes. This defined matrix enabled robust differentiation of hES cells into hepatocytes and their progenitors using chemically defined and xeno-free cell culture media. Progression of hES cells through differentiation stages was analysed by qRT-PCR, immunocytochemistry and flow cytometry. Gene expression studies showed downregulation of pluripotent genes (NANOG, POU5F1 and SOX2) and absence of mesodermal (T, MIXL1) and neuroectodermal cells (PAX6). Efficient differentiation into definitive endoderm was characterized by expression of SOX17 and CXCR4 in 70-90% of cells. Hepatic specification was confirmed on day 8 by AFP, HNF4A, EPCAM, and DLK expression. These progenitor cells were successfully differentiated into hepatocyte-like cells expressing AFP, ALB, and SERPINA1 on day 10. Hepatocyte-specific genes remained highly expressed in 80% of the cells at day 20. Morphological analysis indicated polygonal cells with multiple nuclei and canaliculi-like structures. Protein secretion, cytochrome activity, LDL uptake and glycogen storage assays are underway to determine maturation status of generated hepatocytes.

W-1144

MICRO RNA REGULATION OF FGF9 IN LUNG DEVELOPMENT REVEALS A LINK BETWEEN DICER1 LOSS AND THE PATHOGENESIS OF PLEUROPULMONARY BLASTOMA

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Mice lacking Fibroblast Growth Factor 9 (FGF9) have hypoplastic lungs and die at birth due to respiratory failure. Investigation of the underlying mechanisms identified an early embryonic feed-forward signaling pathway involving mesenchymal FGF and β-catenin-dependent Wnt signaling that regulates lung mesenchymal growth and differentiation. Consistent with this model, induced expression of FGF9 in embryonic lung epithelium resulted in lung mesenchymal hyperplasia and activation of mesenchymal Wnt/β-catenin signaling. Interestingly, the histopathology of lungs overexpressing FGF9 closely resembled that of Pleuropulmonary Blastoma (PPB), a syndromic lung cancer in humans that is associated with heritable mutations in Dicer1. PPB presents in early childhood with multifocal cystic lung lesions. Heterozygosity of DICER1 predisposes to PPB, and loss/mutation of the wild type allele in lung epithelium is hypothesized to initiate cyst formation. In later stages, with additional genetic events, the mesenchymal component of PPB cysts can progress to malignant sarcomas. We hypothesized that loss of DICER1 function in lung epithelium leads to persistent hyperplastic mesenchyme (and subsequent risk for malignancy), implicating a non-cell autonomous mechanism. Here, we show histological and molecular similarity in human Type I PPB, mice lacking epithelial Dicer1, and mice induced to overexpress epithelial Fgf9. We demonstrate that FGF9 is expressed in lung epithelium in human Type I PPB and in mice lacking epithelial Dicer1, and that Fgf9 partially mediates the Dicer1 deficient lung phenotype. Finally, we show that Fgf9 expression and function in lung mesenchymal and epithelial development is regulated by specific lung epithelial miRNAs. These studies identify FGF9 as a biologically active downstream target of DICER1 in lung epithelium that regulates lung development and that functions as an initiating factor for PPB.

W-1145

CRISPR-ON ACTIVATION OF ENDOGENOUS INSULIN GENE IN DIFFERENT HUMAN CELL LINES

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CRISPR-on is a novel and powerful RNA-guided transcriptional activator system. However it is unclear whether the CRISPR-on efficiency could be widely used, or if it depends on the cellular type, target genes or epigenetics context. Our goal was to activate human pancreatic gene INS transcription in 293T, Hela and fibroblasts cells from 2 patients with type 1 diabetes (T1D) using CRISPR-on technology. Informed Consent was approved by the Institutional Ethics Committee. In order to target the INS promoter, we selected 4 sgRNA sequences for the proximal promoter that precedes NGG, the protospacer adjacent motif required for sgRNA targeting. Pairs of 26-mer oligos containing sgRNA target sequences were synthesized, annealed and then inserted into the BbSI site in the sgRNA expression vector pSPgRNA (Addgene 47108). The plasmid pAC94 (Addgene 48226) was used for expression of dCas9-VP160 proteins. For transfection, cells seeded into 6-well plates at 80% confluence and with 8 μg of plasmids (4 μg dCas9-VP160 + 4 μg of sgRNA vectors) were lipofected in each well following the manufacturer's instruction (Lipofectamine LTX). The total amount for sgRNA vector was equally divided by the number of plasmids. Cells were grown for an additional 60h and were sorted for positive GFP using 0.8 μg pCX-EGFP before harvesting for RT-PCR analysis and DNA methylation analysis. Our results showed endogenous INS gene activation in 293T, Hela and fibroblasts from patients with T1D, even using each individual sgRNA (two independent experiments for each cell line). Controls (without CRISPR-on) always rendered negative results, while human pancreas controls were positive. Finally, bisulphite followed by sequencing was used to detect promoter status methylation before and after CRISPR-on treatment. We found that, in spite of previous methylation status (fully-methylated in INS promoters), the CRISPR-on system always activated the target gene in all treated cells. Curiously, the target gene did not change its methylation status after treatment. These novel results showed that the CRISPR-on system for endogenous human INS activation act on different cell types and in fully methylated promoters. Our results support the great potential of the CRISPR-on system to activate the INS gene for future pancreatic-cellular reprogramming strategies.

W-1146

PNEUMACULTTM: AN INTEGRATED CULTURE MEDIUM SYSTEM FOR IN VITRO HUMAN AIRWAY MODELING

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Primary human bronchial epithelial cells (HBECs) can be expanded in monolayer cultures for several passages whilst retaining their ability to differentiate at the air-liquid interface (ALI) to generate pseudostratified mucociliary epithelium that mimic the in vivo airway. Traditional medium formulations for expansion and differentiation of primary HBECs have typically contained undefined components such as Bovine Pituitary Extract (BPE), which may be subject to lot-to-lot variability thus leading to inconsistent performance. Recently, we launched PneumaCult™-ALI and PneumaCult™-Ex, both novel defined BPE-free media for differentiation and expansion of primary HBECs, respectively . Commercially available HBECs (P1; e.g. Lonza's Cat#: CC-2540s) were thawed and seeded into T-25 cm2 flasks containing PneumaCult™-Ex or BPE-containing control medium (BEGM™; Lonza) at a density of 1 x 104 cells/cm2. Media were changed every other day and cultures were passaged once cells reached approximately 80% confluence. At the same time, an aliquot of cells cultured in each medium was plated in 24-well plates for immunocytochemistry (ICC). Air-liquid interface cultures were prepared using samples of HBECs that had been expanding using either PneumaCult™-Ex or control medium for multiple passages, and all differentiation experiments were performed using PneumaCult™-ALI. Differentiated cells were analyzed by qPCR and ICC. The average fold expansion over 4 passages was not significantly different between cells cultured in PneumaCult™-Ex and cells cultured in control medium (7.1 ± 1.4 versus 7.2 ± 1.9; mean ± SD; n=7; p = 0.9). Similar to cells cultured in control medium, cells cultured in PneumaCult™-Ex showed cobblestone morphology and

uniformly expressed the basal cell markers p63, P75NTR and CD49f as assessed by ICC. Furthermore, cells cultured in both media could be successfully differentiated at early passages (P1-3) using PneumaCult™-ALI to generate a functional mucociliary epithelium at 28 days post air-lift, as indicated by mucus production, beating cilia and expression of the goblet cell marker MUC5AC and the ciliated cell markers FoxJ1 and AC-tubulin. Together, PneumaCult™-Ex and PneumaCult™-ALI create a fully integrated, defined and BPE-free tissue culture system for in vitro human airway modeling.

W-1147

CHARACTERISATION OF LUNG-RESIDENT PERICYTES IN A HOUSE DUST MITE-DRIVEN MODEL OF ASTHMA

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Asthma is characterised by structural changes such as airway smooth muscle (ASM) thickening. Our previous studies have shown that, in a murine house dust mite (HDM) model of asthma, pericytes detach from the airway microvasculature and contribute to ASM thickening however the mechanism is not understood. Pericytes are vascular mural cells that maintain vascular homeostasis but are also considered to be tissue-resident mesenchymal stem cells. Although pericytes have been shown to contribute to fibrotic disease in multiple organs including the lung, little is known about their role in asthma. To determine inflammation-driven phenotypic changes in lung-resident perivascular progenitor cells following five weeks of house dust mite (HDM) treatment and to establish markers for further investigation. Chronic allergic airway inflammation will impact the expression of markers related to chemotaxis, stemness and vascular homeostasis. Perivascular cells were defined as CD45-/CD31-/Ter119-, PDGFRβ+/CD146+. A LEGENDScreen™ for flow cytometric analysis was performed on lung structural cells from naïve and HDM-treated mice. The LEGENDScreen™ revealed the expression of chemokine receptors CCR3, CCR9 and CXCR7, stemness markers CD29, CD44, CD73 and CD105, as well as vascular signaling molecules Notch2 and Tie2. Other interesting markers included podoplanin, EpCam and PDGFRα. HDM-treatment appeared to increase CCR9, CXCR7, CD105 and CD73 expression. Lung pericytes represent a tissue-resident population of mesenchymal stem cells, and may become more 'stem-like' in chronic allergy. Chemokines, angiopoietins and Notch signaling may be involved in pericyte accumulation in ASM. These markers are under further investigation.

W-1148

GENERATION OF HUMAN IPS DERIVED HEPATOCYTE LIKE CELL PANEL THAT REFLECTS INDIVIDUAL DRUG METABOLISM CAPACITY AND DRUG SENSITIVITY

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Inter-individual differences in hepatic metabolism have a large influence on individual drug efficacy and adverse reaction. Inter-individual differences in the activity of hepatic drug-metabolizing enzyme, such as cytochrome P450 enzyme (CYP), are mainly due to genetic polymorphism in its gene. Although drug metabolism enzyme-related SNP genotyping could be performed by using human blood samples, it is so hard to accurately predict drug metabolism capacity and drug responsiveness of hepatocytes. Hepatocyte-like cells (HLCs) differentiated from human iPSCs have the potential to predict inter-individual differences in drug metabolism capacity and drug response and thus are expected to be applicable for drug screening. However, it has not been well known whether human iPS-derived HLCs can reproduce the inter-individual difference in hepatic metabolism and drug reaction. First, we generated the 12 individual HLCs (PHH-iPS-HLCs) differentiated from human iPSCs which had been established from 12 individual primary human hepatocytes (PHHs). And then, the CYP metabolism capacity and drug responsiveness were compared between the PHH-iPS-HLCs and parental PHHs. Next, we also generated the HLCs differentiated from human iPSCs which had been established from donors with poor or extensive metabolizer genotype for CYP2D6, and then compared the CYP2D6 metabolism capacity and CYP2D6-mediated drug reaction between the PHH-iPS-HLCs and parental PHHs. CYP1A2, 2C9, and 3A4 metabolism capacity of the PHH-iPS-HLCs were highly correlated with those of PHHs. The drug -induced hepatotoxicity levels in the PHH-iPS-HLCs was highly correlated with that in PHHs. These results suggest that the PHH-iPS-HLCs retained donor-specific CYP metabolism capacity and drug responsiveness. The PHH-iPS-HLCs, which have poor or extensive CYP2D6 metabolizing capacity, could be generated from PHHs with poor or extensive metabolizer genotype, respectively. These results suggest that inter-individual differences of CYP metabolism capacity and drug responsiveness, which are due to the diversity of individual SNP in CYP2D6 gene, could also be reproduced in the PHH-iPS-HLCs. We succeeded in establishing the iPS-HLC panel, which reflect the inter-individual difference of hepatic drug metabolizing capacity and drug responsiveness.

W-1149

DIRECT CONVERSION OF MOUSE AND HUMAN FIBROBLASTS TOWARDS LUNG EPITHELIAL CELLS

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Generation of renewable tissue cells from induced pluripotent stem cells (iPSCs) offer great hope for patient-specific regenerative medicine. However, the process of reprogramming somatic cells and subsequent directed differentiation into the cell lineages is time-consuming and costly. Therefore, direct conversion of fibroblasts to tissue cells through transdifferentiation may provide a faster and economical cell source for organ regeneration. Here we show that transient transduction of fibroblasts with the four reprogramming factors (Oct4, Sox2, Klf4, and c-Myc) in addition to the early lung transcription factor Nkx2.1, can transdifferentiate mouse fibroblast into endogenous Nkx2.1-expressing cells within 14 days when cultured in media that support lung epithelial cell growth. These induced lung-like epithelial cells (iLECs) express markers associated with the epithelial phenotype (cytokeratins and Epcam), some markers associated with lung epithelial cells (Tp63, Sftpc, Sox17, and Pdpn), but no markers associated with thyroid (TG, Pax8) or forebrain (Pax6). Human transdifferentiated cells express pan-

cytokeratin, NKX2.1, TP63, and the tight junction protein ZO1. Inducing transdifferentiation in mouse embryonic fibroblasts from our Nkx2.1-mcherry knock-in mouse reporter, produced cells with bright mcherry expression at early but not later passages suggesting an early activation of the lung progenitor pathway and later conversion of the cells into more differentiated epithelial cell types. No expression of the endogenous pluripotency genes Oct4 and Nanog were detected, suggesting that these cells do not transition through a pluripotent state before converting to epithelial cells. In a mouse model of airway injury, iLECs from passage 5 showed some evidence of engraftment in the respiratory epithelium and expressed the Type II alveolar epithelial cell marker Sftpc. Conversion of fibroblasts into lung-like epithelial cells is a promising strategy to generate cells for in vitro assays and in vivo lung repair.

ENDOTHELIAL CELLS/ HEMANGIOBLASTS

W-1150

THE HEMOGENIC COMPETENCE OF ENDOTHELIAL PROGENITORS IS RESTRICTED BY RUNX1 SILENCING DURING EMBRYONIC DEVELOPMENT

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It has long been proposed that hematopoietic and endothelial lineages share a common mesoderm progenitor. Early studies noted the close proximity of endothelial and hematopoietic cells in the blood island region of the yolk sac. It is now established that blood cells originate from a transient cell population termed hemogenic endothelium (HE). However, it is still not understood how some endothelial progenitors have hemogenic competency while others are not. Here, we use an ETV2:GFP reporter mouse to isolate emerging endothelial progenitors in order to investigate changes in the hemogenic potential during embryonic development. We observed a remarkable decrease of hemogenic potential in E8.5 progenitors compared to their E7.5 counterparts. Microarray and single cell gene expression analysis suggested that this differential hemogenic potential might be due to a lack of Runx1 expression. Ectopic expression of Runx1 was able to redirect E8.5 endothelial progenitors towards hematopoiesis. These data not only highlight the plasticity of ETV2 expressing endothelial progenitors but also suggest that hematopoiesis is a dominant programme that needs to be repressed in E8.5 endothelial progenitors via Runx1 repression to allow endothelial specification.

W-1151

ANGIOGENIC AND ANTITHROMBOTIC ACTIVITIES OF HUMAN PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS

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Transplantation of vascular derivatives of human pluripotent stem cells may provide effective vascular regeneration in ischemic cardiovascular diseases. Here we studied differentiation conditions toward mature endothelial subpopulations and fate of these cells during 3D culturing in vitro and in vivo. Human embryonic (hESC) and induced pluripotent stem cells (hIPSC) were differentiated via embryoid body and monolayer methods. CD31-positive endothelial cells (EC) were sorted by FACS and expanded in endothelial growth medium. Both hESC-EC and hIPSC-EC showed endothelial characteristics such as cobblestone pattern, ac-LDL uptake, and tube formation in vitro. Proteome profiling revealed high abundance of angiogenesis-related proteins. High expression of arterial (EphrinB2, Notch1-2) and venous (EphB4) endothelial markers was shown, suggesting the presence of mixed endothelial population in culture. For engineering 3D vascular constructs, decellularised human aortic slices (300µm) were repopulated with hESC-EC and hIPSC-EC. As shown by vital dyes, both hESC-EC and hIPSC-EC remained viable on slices and bioscaffold activated antithrombotic effects of the cells (as verified by decreased secreted levels of chemokine Rantes, $p<0.01$). Using extracellular matrix Matrigel as scaffold, cells were implanted subcutaneously into athymic nude rats. High-resolution small animal imaging systems, NanoSPECT/CT and PET/MRI were used to acquire multimodal whole-body images. By using 99mTc human serum albumin, a significant increase in local perfusion was detected at the grafted sites of both cells after 2 weeks, suggesting the functional incorporation of EC into the microvasculature. PET/MRI using $\alpha v\beta 3$ integrin-radiolabelling with 68Ga-labelled NOTA-RGD2 tracer showed induced angiogenesis. Post-mortem 3D histology confirmed that hESC-EC and hIPSC-EC survive, engraft successfully and form capillary-like structures in vivo. As assessed by real time PCR, expressions of angiogenic, arterial and venous markers were induced as compared with preimplanted cells, suggesting that cells may undergo an in vivo maturation. Vascular cell replacement therapy by using pluripotent stem cell derived endothelial cells may be a promising future approach to induce formation of blood vessel networks.

W-1152

RAT MODEL OF SHEATH GUIDED STENT IMPLANTATION FOR CELL THERAPY

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Delay or failure of endothelialization of intracoronary stents has been associated with increased stent thrombosis and recurrent symptoms. Facilitated endothelialization of stents has the potential to improve clinical outcomes. Delivery of magnetically-labeled autologous endothelial cells can lead to improved endothelialization of magnetized coronary stents within 3 days of implantation. Objective: Describe the surgical procedure of stent implantation into the descending aorta of rats with the perioperative/peri-implantation application of cells into the lumen of the stent. Material and methods: 10 male outbred Wistar rats, weight 350-550 g. Implantation under diazepam (2 mg/kg bw), ketamine (35 mg/kg bw) xylazine (5 mg/kg bw) anesthesia (IM induction). Anesthesia was maintained with isoflurane (1-2%) according the effect. Laparotomy was performed from the median line incision, abdominal aorta were carefully dissected distal to the renal arteries. Distal clamp was located on the aorta at first. Then proximal clamp was placed so as not to compress renal artery. The aorta was cannulated with IV Catheter G18 1,3x35 mm. Aorta was flushed with salt solution after removing catheter´s metal needle. Adapted 5F sheath was introduced over puncture hole into the aorta and the proximal clamp was released. Balloon was introduced with crimped stent (3 mm x 3mm) through the sheath and the balloon (3mm x 15mm) was inflated to 8 atm for 10 sec. Then the arterial catheter with guide wire was introduced into the lumen of the stent and the cells or control solution were applied. Standard closure of aorta and abdominal cavity was performed. Antibiotic (enrofloxacine) and antiaggregant + antiinflammatory treatment (tolfetamic acid) was used for 7 days. Results: Implantation into suitable position was successful in all cases. Bleeding from aortotomy suture was the main complication which could be fatal. Minor complications were reversible (max 7 day), paresis of the hind legs were always fully resolved within seven days after surgery. Conclusion: Stent implantation into the rat abdominal aorta with sheath was described. Sheath guided implantation allows targeted application of cells suspension or other solutions into the lumen of the stent without the risk of blood loss and unnecessary trauma to the vessels.

W-1153

DEFINING A NOVEL IN VIVO HIERARCHY AMONGST VESSEL RESIDENT ENDOTHELIAL PROGENITORS USING THE HUMAN TERM PLACENTA

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Endothelial progenitor cells (EPCs) have been used for the treatment of ischemic diseases in previous clinical trials with promising but modest results. Major limitations of these trials were the lack of proper definition of EPCs and the incapacity to deliver large numbers of cells. We aimed to define EPC populations in vivo using a highly vascularised organ, the human term placenta. Human term placenta was obtained and enzymatically digested to obtain a single cell suspension. Upon digestion and single cell suspension, significant heterogeneity could be observed within placental endothelial cells using common markers such as CD34 and CD31, after exclusion of CD45+ cells. After CD45+ hematopoietic cell depletion and CD34 enrichment via magnetic sorting, CD45-CD34+ were flow sorted in different populations based on CD31 expression levels. As expected most high proliferative endothelial colonies were in the CD45-CD34+ population. Further examination identified the high-proliferative potential endothelial colony forming EPCs to be almost exclusively in the CD31-low population as opposed to CD31hi and CD31neg populations. Only colonies emanating from the CD31-low population could be further passaged and expanded. We also showed that these progenitors were vascular in origin as labelled with VE-Cadherin in vivo. Interestingly, intra-arterial injection of placenta tissue with FITC-conjugated Ulex europaeus agglutinin 1 (UEA-1) showed vessel resident progenitors in vivo that had colony forming ability to be UEA-1-negative. Upon colony formation and expansion in vitro UEA-1 as well as other endothelial specific markers were expressed. The CD45-CD34+CD31neg, low and high populations were of fetal origin, and in culture displayed no hematopoietic potential. Finally, in single cell seeding experiments, quantitative analysis of colony formation revealed a clear hierarchy between the CD31low and CD31hi cells, as the latter gave rise to only small endothelial colonies with limited proliferative potential. We have established a novel in vivo hierarchy amongst vessel resident endothelial cells narrowing the identity of EPCs. Additionally, this may potentially provide an avenue for the isolation of pure EPCs in large quantities that could be used for future cell therapies.

W-1154

DIPROTIN-A INCREASES VASCULAR PERMEABILITY IN HUMAN ENDOTHELIAL CELL AND AGGRAVATES VASCULAR LEAKAGE IN DIABETIC RETINOPATHY

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Diabetic retinopathy is a major cause of blindness among working age adults. The pathophysiologic mechanism of non-proliferative diabetic retinopathy involves increased retinal vascular permeability and abnormal retinal microvasculature all of which lead to retinal ischemia. The appearance of neovascularization in response to retinal hypoxemia is the hallmark of proliferative diabetic retinopathy. SDF-1α expression was higher in human vascular smooth muscle cells than in human endothelial cells, which was augmented by hypoxia/reoxygenation (H/R). H/R increased phosphorylation of Src and vascular endothelial-cadherin (VE-cadherin) in human endothelial cells and then disrupted cell-to-cell junctions. Similar to H/R, Diprotin-A (DPP4-inhibitor) increased phosphorylation of Src and VE-cadherin in human endothelial cells and then disrupted cell-to-cell junctions, which was augmented by H/R or addition of exogenous SDF-1α but attenuated by CXCR4-blocker or Src-inhibitor. Disrupted endothelial cell-to-cell junction in immuno-fluorescence pictures was correlated with the actual leakage of endothelial monolayer in transwell endothelial permeability assay. We also tested in-vivo effect of Diprotin-A on retinal vascular permeability using the retinopathy of prematurity in a mouse model. Systemic administration of Diprotin-A increased not only vascularity but also vascular leakage, which was prevented by CXCR4-blocker (AMD3100). Streptozotocin (STZ) induced diabetic retinopathy model was used to elucidate in-vivo effect of Diprotin-A in actual diabetic animal model. The retinas of STZ-induced diabetic mice showed more evident vascular leakage compared to the control group. Diprotin-A further aggravated retinal vascular leakage in the diabetic retinopathy model. CXCR4-blocker (AMD3100) and Src-inhibitor (PP2), however, reversed the increased vascular leakage induced by Diprotin-A. Clinical implication of this study is

that our findings suggest one potential dark side of DPP4-inhibitor, an aggravation of diabetic retinopathy via increasing vascular permeability in retina.

W-1155

IN VITRO PRIMING WITH SEVOFLURANE ENHANCES PROLIFERATION AND FUNCTIONAL ABILITIES OF ENDOTHELIAL PROGENITOR CELLS ISOLATED FROM HUMAN UMBILICAL CORD BLOOD

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We have previously shown that anesthetic preconditioning (APC) increases plasma and tissue levels of endothelial progenitors (EPCs) in a rat model of ischemia/reperfusion. As a pursuit of this finding, we investigate here the effects of sevoflurane on proliferation and function of EPCs. Human EPCs isolated from umbilical cord blood were exposed to sevoflurane 2% or 4% in air/5% CO_2, or only to air/5% CO_2 (sham control). The number of adherent DilAcLDL/ UEAI-FITC double positive cells was increased following exposure to sevoflurane 2% (180.62 ± 19.09, n = 4, $p<0,05$) and 4% (167.36 ± 7.95, n = 4, $p<0,05$) versus control (123.98 ± 11.17). A tetrazolium salt test indicated raised proliferation 24 hrs post-exposure to sevoflurane 4% (0.156 ± 0.024 vs. 0.124 ± 0.011, n=4, $p<0.05$), and LDH cytotoxicity assay showed no significant effects of the anesthetic on EPCs. Apoptosis was lowered following the in vitro priming, without statistical significance though. Compared to untreated cells, adherence of Dil labelled EPCs to monolayers of human umbilical vein endothelial cells (HUVEC) was substantially increased 24 hrs postexposure to sevoflurane 2% (58.28 ± 13.39 vs. 27.67 ± 5.15, n=4, $p<0.05$) and 4% (65.22 ± 13.16 vs. 23.35 ± 3.75, n=4, $p<0.05$), and incorporation of EPCs into HUVEC networks was increased as well (sevoflurane 4%: 97.12 ± 3.61 vs. 47.6 ± 1.94, n=3, $p<0.01$). Thus, augmented EPCs proliferation and functional abilities are candidate mechanisms underlying the regenerative and anti-ischemic processes of APC. In addition, these results indicate a good potential of the ex-vivo priming with sevoflurane for supporting cell replacement therapies. Financial support: research grant PN-II-RU-TE-2012-3-0463, UEFISCDI Romania.

EPIDERMAL CELLS

W-1156

IMPACT OF CALORIC RESTRICTION ON HAIR FOLLICLE STEM CELL-MEDIATED FUR REMODELLING

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Caloric restriction (CR), or the limitation of dietary calories without lack of essential nutrients, extends lifespan in a variety of species, including yeast, worms, flies and mice. There are several theories about the biological mechanism responsible for the increase not only in lifespan but also in healthspan observed in response to CR, amongst them the latest involves the participation of stem cells, as they are responsible for maintenance and replacement of tissues throughout life in mammals. It is also a consensus that many aspects of mammalian aging can be related to a decline associated with age in replicative function of stem cells in specific tissues. 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 amount of food (40% CR) and observed the impact on skin and associated stem cells. The first striking observation was that backskin fur coat was phenotipically different, namely, CR animals developed a more even and long coat. An increase in guard hair follicle density and length was observed ($p\leq0.001$). The thickness of epidermis was also increased in CR group in detriment of the hypodermis ($p\leq0.05$). Hair clipping experiments also shown that both synchronized (P40) and unsynchronized (6 month) hair follicles show increased rates of growth ($p\leq0.05$), most probably due to early recruitment of stem cells, as suggested by BrDU pulse incorporation. The pool of interfollicular and hair follicle associated epidermal stem cells was evaluated through flow cytometry. The number of $\beta1$ integrin+ cells was significantly increased ($p\leq0.001$) as the number of $\beta1$ integrinHIGH/CD34+ ($p\leq0.05$) indicating an expansion of these SC pools. Using realtime measurements of O_2 consumption (OCR) and acidification (ECAR) in primary cultures, the bioenergetic profile of epidermis and dermis were evaluated. CR epidermis presented increased ECAR while dermis displayed a significant increase in spare and maximal respiration (mitochondrial biogenesis). In brief, CR induces a complete remodeling of backskin fur through the expansion of IFSC and HFSC pools associated with a significant metabolic shift both in epidermis and dermis. These findings lead us to propose a central role for calorie restriction in the maintenance/ commitment of hair follicle stem cells.

W-1157

STUDY OF TUMORIGENESIS DERIVED FROM HAIR FOLLICLE STEM CELL BY COMBINED ACTIVATION OF HRAS AND AKT IN A MOUSE MODEL

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Cutaneous squamous cell carcinoma is a common skin cancer which derived from the epidermal keratinocytes. Most mouse model for cutaneous squamous cell carcinoma is based on the chemical-induced carcinogenesis, a two-stage induction by applying DMBA followed by TPA on the mouse skin. The chemical carcinogenesis model is successful to develop skin benign papilloma and squamous cell carcinoma. However, the chemical application on the skin hinders our understanding of the tumorigenesis derived from different parts of the epidermis since the chemical application affects all the epidermal components. Hair follicle stem cell is a quiescent population of stem cells located at the hair bulge that cyclically regenerate the hair follicle. Since the chemical-induced carcinogenesis

can not specifically apply to the hair follicle stem cell, tumorigenesis derived from these stem cells has not been well characterized. Skin carcinogenesis is known to associate with oncogenic HRAS mutation and AKT activation. Our lab generated transgenic mice with Cre-inducible overexpression of both myr-AKT and HRAS-G12V. We activated myr-AKT and HRAS-G12V overexpression in the hair follicle stem cell without chemical application and multiple skin papilloma were noticed later. The expression of both myr-AKT and HRAS-G12V was confirmed in the tumor by immunostaining. The histological section of the papilloma revealed its unique feature of hyperplasia of both skin epidermis and sebaceous gland. These findings suggested that the sebaceous differentiation from hair follicle stem cell during tumorigenesis could be enhanced by myr-AKT and HRAS-G12V overexpression. In summary, we have established an inducible skin tumorigenesis model without chemical application that is useful to study tumorigenesis derived from stem cells. Our study also showed that the tumorigenesis derived from hair follicle stem cell is characterized as simultaneous epidermal and sebaceous hyperplasia.

W-1158

12C IS THE OPTIMAL STORAGE TEMPERATURE FOR RETENTION OF VIABILITY AND UNDIFFERENTIATED CELL CHARACTER OF CULTURED EPIDERMAL CELLS

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Cultured epidermal cells are used to treat large area burns and are a source of autologous epithelial cells for treating limbal stem cell deficiency, as shown in animal models. Retention of undifferentiated phenotype has proved important to clinical success in both applications. Storage is critical to provide worldwide distribution of transplants. Based on previous work, the temperatures 4oC, 8oC, 12oC, 16oC and 24oC were chosen for in-depth analysis following one-week storage of cultured epidermal cells. Cell viability, as inferred by calcein uptake, was similar to control at 12oC (99±3%; p=0.697), and increased at 16oC (108±2%; p=0.012), which coincided with significantly increased PCNA expression. Morphology was also best preserved at 12oC and 16oC. The integrity of mitochondrial DNA was maintained at 12oC, but impaired at 4oC and 24oC (p<0.05). Lactate dehydrogenase release occurred upon storage, with a tendency to increase with temperature. Expression of caspase-3 was similar between the groups, indicating that storage did not predispose cells to apoptosis. Expression of progenitor cell marker p63 was only maintained at 12oC (92±2%; p=0.801) and 16oC (97±1%; p=0.166). The transporter ABCG2 significantly increased from 59±6% in control to above 80% in all groups stored at 8oC – 16oC (p<0.05). This could suggest a cell protective effect by this transporter against accumulation of toxic metabolic byproducts. Signs of differentiation were seen at 16oC, where CK10 expression increased to 56±11% from 10±5% in control (p<0.05). Only 16oC showed a significant increase in C/EBP-δ expression, which is associated with initiation of epidermal cell differentiation. Cells at 24oC demonstrated pronounced cytokeratin bundles and significantly increased CK10 expression, indicative of differentiation. Importantly, all temperature groups resumed growth following storage; 8oC – 16oC performed best and were passaged twice in a 10-day period compared to 4oC and 24oC that were passaged only once, thus supporting the findings from the viability/differentiation analyses. Our results indicate 12oC was the only temperature where retention of viability and undifferentiated cell character were maintained and should be the preferred temperature for one-week storage and transportation of cultured epidermal cells.

W-1159

DYNAMICS OF EPIDERMAL LGR6+ PROGENITORS IN MURINE SKIN HOMEOSTASIS

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The dynamics and interactions between stem and progenitor cell populations in the hair follicle (HF), sebaceous gland (SG) and interfollicular epidermis (IFE) of murine skin are still poorly understood. Here, we used multicolor lineage tracing to demonstrate that Lgr6 marks independent progenitor populations in the HF isthmus, SG and IFE. While there is no cellular exchange between IFE and isthmus during homeostasis, the SG is maintained by resident SG basal cells in conjunction with isthmus cells. Clonal dynamics revealed that Lgr6+ progenitors compete neutrally in the IFE, isthmus and SG, indicating that population asymmetry is the underlying mode of tissue renewal in all three compartments. On the molecular level, Lgr6+ IFE and HF cells present distinct signatures, underlining their independence. Our results elucidate the interrelation between IFE, HF and SG progenitors and suggest population asymmetry as a common mechanism for homeostasis of the permanent epidermal compartments.

W-1160

BNIP3 PLAYS CRUCIAL ROLES IN THE DIFFERENTIATION AND MAINTENANCE OF EPIDERMAL KERATINOCYTES.

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Recent studies have revealed that autophagy is involved in differentiation of erythrocytes, lymphocytes, and adipocytes. Keratinocyte differentiation is also going along with activation of lysosomal enzymes and organelle clearance, expecting the contribution of autophagy in this process. Previously, by integrating both loss- and gain-of-function studies of Notch receptors and their downstream target Hes1, we show multiple roles of Notch signaling in the regulation of transit amplifying cells in suprabasal layers. Notch signaling induces differentiation of suprabasal cells via Hes1 independent manner, whereas Hes1 is required for maintenance of the immature status of suprabasal cells by preventing premature differentiation. In this study, we found that Hes1 directly suppressed the expression of Bnip3, whose expression is sufficient to induce terminal differentiation of keratinocytes by induction of autophagy. Our data showed 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. Furthermore, dead cells were increased in human epidermal equivalent from BNIP3 knockdown keratinocytes, which gave us the idea that BNIP3 is also indispensable for maintenance of skin epidermis. To test the hypothesis, HPEK were irradiated with UVB. UVB irradiation stimulated BNIP3 expression and cleavage of caspase3. Surprisingly, suppression of BNIP3 expression induced by UVB irradiation caused a further increase of the cleaved caspase3 protein level, suggesting that BNIP3 has a protective effect against UVB-induced apoptosis in keratinocytes. Overall, our data shed light on functions of BNIP3, an inducer of autophagy, in both differentiation and maintenance of epidermal keratinocytes.

W-1161

CHEMOTHERAPY USED IN CONDITIONING REGIMEN FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN WITH RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA (RDEB) ALTERS EXPRESSION OF TYPE VII COLLAGEN

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Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a severe inherited skin disorder characterized by loss of skin integrity and chronic blistering due to mutations in type VII collagen (C7). Complications such as systemic infections and aggressive squamous cell carcinoma are common and life-threatening for individuals with this disorder. Recently, cell-based therapies including hematopoietic stem cell transplantation (HCT) have shown to be effective in treating this disorder, but certain aspects of this treatment are not well characterized. Following HCT, there is an increase in C7 deposition in RDEB patient skin, an improvement of symptoms, as well as a substantial amount of donor-derived cells in both the skin and blood. Although, in cases of unsuccessful transplantation where no donor cells were found in the blood or the skin of these patients, there was an increase in mutant C7 expression in the recipient's skin. We hypothesized that exposure of RDEB patient skin to drugs in the HCT preparative regimen resulted in an increase in mutant C7. We determined that RDEB patient fibroblasts express significantly higher levels of C7 following exposure to fludarabine (6.90±0.06 48h-post exposure, $p<0.05$). To elucidate the mechanism by which fludarabine exposure results in an increase in C7 expression, we investigated the role of various pathways known to activate C7 transcription. We determined via western-blot that fludarabine exposure results in activation of the MAPK/ERK pathway. We were able to demonstrate that chemical inhibitors of the MAPK/ERK pathway (PD98095 and U0126) abrogated the increase of C7 expression in fibroblasts following exposure to fludarabine. Furthermore, we demonstrated that fludarabine exposure resulted in activation of the AP1 complex, a known transcriptional activator of C7. These results suggest a mechanism by which fludarabine exposure leads to an increase in C7 expression in RDEB fibroblasts, which likely explains our clinical findings in RDEB patients who did not have a successful transplant yet showed an increase in mutant C7. In the context of novel uses for stem cells to treat genetic disorders, this study demonstrates the importance of understanding how the HCT process influences expression levels of mutant proteins in patients with genetic disorders.

EPITHELIAL CELLS (NOT SKIN)

W-1162

MESENCHYMAL WNT/BETA-CATENIN SIGNALING CONTROLS EPITHELIAL STEM CELL HOMEOSTASIS IN TEETH BY INHIBITING THE ANTIAPOPTOTIC EFFECT OF FGF10

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Continuous growth of rodent incisors relies on epithelial stem cells (SCs) located in the SC niche called labial cervical loop (LaCL). Here we found a population of apoptotic cells residing in a specific location of the LaCL in mouse incisor. Activated Caspase3 and Caspase 9, expressed in this location colocalized in part with Lgr5 in putative stem cells. The addition of Caspase inhibitors to incisors ex vivo resulted in concentration dependent thickening of LaCL. To examine the role of Wnt signaling in regulation of apoptosis we exposed the LaCL of postnatal day 2 (P2) mouse incisor ex vivo to BIO, a known activator of Wnt/beta-catenin signaling. This resulted in marked thinning of LaCL as well as enhanced apoptosis. We found that Wnt/beta-catenin signaling was intensely induced by BIO in the mesenchyme surrounding the LaCL, but, unexpectedly, no beta-catenin activity was detected in the LaCL epithelium either before or after BIO treatment. We discovered that the expression of Fgf10, an essential growth factor for incisor epithelial SCs was dramatically down regulated in the mesenchyme around BIO-treated LaCL, and that exogenous Fgf10 could rescue the thinning of the LaCL caused by BIO. We conclude that the homeostasis of the epithelial SC population in the mouse incisor depends on a proper rate of apoptosis and that this apoptosis is controlled by signals from the mesenchyme surrounding the LaCL. Fgf10 is a key mesenchymal signal limiting apoptosis of incisor epithelial SCs and its expression is negatively regulated by Wnt/beta-catenin.

W-1163

TARGETING THYMIC EPITHELIAL STEM CELLS FOR REGENERATING THE AGED OR DAMAGED THYMUS.

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Endodermally derived thymic epithelial cells (TEC) are critical for T cell development and self-tolerance. However, they are gradually lost with age, leading to >75% loss of thymus function by mid-life and hence profound decrease in naïve T cell production. This becomes a problem when cytoablative therapies for cancer treatment, such as chemotherapy, deplete mature T cells and damage the residual aged

thymus, further compounding its atrophy. The ensuing protracted period of immune deficiency leaves patients highly susceptible to infections. Understanding the basis for TEC loss with age will assist in developing strategies for thymus repair and hence restoring immune competence. While the fetal thymus has been widely investigated, progress in the adult has been hindered due to difficulties in isolation techniques of TECs, which make up only 1% of total adult thymus cellularity. Through a combination of comprehensive cellular and molecular characterisation, in vivo turnover and functional analysis by 3D in vivo reaggregate organ culture and an in vitro 3D culture system, we have identified an adult thymic epithelial progenitor cell population residing in a subset of phenotypically immature cortical thymic epithelium. We have demonstrated their capacity for self-renewal, colony forming potential and importantly, the generation of mature cortical and medullary cell lineages over 3 months, including the autoimmune regulator (Aire)+ medullary subset. A profound loss in thymic medulla cell lineages with aging will be discussed in relation to TEPC function. The phenotypic identification of adult thymic epithelial stem cells will provide an important catalyst for investigations into the molecular regulation of these critical cells during homeostasis, differentiation and regeneration following damage.

W-1164

AN INCISIVE LOOK AT THE DENTAL EPITHELIAL STEM CELL POPULATIONS

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The mouse continuously growing front tooth serves as a valuable model to approach the adult tissue homeostasis and stem cell regulation at the molecular, cellular and evolutionary levels. Moreover, the incisor stem cells can be evaluated to develop regenerative medicine protocols based on the dental stem cell biology. To develop such protocols, we analyzed the genetic network involved in epithelial stem cell segregation during tooth development, and their maintenance in adult incisor renewal. We succeeded to reveal the importance of Sox2 during incisor formation and the role of Sox2+ stem cells in incisor renewal. In order to understand the role of the Sox2+ dental stem cell population, we selectively removed this cell population by the transitory expression of DTA in these cells. Strikingly, 4 to 6 weeks after the ablation, the tooth did not exhibit any renewal defect or anomaly in the stem cell niche. To study the function of Sox2 transcription factor during the tooth formation and renewal, we generated Sox2 cKO mouse and analyzed their teeth. While the incisors were able to form, the tooth orientation and cell differentiation were largely impaired. Interestingly, our data combined with the literature is pointing out a complex hierarchy in a stem cell niche housing four populations able to renew the dental epithelium. Moreover, the expression of Sox2 before exiting the niche seems necessary. Our recent results present the mouse incisor epithelial stem cell niche as an interesting model to study several stem cell populations interacting in a confined environment. We are currently grouping these results in our tooth reconstruction protocol, serving as a proxy to test cell populations in organ bioengineering method, first step on the way of clinical applications.

W-1165

EPITHELIAL STEM/PROGENITOR MAEKER K15 EXPRESSION PATTEN IN AN IN VITRO EPITHELIAL CELL NICHE MODEL

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Cultured epithelial cell sheets are used for regenerative medicine and the study of stem cell biology. However, it is unclear whether stem/progenitor cells survive in vitro, and if they form a niche structure similar to in vivo. Using a primary cultivated human limbal epithelial sheet maintained for 1 year, we showed that epithelial stem/progenitor marker K15 was homogenously expressed in the first month, whereas K15 was limited to cell clusters from 3 months to 1 year. Label retaining cells were located in and around K15 positive cell clusters after 6 months chasing, whereas pulse labeling cells were rarely found within K15 positive cell clusters, suggesting that K15 positive cell clusters are quiescent. Melanocytes, one of the limbal niche candidate cells, were co-localized with several K15 positive cell clusters, suggesting a role for these cells the maintenance of the niche during 1-year culture. In conclusion, stem/progenitor cells are homogenously distributed in early primary culture, whereas located in specific niche-like structures in long term primary cultures, providing a new tool for stem cell study.

W-1166

SOX10 POSITIVE NEURAL CREST CELLS MIGRATE TO PITUITARY GRAND AND CHARACTERISTICS OF PITUITARY STEM/PROGENITOR CELLS.

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The pituitary gland is an important endocrine tissue to regulate many biological roles by hormones secreted from endocrine cells, of which functions are maintained and supplied by neighboring non-endocrine cells such as vascular endothelial cells, supporting cells and stem/progenitor cells. Pituitary organogenesis is progressed by invagination of the oral ectoderm, adenohypophyseal placode. Recently, we observed that cells of extrapituitary origin invade the developing embryonic (E) anterior lobe of the pituitary gland. Immunostaining showed that invading cells are heterogeneous and non-endocrine cells such as vascular endothelial cells, pericyte/smooth muscle cells, p75-positive cells, nestin-positive cells, PRRX1- and PRRX2-positive mesenchymal stem/progenitor cells, and S100β-positive cells which are characteristic for vessel and pluripotent/multipotent cells. Notably, p75-psotitive cells and pericytes are postulated to be originated from neural crest, a fourth germ layer. The present study aimed whether neural crest marker SOX10-positive cells are present in the rat pituitary gland. Sox10-positive cells first appeared in the rostral part of the posterior lobe on E21.5. After postnatal day 15 (P15), SOX10-positive cells localized in the parenchyma and marginal cell layer (MCL), a stem/progenitor cell niche, of the intermediate lobe. Although SOX10-positive cells on E21.5 were negative for S100β, most of SOX10-positive cells in

the intermediate lobe turned to S100β-positive by P30. In addition, stem/progenitor cell marker SOX2 and cell division marker Ki67 colocalized in the SOX10-positive cells. We further observed S100β signals in the SOX10-positive cells in the postnatal anterior lobe. Finally, a pituitary-specific transcription factor PROP1 (Prophet of Pit1), which is expressed by pituitary stem/progenitor cells, was present in the SOX10-positive cells of the postnatal anterior lobe. Thus, the present study demonstrated that the neural crest derived SOX10-positive cells migrate into three lobes of the pituitary gland and acquire characteristics of the pituitary stem/progenitor cells. These data indicate that the pituitary stem/progenitor cells are composing of not only adenohypophyseal placode but also neural crest derived cells.

W-1167

PROBING MOUSE MAMMARY GLAND STEM CELLS BY LINEAGE TRACING AND CLONAL ANALYSIS

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For years, the cellular hierarchy governing the postnatal development of the mammary gland has been the focus of extensive research. Conflicting results have been obtained concerning whether unipotent or multipotent stem cells drive mammary gland expansion during puberty, adult homeostasis and lactation. Here, we performed extensive lineage tracing using a Doxycycline-inducible transgenic approach to label all basal cells during pubertal development and assess their fate at the end of the postnatal development and during pregnancy using confocal analysis of whole mount of mammary glands and FACS analysis. Using this approach, we labeled almost all K5/K14+ basal cells (95%) after 4 weeks of Dox administration. However, despite the almost complete labeling of basal cells, none of the luminal cells were labeled. We performed lineage tracing of luminal cells using another Doxycycline-inducible transgenic model that allowed us to label almost all luminal cells (98%) during pubertal development and we assessed their fate during adult homeostasis and demonstrated that luminal cells are a self-sustained unipotent lineage that is not replaced by unlabeled bipotent basal cells overtime. We tested different transgenic Cre lines that were previously described to target bipotent basal cells and we performed rigorous clonal and statistical analyses of the clonal data. We demonstrated that these Cre labeled initially and independently basal and luminal cells, as demonstrated by the presence of isolated basal or luminal cells, and that the initial and independent labelling of basal and luminal cells can give the false impression of bipotency at high level of chimerism. Furthermore, we found that many other Cre lines including K19CreER, Sox9CreER and Lgr6CreER also result in the non-specific labeling of basal and luminal cells. In conclusion, these results rule out any significant contribution of K5/K14+ bipotent stem cells during mammary gland postnatal development, homeostasis, pregnancy and lactation.

W-1168

EYA1 IS REQUIRED TO MAINTAIN THE BRANCHIAL EPITHELIAL PROGENITOR SIGNALING CENTRES

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Mutations in the Eya1 gene will lead to human Branchio-Oto-Renal (BOR) syndrome. The patients have a series of craniofacial defects. Using the Eya1-/- mutant mouse as a disease model, we investigated the role of Eya1 in the facial and branchial epithelium in early developmental stages. By cell lineage tracing experiments using the Sox2-CreER mice, we identified a group of Sox2 and Sox3-positive branchial epithelial progenitors at E8.5 in the Eya1 expressing regions of normal embryos. These epithelial progenitors express signaling molecules including Fgf and Shh, which are essential for branchial arch patterning and differentiation. Analysis of the Eya1-/- mutants showed that at E8.5, expression of both Sox2 and Sox3 in the branchial epithelial progenitors was significantly down regulated. By E9.5, Sox2 and Sox3 could be detected in the epithelium of the proximal second branchial arch. However, these progenitors had morphological and cell polarity defects. Furthermore, they did not express Fgf3 or Fgf8, and failed to form branchial clefts and other branchial arch derived structures. Interestingly, Notch signaling was also down regulated in these epithelial progenitors in the Eya1-/- mutants. To test whether Notch signaling is involved in regulating the normal function of these progenitors, by genetic approach using the Rosa-NICD mice, we overexpressed a stable and activated form of the Notch1 receptor (NICD) in the Eya1-/- mutants. We found that over-expression of NICD could rescue the signaling centre defects and that expression of Fgf3 was restored. Taken together, our results indicate that Eya1 is required for maintaining the normal function of the branchial epithelial progenitors. Deficiency of Eya1 leads to attenuated Notch signaling and branchial epithelial progenitors defects, which may contribute to the craniofacial anomalies in BOR syndrome.

EYE OR RETINAL CELLS

W-1170

USING ZINC FINGER NUCLEASE TECHNOLOGY TO GENERATE REPORTER-LABELLED HUMAN PLURIPOTENT STEM CELLS AS A TOOL TO OPTIMISE PHOTORECEPTOR TRANSPLANTATION

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The purpose of this study is to generate human pluripotent cell lines harbouring the green fluorescent protein (GFP) gene at the endogenous loci of a key transcription factor, namely Cone-Rod Homeobox (CRX), which is expressed in post-mitotic photoreceptor precursors. Sixteen Zinc Finger Nuclease (ZFN) pairs were designed to target the 3'UTR of human CRX gene without disrupting its coding sequence. The most efficient ZFN pair was selected using a mismatch sensitive enzyme assay and subsequently

transfected into human embryonic stem cell line (H9) along with a donor construct containing homology to the target region and a puromycin selection cassette. Following selection, analysis of six resistant clones indicated integration of the reporter cassette into the 3'UTR of CRX gene. Sequencing of the insertion site showed targeted integration and copy variant analysis suggested that a homozygous clone contained no other copies inserted elsewhere within the genome or as tandem repeats at the 3'UTR. This clone was selected for further analysis and differentiation towards the retinal lineage using a 3D method developed by our group. Analysis of the homozygous CRX-GFP labelled clone showed that insertion of the reporter cassette does not interfere with maintenance of pluripotency and genomic stability. Flow activated cell sorting was used to purify GFP+ and GFP- cell fractions, which were further analysed by quantitative RT-PCR. This analysis and immunocytochemistry of sections obtained from embryoid bodies indicated a significant correlation between GFP expression and endogenous CRX expression. Immunocytochemistry analysis with various markers labelling photoreceptors precursors, RPE, retinal ganglion cells and inner nuclear layer retinal neurones indicated clear expression only in the photoreceptor precursors throughout the 90 day differentiation time course. This approach could enable the isolation of stage-specific photoreceptor precursors from human pluripotent stem cell differentiations, allowing characterisation of their transcription and proteomic profile and subsequent testing of their transplantation potential.

W-1171

DERIVATION OF RETINAL NEURONS AND (RETINAL) PIGMENTED EPITHELIAL CELLS FROM HUMAN JNCL PATIENTS' IPSC

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Neuronal Ceroid Lipofuscinosis (NCL) is a group of progressive neurodegenerative lysosomal storage disorders. The most common form is the juvenile (JNCL or Batten disease), caused by a mutated version of CLN3 gene. JNCL leads to retina degeneration, followed by CNS neurodegeneration and premature death. Interestingly, whereas in JNCL patients retina is affected first, retinal defects are not severe and appear only later in JNCL mouse models. Therefore, human cell based-models might enable new insight into pathomechanisms and therapy development. hiPSC have previously been generated from fibroblasts of two JNCL patients, one healthy donor carrier for the common mutation and one healthy donor not carrier for the mutation. Taking advantage of protocols established in the last years, we investigated JNCL hiPSCs potential to differentiate into neuronal lineage, retina and (retinal) pigmented epithelium. Here we show that, following dissociation to single cells, control and JNCL hiPSCs efficiently reaggregated when plated onto low adhesion 96-wells plates. During the following days the aggregates became more compact, acquired patterning and formed potential optic vesicle-like epithelial evaginations, which upon isolation and further culture continued to grow and express eye-field transcription factor Rax. After one month in culture immunostaining analysis suggest formation of neural retina (including ganglion cells [Brn3+] and photoreceptor precursor [Crx+]) and pigmented epithelial structures (potentially RPE or ciliary epithelium). After two months the photoreceptors-specific marker Recoverin was also clearly expressed. We conclude that JNCL hiPSCs can be used to obtain JNCL patients'-specific neural retina and pigmented epithelium. Because of its early retinal disease onset, progressive neurodegeneration and potential involvement of different retinal cell types, we hypothesize that iPSCs-derived retinal cells will be a great tool to model JNCL disease, study retinal cell specific pathomechanisms and possibly discover therapies.

W-1172

COMPARATIVE PROTEOMIC ANALYSIS OF HUMAN EMBRYONIC STEM CELL-DERIVED RETINAL PIGMENT EPITHELIAL CELLS TO PRIMARY HUMAN RPE

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Human embryonic stem cell-derived retinal pigment epithelial (hESC-RPE) cells have the potential to provide an infinite cell source for cellular replacement therapies. Clinical trials with hESC-RPE to treat retinal degenerative diseases such as age-related macular degeneration (AMD) have been initiated. Many groups, including ours, have demonstrated that hESC-RPE show features similar to native RPE including morphology, relevant gene and protein expression, epithelial barrier properties, and functionality commonly demonstrated as key cytokine secretion and phagocytosis capacity. To extensively characterize the proteomics of hESC-RPE we compared the protein expression profiles of hESC-RPE cells generated from two different hESC lines to primary human RPE using isobaric tags for relative quantitation (iTRAQ) technology. Two hESC lines (Regea 08/017 and Regea 08/023) previously derived at our laboratory were differentiated to RPE using spontaneous differentiation method as floating cell aggregates, followed by enrichment and maturation of pigmented cells on collagen IV. Mature RPE cells from three separate differentiation experiments for both hESC lines were collected for protein extraction. For comparison, primary human RPE was collected from three cadaver donors. Total protein was extracted and then digested with trypsin. The peptides were labeled with iTRAQ labels and analyzed using Nano-RPLC- TripleTOF instrumentation (Ab Sciex). 1945 proteins in total were identified in both hESC-RPE lines and human RPE. Overall expression profiles between the hESC-RPE lines were very similar, showing that there was little cell line specific variation. Most proteins were expressed in hESC-RPE cells at levels similar to human RPE. 32 % of proteins

were differentially expressed at the 08/017 hESC-RPE compared to human RPE and 26 % at 08/023 hESC-RPE. Interesting differentially expressed proteins in hESC-RPE and human RPE included proteins involved for example in eye development, retinal homeostasis, cell adhesion, autophagy, phototransduction, and immune system. This study provides an interesting new insight into in-depth proteomic characterization of hESC-RPE cells.

W-1173

DIFFERENTIATION AND TRANSPLANTATION OF EMBRYONIC STEM CELL-DERIVED CONE PHOTORECEPTOR PRECURSORS

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High acuity colour vision in mammals depends on cone photoreceptors. Their death leads to untreatable blindness, for which a promising treatment strategy would be replacement of lost cones by cell therapy. A number of proof-of-concept studies have shown that precursors of cone and rod photoreceptors, mediating day and night vision respectively, isolated from donor mice are able to integrate into adult host retina after transplantation. More recently, it has been demonstrated that rod photoreceptor precursors can be differentiated in large numbers from mouse embryonic stem cells (ESCs) using a 3D protocol that results in recapitulation of in vivo eye development and these cells can integrate into adult recipient retinas after transplantation. Given the importance of cone-mediated vision, a thorough analysis of cone differentiation from mouse ESCs using various methods has been performed. The expression of transcription factors involved in cone genesis and differentiation has been examined using PCR and immunohistochemistry. Increasing numbers of early cone precursors expressing the nuclear receptor Trβ2 appear from day 12 to 18 of differentiation, at which stage they constitute approximately 18% of cells in ESC-derived retinas. These precursors persist in high numbers until later stages of differentiation (day 26) with some beginning to show immmunoreactivity for later markers of cone differentiation such as blue opsin and cone arrestin (at days 23-29). Furthermore, cone precursors label with an adeno-associated viral vector encoding a GFP reporter under the control of cone opsin promoter. When isolated using flow sorting and transplanted into adult mouse retinas by subretinal injection, these form cell masses in the subretinal space that express a range of cone markers. A fraction of the transplanted cells integrate into the host photoreceptor layer and express mature cone markers. In conclusion, in this study we have developed a method of obtaining large numbers of cone precursors, which develop in a fashion mimicking the in vivo developmental process, and might be used for establishing cell therapies for daytime vision.

W-1174

PRODUCTION OF RPE FROM AGE-RELATED MACULAR DEGENERATION PATIENT-SPECIFIC PLURIPOTENT STEM CELLS USING SOMATIC CELL NUCLEAR TRANSFER AND INDUCED REPROGRAMMING

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Autologous cell replacement therapy using patient-specific pluripotent stem cells might be the best choice for patients in cell therapy and regenerative medicine. In this study, we reported the establishment of human SCNT-ES and hiPSC from dermal fibroblast cells of 73 years-old female patient with Age-related Macular Degeneration (AMD). Human dermal fibroblast (DFB-NT5) cells were primarily cultured from the skin biopsy of a female patient with AMD (46, XX), and then used for SCNT and induced reprogramming after the approval by the Institutional Review. SCNT embryo production and ES derivation were performed as described previously. For iPSCs production, we expressed Yamanaka's 4 factors in dermal fibroblasts using Sendai virus. We have successfully derived one SCNT-ESC line (CHA-NT 5) and several iPSC lines, and then characterized CHA-NT 5 and two iPSC lines (iPS-NT5-S1 and iPS-NT5-S9). All of them showed normal karyotype (46, XX), the same STR marker expression compared with dermal fibroblasts, undifferentiated pluripotent stem cell characteristics, and differentiation ability to three-germ layer in vitro. To testify the differentiation ability of AMD patient-specific pluripotent stem cells to retina pigment epithelim (RPE) cells, we differentiated CHA-NT5, iPS-NT5-S9, and MA09 (ACT) as a hES control to RPE cells as described previously with one exception of mechanical EB formation. CHA-NT5 and iPS-NT5-S9 were successfully differentiated to RPE cells, and the characteristics of RPE cells derived from AMD patient-specific pluripotent stem cells were same as those of RPE cells from hESCs. From the results, we successfully established SCNT-ES and iPSCs from a 73 years old female AMD patient, and produced RPE cells from these patient-specific pluripotent stem cells.

W-1175

TGF-BETA1 INHIBITS THE CANONICAL WNT SIGNALLING PATHWAY RESPONSIBLE FOR THE DIFFERENTIATION OF ADULT HUMAN MüLLER GLIA INTO PHOTORECEPTOR NEURONS

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Although the human retina harbours Müller glia with neural stem cell characteristics (hMSC), there is no evidence of regeneration occurring in humans following retinal injury. TGF-β and Wnt signalling are known to regulate retinal neurogenesis and inflammation, but their role in the neural differentiation of hMSC is not known.

We investigated the expression of Wnt signalling components and the effect of TGF-β1 on these molecules, as well as on the photoreceptor differentiation of hMSC. Amongst others, hMSC express WNT2B, WNT5B and the canonical Wnt signaling protein β-catenin. TGF-β1 decreased mRNA and protein expression of WNT2B whilst increasing WNT5B. Although it did not modify β-catenin expression, it significantly increased the ratio of phospho-β-catenin/β-catenin, indicating inactivation of the canonical Wnt signalling pathway. Factors that induce photoreceptor differentiation of hMSC (FGF2, taurine, retinoic acid and Insulin growth factor; FTRI) markedly upregulated WNT2B and decreased the ratio of phospho-β-catenin/β-catenin, but did not modify WNT5B expression. Interestingly, inhibition of β-catenin by XAV-939 prevented FTRI-induced photoreceptor differentiation, as judged by NR2E3 expression. Similarly, TGF-β1 inhibited FTRI-induced photoreceptor differentiation and upregulation of WNT2B. Inhibition of TGF-β1 signalling by ALK5 and SMAD3 inhibitors, but not a JNK inhibitor, prevented TGF-β1 induced downregulation of WNT2B. WNT5B upregulation by this cytokine was only inhibited by an ALK5 inhibitor. These observations suggest that upregulation of TGF-β1, a factor produced during retinal gliosis, may play a key role in preventing neural differentiation of hMSC and may constitute a potential target for induction of endogenous regeneration of the human retina.

W-1176

TRANSPLANTATION OF HUMAN NEURONAL PROGENITOR CELLS EXPRESSING IGF-1 FUSION PROTEIN PREVENTS STRESS-INDUCED RETINAL GANGLION CELL DEATH

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We have shown that human neuronal progenitor (hNP) cells can be differentiated into retinal ganglion-like cells (RGCs) by integrating into the host retina after intravitreal transplantation. Based on these observations we examined whether hNP cells expressing a neurotrophic factor (NTF), IGF-1 fused to a double-fluorescent reporter protein could prevent stress-induced loss of RGCs in vitro and in a mouse model of glaucoma. Methods: IGF-1 cDNA was spliced into a fluorescent reporter, tdTomato (TD). IGF1-TD or TD alone was cloned into pJ603-neo vector and transfected into hNP cells to create hNPIGF-1/TD and hNPTD cells. A model of glaucoma was created by injecting microbeads in the anterior chambers of C57BL/6J mice. Mice were intravitrealy injected with 2 µl (~ 50000 cells) of hNPIGF-1/TD, hNPTD or hNP cells. Intraocular pressures (IOP) were measured for 4 weeks. The population of RGCs and axons were analyzed on retinal flatmounts and optic nerve cross sections, respectively. hNPIGF-1/TD cells expressed active IGF-1 (0.25 - 0.75 ng/ml by day 3) and increased survival of primary RGCs in co-culture. Bead injection induced IOP elevation from baseline of 8.17 ± 1.04 to 32.42 ± 3.44 mmHg (by day 11 - 4 weeks). Analysis of retinal flatmounts and axons indicated that hNPIGF-TD cells effectively protected from RGC loss. RGC (5293 ± 645 RGC/mm2) and axon (52.5 ± 3.9 × 104 axons/mm2) densities in hNPIGF-TD injected group were comparable to non-glaucomatous group (5352 ± 652 RGCs/mm2 and 55.5 ± 4.4 × 104 axons/mm2, both P > 0.05). Significant RGC and axon loss were observed in all 3 glaucoma groups: saline (3196 ± 397 RGCs/mm2; 39.4 ± 4.0 × 104 axons/mm2), hNP (2965 ± 360 RGCs/mm2; 42.0 ± 3.5 × 104 axons/mm2) and hNPTD (3173 ± 386 RGCs/mm2; 4.1 ± 3.5 × 104 axons/mm2). RT-PCR analysis indicated that IGF-1 induced down-regulation of inflammatory and angiogenic pathways. This study shows that hNPs can effectively deliver a desired NTF, such as IGF-1 locally to the inner retina. IGF-1 enhances survival of cultured RGCs. Transplantation of hNPIGF-TD cells into glaucomatous eyes protected against RGC and axon loss. This model provides the experimental basis for applying cell-based strategies for local delivery of NFT into the retina for treatment of RGC loss.

W-1177

COMPARATIVE ANALYSIS OF HUMAN PLURIPOTENT STEM CELL DERIVED LIMBAL STEM CELLS AND PRIMARY OCULAR SURFACE EPITHELIAL CELLS USING ITRAQ PROTEOMICS

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Corneal epithelium is renewed by limbal stem cells (LSCs), a type of tissue-specific stem cells located in the basal layer of the limbus. Acute trauma or chronic disease affecting the LSCs may disrupt corneal epithelial renewal, resulting in ocular surface disorders which are painful and difficult to treat with conventional corneal transplantation. Human pluripotent stem cells (hPSCs) are readily available in limitless supply, and have a vast differentiation potential, offering novel opportunities for cell-based therapy of ocular surface disorders. Thorough characterization of hPSC-derived LSCs is crucial before considering clinical applications. In this study, we compared protein expression in primary corneal epithelial cells (CECs) and limbal epithelial cells (LECs) to that in hESC-LSCs and hiPSC-LSCs using isobaric tag for relative and absolute quantitation (iTRAQ) technology. Primary CECs and LECs were collected from the ocular surface of three separate cadaver donors. Two hPSC lines (one hESC and one hiPSC line) were differentiated towards LSC-like cells. Total protein was extracted from the cell and tissue samples, and digested with trypsin. The peptides were then labeled with iTRAQ 4-plex reagents and analyzed using Nano-RPLC-TripleTOF instrumentation. We identified 1004 proteins present in all samples, including various limbal and corneal epithelial markers, cell adhesion and junction proteins, calcium-binding proteins, and proteins involved in angiogenesis. Primary CECs and LECs had similar expression profiles, suggesting that they were likely mixed populations of corneal epithelial cells at various stages of maturity. Protein expression levels were very similar between hESC-LSCs and hiPSC-LSCs, meaning that the differentiation protocol is reproducible, yielding homogeneous cell populations. However, as the cells are cultured in vitro, they naturally differ from primary samples, with generally lower expression levels than in primary cells. Their expression profile suggests that hPSC-derived cells likely fall somewhere in between LSCs and terminally differentiated cells. Overall, this is the first study utilizing relative quantitative proteomics to compare hPSC-LSCs to their in vivo counterparts, and an important step towards clinical applications.

W-1178

MODELING LATE-ONSET DISEASE PHENOTYPES IN AGE-RELATED MACULAR DEGENERATION USING HUMAN IPSC-DERIVED RETINAL PIGMENT EPITHELIAL CELLS AND PROGERIN

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Age-related macular degeneration (AMD) is the major cause of blindness in older adults, affecting 1 in 5 people over age 75. AMD results in the loss of central vision later in life due to the progressive dysfunction and degeneration of the retinal pigment epithelium (RPE) and the subsequent degeneration of the underlying photoreceptor cells. Dry AMD, the most prevalent form, is characterized by extracellular lipid aggregates (termed drusen) beneath the RPE layer. While a number of genetic and environmental risk factors have been identified in AMD patients, the disease mechanism is still unclear. We have established a collection of RPE-derived hiPSC lines from age-matched AMD and non-AMD donors. Following differentiation to RPE cells (hiPSC-RPE), we observe a significant upregulation of key drusen-related transcripts such as BACE-1, APP, and APOJ and increased secretion of drusen-related proteins including Aβ42, VEGF-A, and clusterin in AMD compared to non-AMD hiPSC-RPE, demonstrating the ability to model aspects of drusen formation. These findings indicate that despite known environmental contributors, hiPSC-RPE and thus the AMD patients themselves have a genetic propensity toward drusen formation as demonstrated in vitro. Given the late-onset of AMD and the recent studies supporting a rejuvenation of somatic cells from aged donors back to a young state, we sought to accelerate aging in the hiPSC-RPE. AMD and non-AMD hiPSC-RPE were treated with progerin, a protein which accumulates during normal aging and is known to cause the premature aging disorder Hutchinson Gilford progeria syndrome. This novel disease-in-a-dish model is being used to investigate the cellular, molecular, and functional changes that require the synergistic interaction of genetics and aging to induce RPE dysfunction and degeneration in AMD with the goal of identifying novel targets that can be used to accelerate therapy development for this highly prevalent disease.

W-1179

XENO-FREE DIFFERENTIATION OF HESC INTO RETINAL PIGMENT EPITHELIAL CELLS AIMED TOWARDS THERAPY FOR AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration is one of the most important causes of vision loss in the Western world. Recent clinical trials have suggested that transplantation of human embryonic stem cell (hESC) derived retinal pigment epithelial (RPE) cells, could be used to replace the tissue lost in the degenerative form of macular degeneration. Production of functional RPE cells have however not been produced in a xeno-free and defined manner which is critical for clinical compliance and to reduce immunogenicity. Building on our recently developed methodology for xeno-free derivation of hESC, we now present in vitro differentiation into RPE cells by using a human recombinant laminin-based matrix and a xeno-free, chemically defined medium. Briefly, hESC are differentiated as embryoid bodies in suspension. Optic vesicle-like structures containing pigmented cells emerged following three weeks of differentiation and were manually isolated two weeks later. Enzymatically dissociated single cells are subsequently cultured in 2D cultures on laminin. This approach yields highly homogeneous populations of mature hESC-derived RPE cells that exhibit characteristics of native RPE cells such as morphology, pigmentation, expression and polarization of specific markers and phagocytic activity. Furthermore, upon transplantation into the retina of albino immunosuppressed rabbits, these cells form an organized, polarized and pigmented epithelial layer with maintained expression of RPE markers. Altogether, these findings proof that our xeno-free and chemically defined differentiation method could serve as a potential source of clinically compliant RPE cells for the development of a safe and efficient cell replacement therapy for age-related macular degeneration.

W-1180

BIOENGINEERED CARRIERS FOR HUMAN PLURIPOTENT STEM CELL DERIVED LIMBAL STEM CELLS

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Limbal stem cell deficiency (LSCD) is a major cause of corneal blindness worldwide, and its treatment remains a challenge. In healthy cornea, epithelium is renewed by limbal stem cells (LSCs), a type of tissue-specific stem cells located in the basal layer of the limbus. We and others have previously demonstrated that human pluripotent stem cells (hPSCs) provide new opportunities for corneal regeneration. In this study, we investigated the use of bioengineered collagen matrices as substrate carriers for hPSC-derived LSC-like cells, aiming for clinical applications. Differentiation of hPSCs towards LSC-like cells was directed using small-molecule induction followed by maturation in corneal epithelial medium. After four weeks of culture, differentiated cells were seeded onto the bioengineered transparent collagen matrices. Cell attachment and morphology were monitored regularly using phase-contrast microscopy, and protein expression of several key markers (p63, CK15, Ki67, CK3 and CK12) was evaluated using immunofluorescence microscopy. Cell viability and proliferation activity were assessed after 15 days of culture using the WST-1 Cell Proliferation Assay. Our results demonstrate that differentiated LSC-like cells on collagen matrices had the appropriate cuboidal morphology expressing the LSC marker p63, but not CK3, a marker of terminally differentiated corneal epithelium. After

two weeks of culture, cell proliferation was significantly higher on bioengineered collagen matrices than in control wells. In addition, LSC markers CK15 and p63, along with proliferation marker Ki67 were highly expressed even after 30 days in culture. Moreover, these cells were able to terminally differentiate upon stimulation, as suggested by protein expression of CK3 and CK12. In conclusion, our results suggest that the use of a bioengineered collagen matrix as a substrate scaffold enhances proliferation and differentiation of hPSC-derived LSC-like cells, applicable to cell replacement therapy.

NEURAL CELLS

W-1181

HELPER-DEPENDENT CANINE ADENOVIRUS PRE-CLINICAL EVALUATION IN A HUMAN CNS 3D IN VITRO MODEL

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Neurodegenerative diseases represent a burden both in terms of patient suffering and economic cost. Viral vectors derived from canine adenovirus serotype 2 (CAV-2), due to their attractive features to bypass the clinical disadvantages associated with the use of human adenoviruses, presenting a preferential neuronal tropism and high level of axonal retrograde transport, are considered as potential tools for the treatment of neurodegenerative disorders. However, accurate pre-clinical evaluation of efficacy and safety is critical for clinical translation. Human stem cells have great potential as complementary tools, bridging the gap between animal models and clinical trials. Herein we describe the assessment of helper-dependent CAV-2 (hd-CAV-2) efficacy and safety for gene delivery in a human stem cell derived 3D neural in vitro model. Human midbrain-derived neural progenitor cells (hmNPC) were cultured as neurospheres in dynamic culture systems. Differentiated neurospheres enriched in neurons, astrocytes and oligodendrocytes, presented increased expression of mature neuronal markers, along with synaptic functionality, neurotransmitter synthesis/release and were able to elicit voltage- and ligand-activated currents. Assessment of hd-CAV-2 transduction was performed at different MOIs, by evaluation of transgene expression and toxicity by cell viability and impact on neuronal gene expression. Under optimized conditions, hd-CAV-2 transduction led to stable long-term (up to 30 days) transgene expression with low toxicity. The evaluation of vector specific tropism showed that hd-CAV-2 has an increased neuronal tropism in contrast to hAd5 that preferentially transduced the glial cell population. This work demonstrates in a physiologically relevant 3D human cell model, that hd-CAV-2 vectors represent an efficient vehicle for gene therapy of human neurodegenerative diseases, with stable long-term transgene expression and minimal cytotoxicity.

W-1182

METABOLIC REMODELING DURING OLIGODENDROCYTE PRECURSOR CELL DIFFERENTIATION

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Oligodendrocytes wrap myelin sheaths around central nervous system (CNS) axons to facilitate saltatory signal conduction and to maintain axonal integrity. They derive from oligodendrocyte precursor cells (OPCs) during development, which remain in the adult brain as a self-renewing and proliferating stem/precursor cell population responsible for the repair of demyelinated areas. Despite recent studies suggesting that mature oligodendrocytes play a fundamental trophic support role to axons via transfer of lactate, the metabolic properties of oligodendrocyte lineage cells remain poorly understood. It is also unclear whether or not metabolic changes occur during differentiation, as it has been reported for other stem cell lineages, and what might be their role in myelination. We investigated the metabolic profiles associated with specific lineage stages using isotope-labelled glucose and mass spectrometry detection of glucose-derived metabolites combined with oxygen consumption measurements using the Seahorse Flux Analyser. We observed that immature OPCs have relatively low glycolytic rates and low levels of mitochondrial respiration compared to mature oligodendrocytes, which present higher rates of both aerobic glycolysis and mitochondrial oxidation. Mitochondria-specific staining indicated expansion of mitochondria from the perinuclear space in OPCs to the whole cell, including processes, in mature oligodendrocytes. Finally, inhibition of mitochondrial activity with rotenone or sodium azide inhibited OPC differentiation and myelin sheath formation in vitro in a dose-dependent manner, whereas inhibition of glycolysis with sodium oxamate did not affect differentiation, suggesting an essential role of mitochondria biogenesis and function in OPC differentiation and myelination. Our data suggests that a significant metabolic remodeling occurs during OPC differentiation and could regulate other mechanisms involved in OPC differentiation. Further studies will help to understand the role of such metabolic remodeling in oligodendrocyte maturation, myelinating capacity, axonal trophic support, and CNS remyelination - a natural repair mechanism in the adult CNS.

W-1183

ULTRASONIC ASPIRATE SAMPLE OFFER NEW SOURCE FOR ADULT NEURAL PROGENITORS WITH MULTIPOTENT DIFFERENTIATION POTENTIAL

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Adult neural progenitor cells (aNPCs) are a potential source for cell based therapy for neurodegenrative diseases and traumatic brain injuries. These cells have been isolated from different regions of adult human brain such as hippocampus, subventrical zone and white matter. However, there is still a need for easy accessible source with better yield to cover the limitation of small surgical sample of previous characterized aNPCs. Here we show that

ultrasonic aspirate (UA) sample, that are considered as "biological waste after surgery", offer big source for aNPCs. We have isolated and expanded these cells in both serum containing and serum free culture conditions. We show that UA-NPCs expanded in 10% and 1 % serum resembles previously characterized mesenchymal stem cells from two different regions in the adult human brain, the subventricular zone and the neocortex. UA-NPCs show neural differentiation potential. For the first time, we show a wide range comparison between fresh and culture expanded cells from 10 adult human brain samples by flow cytometry. Our data show that UA-NPCs are multipotent NPCs with long-term in vitro expandability equal to NPCs from hippocampus and subventrical zone. As well, we show the phenotype of fresh and culture expanded cells from adult human brain.

W-1184

IDENTIFICATION OF A UNIQUE NEURAL STEM CELL POPULATION IN THE HYPOTHALAMUS OF MAMMALIAN BRAIN

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The hypothalamus is a small region in the brain which is responsible for many vital events for survival and reproduction of the organism. Therefore, identifying the molecular mechanisms in the hypothalamus is essential for understanding diseases related to hypothalamus dysregulation. In the adult mammalian brain, active neurogenesis under normal conditions has been found in two neurogenic regions which are the subgranular zone (SGZ) of the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles. Despite being limited under physiological conditions, neurogenesis was suggested to exist in other CNS regions like the hypothalamus. Hypothalamic tanycytes have been considered as neural stem cells (NSCs) in the adult mouse brain. The nuclear receptor tailless (Tlx) 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 (3V) of the adult mouse brain. These cells are active during early postnatal development but become quiescent in adulthood. FGF2 infusion activates these cells in vivo. Loss of Tlx leads to depletion of these cells but overexpression leads to increase of NSCs and FGF2 mediated neurogenesis. Hypothalamic Tlx positive cells that are isolated at postnatal stage are transplantable into adult mice. Transplanted cells are able to integrate themselves into the existing network and differentiate into both glial and neuronal lineages. Examination of postnatal human sections indicates the existence of active neurogenesis in human 3V at postnatal stage. This suggests that similar to mice, Tlx dependent mechanism may exist in the human hypothalamus. This study identifies a unique population of hypothalamic NSCs and provides insight into their potential roles in regulating hypothalamus related physiological processes.

W-1185

PRION LIKE INVOLVEMENT OF SUPEROXIDE DISMUTASE 1 AGGREGATES IN MOTOR NEURONS DERIVED FROM ALS PATIENT IPSCS

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Amyotrophic Lateral Sclerosis (ALS) is a fatal and incurable neurodegenerative disease in which selective loss of motor neurons leads to progressive paralysis and ultimately death within 2-5 years of symptom onset. Protein aggregation is a hallmark of many neurodegenerative diseases including ALS, as well as Parkinson's disease, Alzheimer's disease and Huntington's disease. In ALS, aggregation of mutant or misfolded SOD1 and, especially, the potential transfer of SOD1 aggregates from one neuron to another, are poorly understood components of the disease pathophysiology. In particular, aggregate transmissibility may account for the anatomical spread of disease pathology within the CNS. To study that process in greater detail, we used induced pluripotent stem cells (iPSCs) from healthy controls and ALS patients to generate an unlimited supply of motor neurons, the cells that are selectively lost in ALS. These cells are able to recapitulate some key aspects of motor neuron degeneration during disease progression in patients. To elucidate mechanisms of protein aggregation and prion-like aggregate spread, we used synthetically produced mutant SOD1 aggregates, as well as naturally formed SOD1 aggregates obtained from the spinal cords of mice that highly overexpress a mutated form of SOD1 (SOD1G85R) and that model some aspects of ALS. We found that SOD1 aggregates are taken up by motor neurons, although not as readily as by other cells in the cultures. Surprisingly, although the uptake of SOD1 aggregates is not limited to motor neurons, both synthetic and endogenous aggregates appear to be selectively toxic to motor neurons. Furthermore, both control and patient derived cells died following uptake. This suggests that the differential response of motor neurons to SOD1 aggregates is downstream of the uptake. The emergence of protein aggregation as a key pathogenic feature in ALS suggests new therapeutic targets, and new drug discovery strategies based on interfering with the process of neuron-to-neuron transfer of protein aggregates. Valuable insights concerning this process can be obtained using human iPS-derived motor neurons.

W-1186

STUDIES OF THE MOLECULAR PATHWAYS BEHIND DYSLEXIA

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Dyslexia is a complex learning disability characterized by deficits in reading despite adequate intelligence, normal senses and proper socio-cultural opportunities, affecting 5-10% of the population. Dyslexia has a strong genetic component and many susceptibility genes have been identified among which DYX1C1, DCDC2 and KIAA0319 are the most replicated. The molecular functions of these

genes are little investigated, yet all the above have been implicated in neuronal migration and development. More recently, our group and others have shown a link between dyslexia candidate genes and cilia. We are thus performing assays in a human ciliated cell line (RPE1, retinal pigment epithelial cells) and in different tissues for sub-cellular localization by immunofluorescence (IF). In addition, we are looking for common molecular pathways of the candidate genes DYX1C1, DCDC2 and KIAA0319 by functional assays. We are using neuroepithelial-like stem cells (NES cells) derived from induced pluripotent stem cells (iPSCs) as a model to further dissect the molecular mechanisms involved in dyslexia. NES cells are self-renewing and can be differentiated along the neural and glial lineages. We have shown by qRT-PCR and IF that DYX1C1, DCDC2 and KIAA0319 gene expression is increased in NES cells during their differentiation to neurons and glia. In addition, they are growing cilia during differentiation. NES cells are thus a valid model system to study the function of dyslexia candidate genes. Using lentiviral expression constructs, we are overexpressing DYX1C1, DCDC2 and KIAA0319 in NES cells in order to study the downstream pathways of these genes.

W-1187

AKNA ACTS AS A POTENT NOVEL REGULATOR FOR NEURAL STEM CELLS

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Understanding mechanisms regulating neural stem cell homeostasis and their fate commitment is fundamental for their efficient manipulation and future usage in regenerative medicine. To contribute to this goal, our laboratory interrogates novel neurogenic molecular pathways by identifying common regulators in neural stem cells of the developing and adult brain. These studies have proved successful for the identification of new factors essential for embryonic and adult neurogenesis and key in direct neuronal reprogramming. Here we show the functional and molecular analysis of another novel factor common to embryonic and adult neurogenesis, the AT-hook containing protein Akna. Besides it significantly elevated expression in the adult and embryonic neurogenic sites, Akna is of significant interest as it localizes to chromosomal fragile sites common to mouse and human linked to neoplastic diseases. Akna function was first probed by means of gain and loss of function experiments in the developing mouse cerebral cortex. Strikingly, over-expression in neural stem cell provokes delamination of neural stem cells and a consequently premature neuronal differentiation, while 2 different short hairpin RNAs directed against Akna potently interfere with neuronal differentiation. In addition, in both scenarios proper migration of neuronal progenitors is also affected. Thus, Akna is both necessary and sufficient for neurogenesis in the developing cerebral cortex. To investigate the cellular and molecular mechanisms of how Akna regulates neurogenesis we generated monoclonal antibodies and discovered a surprising cellular localization of Akna that help to elucidate its mode of action in embryonic and adult neurogenesis and will be presented.

W-1188

REGIONAL AND STAGE SPECIFIC EFFECTS OF PROSPECTIVELY PURIFIED VASCULAR CELLS ON THE ADULT V/SVZ NEURAL STEM CELL LINEAGE

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Adult neural stem cells reside in specialized niches. The ventricular-subventricular zone (V/SVZ) is an extensive germinal niche adjacent to the lateral ventricles. Stem cells in the V/SVZ continuously generate olfactory bulb interneurons, as well as small numbers of oligodendrocytes. Quiescent neural stem cells (qNSCs) become activated (aNSCs), and generate transit amplifying cells (TACs), which in turn give rise to neuroblasts that migrate to the olfactory bulb. The vasculature is an important component of the adult neural stem cell niche, but whether vascular cells in neurogenic areas are intrinsically different than those elsewhere in the brain is unknown. Moreover, the contribution of pericytes to the neural stem cell niche has not been defined. Here we describe a rapid FACS purification strategy to simultaneously isolate primary endothelial cells and pericytes from brain micro-regions of non-transgenic mice using CD31 and CD13 as surface markers. We compared the effect of purified vascular cells from a neurogenic (V/SVZ) and non-neurogenic brain region (cortex) on FACS-purified cells from each stage of the V/SVZ stem cell lineage in vitro. Endothelial and pericyte diffusible signals from both regions differentially promote the proliferation and neuronal differentiation of qNSCs, aNSCs and TACs. Unexpectedly, diffusible cortical signals had the most potent effects on V/SVZ proliferation and neurogenesis, highlighting the intrinsic capacity of non-neurogenic vasculature to support stem cell behavior. Using antibody arrays, we identify PlGF-2, a VEGFR1 ligand, as an endothelial-derived mitogen secreted at high levels by cortical endothelial cells and at lower levels by V/SVZ endothelial cells, but not by pericytes. PlGF-2 robustly promotes V/SVZ cell proliferation independent of other growth factors. In sum, this purification strategy provides a platform to define the functional and molecular contribution, as well as regional differences, of vascular cells to stem cell niches and other brain regions under different physiological and pathological states.

W-1189

CONSECUTIVE BUILDING BLOCKS OF HUMAN NEURAL STEM CELL ONTOGENY DERIVED FROM PLURIPOTENT STEM CELLS: FUNDAMENTALS AND IMPLICATIONS

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Modeling key cell fate decisions and heterogeneity in neural progenitors is fundamental for revealing origin of diverse lineages, identifying molecular forces regulating distinct potencies, and generating homogeneous neural stem cell (NSC) populations for

regenerative medicine. Here we report our recent progress in developing such approaches and their implications. We isolated consecutive neural progenitors derived from human PSCs differentiated along cortical development based on their Notch activation state. We first isolated Notch active CNS neuroepithelial cells exhibiting high proliferation and broad potential. These successively yield early and mid cerebral neurogenic radial glia followed by gliogenic radial glia, together recapitulating hallmarks of NSC ontogeny, cortical lamination and glial transformation in Notch dependent manner. We used isolated stages as modules to identify forces driving cell fate transitions. We employed gene expression analysis and epigenetic profiling combined with computational approaches to infer key regulators progressively remodeling the epigenetic landscape, and followed by shRNA functional validation. This allowed uncovering a core gene regulatory network of stably expressed transcription factors that dynamically interacts with stage specific factors to regulate cortical NSC fate transition. We further identified dynamics of pathway activation during this process and developed a streamlined and robust protocol for efficient cortical cell fate conversion from naïve and primed PSCs using small molecules. Finally, we found that transition through neurogenic phases in our experimental paradigm is characterized by extensive remodeling also of neural rosette formation, radial organization, and interkinetic nuclear and centriole migration dynamics, which all correlate with progressive changes in neurogenic NSC capacity in vitro. We hence developed a quantitative live imaging framework combined with reporters for these organelles to objectively measure these features during corticogenesis in vitro and further used these reporters to isolate distinct NSCs based on their chosen cell fate immediately following their division, allowing new cellular and molecular insights into cell fate decision of NSCs during human corticogenesis in vitro.

W-1190

INCREASED PRODUCTION OF NEURAL STEM CELLS AND DERIVED OLIGODENDROCYTES IN SIMULATED MICROGRAVITY: IMPLICATIONS FOR MYELIN REPAIR

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It has been shown that microgravity induces apoptosis, alters the cytoskeleton and affects signal transduction, cell differentiation, proliferation, migration and adhesion differently depending on the cell type. The nervous system of all animal species has evolved in a one-g (1G) environment and is functionally influenced by the presence of gravity. The absence of gravity presents a unique opportunity to gain new insights into basic neurological functions as well as to understand if lineage commitment would be affected by simulated microgravity (sim-0G). We have previously designed a developmental stage-specific culture medium that allows for the modulation of proliferation of neural stem cells (NSC) maintaining their pluripotency. We also have another culture medium that induces NSC commitment to the oligodendrocyte (OL) lineage. During the past five years we have developed a culture system to study the impact of simulated microgravity (sim-0G) produced by a 3D-clinostat (Mitsubishi) on neural cells. We subjected NSC and OL progenitors (OLP) 24 and 48h, to sim-0G to ascertain potential differences. We determined that NSC and OL (that myelinate the central nervous system) survived and gave rise to more progenies during and after Sim-0G exposure than cells kept in 1G with concomitant shortening of their cell cycle. We also sought to ascertain if NSC would become OL while in sim-0G and if microgravity would impact the expression of OL lineage-specific markers. The sequential mRNA expression of OL markers remained unaltered while immunocytochemistry showed that more cells expressed early markers. Sim-0G decreased the proportion of cells that expressed mature OL markers like MBP. Nonetheless, when cells were allowed to become OL in the appropriate culture conditions they showed lineage progression. Thus, maturation of these cells was delayed but not impaired by exposure to 0G. Our long-term goal is to be able to produce healthy, functional and transplantable OL to be used for cell replacement therapies and, treat myelin deficiency whether in the premature neonate or due to neurodegenerative disorders like multiple sclerosis. Our data demonstrates that in sim-0G OL are produced faster without losing their ability to mature and synthesize the myelin components necessary for myelination.

W-1191

NEURAL DIFFERENTIATION DEFECTS IN LISSENCEPHALIC PATIENT CELLS

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Lissencephaly (Liss) is characterised by a smooth cerebral surface, mental retardation and seizures. Liss is the result of mutations in one of several genes involved in cytoskeletal regulation and the most common are mutations in LIS1 or in the X-linked DCX. The proposed primary defect underlying the smoothening of the brain is impaired neuronal migration. However, in this study we also demonstrate defects in the neural differentiation of patient derived neural stem cells. We have taken advantage of the iPS technology and neural differentiation to derive a cellular model of Liss using cells from male patients with a truncated DCX gene. Using the integration free Sendai virus we have established iPS cells from two patients with DCX mutations in addition to iPS cells from healthy persons. Further derivation of Neuroepithelial stem (NES) cells from the iPS cells did not indicate any phenotype in the patient cells at that stage. Proliferating NES cells don't express DCX however, upon differentiation the expression increase more than 100 fold. In contrast, patient NES cells did not show any DCX protein in differentiating neuroblasts. The upregulation of DCX seem to be a key not only for migration but also for proper differentiation. Our data show dramatic differentiation phenotypes in patient NES cells, which appear to be partly resistant to differentiation. Transcriptome analyses strengthen these observations and genes connected to proliferation stay upregulated in patient's cell cultures during differentiation compared to healthy cells. The differentiation of patient cells is delayed and they sprout neurites that are half the length of neuritis from healthy neurons. Genes important for neurite outgrowth and synapse maturation are accordingly under-expressed in the patient cells. Taken together, we have shown that NES cells from Liss patients that fail to up-regulate DCX upon induction of differentiation seem to initially be resistant to differentiation. The impaired differentiation results in continued proliferation and defect migration by the patient cells, in addition to underdeveloped neurites.

W-1192

FOXJ1 IS TRANSIENTLY EXPRESSED IN SPECIFIC CELL POPULATIONS IN THE DEVELOPING AND POST-NATAL SPINAL CORD IN A TIME AND SPACE SPECIFIC MANNER

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Forkhead box protein J1 (FoxJ1) - a transcription factor classically involved in ciliogenesis - has recently risen interest in neural stem cell research and regenerative medicine. Indeed, during adulthood, FoxJ1 is expressed by the ependymal cells surrounding the central canal of the spinal cord. FoxJ1+ ependymal cells show their stem cell potential when activated by injury and contribute to mechanisms of self-repair. In the postnatal brain, the expression of FoxJ1 is required for the differentiation of radial glia into ependymal cells and astrocytes, suggesting a crucial role for FoxJ1 in stem cell niche formation. However, we have very limited knowledge of FoxJ1 expression and its roles in the developing and postnatal spinal cord. We observed that FoxJ1-YFP inducible Cre and non-inducible mice transiently express this transcription factor during different developmental stages in subtypes of neurons, astrocytes, and ependymal cells clustered in specific areas of the developing and post natal spinal cord. Floor plate cells and neurons in the ventro-lateral grey matter are the first cell populations expressing FoxJ1 from early developmental stages. Astrocytes in the ventro-lateral white matter and dorsal columns express FoxJ1 from E14.5 and E16.5, respectively. From E16.5, ependymal cells around the central canal also start expressing FoxJ1. Interestingly, the neuronal and astrocytic populations stop expressing FoxJ1 post-natally, while ependymal cells express it throughout adulthood. The time and space specific FoxJ1 expression suggests that this transcription factor can have important roles in maintaining a precursor phenotype, cell fate commitment, migration, or integration. We aim to further identify the FoxJ1+ neuronal and astrocytic subpopulations and to elucidate the functional role of FoxJ1 in the developing and post-natal spinal cord taking advantage of additional transgenic mouse models.

W-1193

STEM CELL INTEGRATION INTO FUNCTIONAL NEURAL NETWORKS STUDIED IN RESPIRATORY ORGANOTYPIC BRAINSTEM SLICE CULTURES

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Gap junctions are crucial in corticogenesis, generation, migration as well as differentiation of newborn subventricular cells. Our data indicate that gap junction mediated integration of neural stem cells (NSCs) into the CNS is both a requisite for stem cell function and also provides a novel mean by which these cells may provide benefit to host cells. Recently we showed that human fetal stem cells as well as primary adult neural stem cell derived neural cells express connexins and interact with striatal organotypic culture cells via formation of intercellular gap junction bridges. Formation of neural networks that generate patterns underlying breathing and its response to environmental changes is modulated by neural activity partly propagated via gap junctions. Interference of this activity, during development or by an insult, may have acute as well as long-term consequences. Therefore we explore the role of ongoing neural activity, gap junctions and stem cells in the formation, and possibly repair, of functional neural networks. We have established a method that keeps respiratory brainstem slices alive for several weeks, with maintained synchronized neural network activity and respiratory-related motor output. Compared to other types of neural slice cultures, our model contains functional pacemaker neurons and small world-organized networks with robust endogenous network activity that also generates rhythmic behavior-respiratory activity. To these slices, we transplant neural stem cells and direct their differentiation through explicit control of the microenvironment. Using a network analysis tool we then examine the correlated intercellular signal activity and network topology, after measuring multiple cells' activity with time-lapse calcium imaging. Ongoing experiments try to establish the mechanisms behind and optimal conditions for repair of host and integration of transplanted NSCs into the active neural networks in "breathing" brainstem cultures. Engrafted NSCs survive as well as interact with the "breathing" organotypic culture system. Grafted neural stem cells rapidly establish gap junctional connections to host neural networks reducing the reactive gliosis after injury but also affect the respiratory center network structure.

W-1194

PAX6 AND THE H3K4 DEMETHYLASE SMCX/KDM5C CO-OCCUPY AND REGULATE A SUBSET OF GENES, INCLUDING DLK-1, IN NEURAL STEM CELLS

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It has been shown that ablation of the key transcription factor Pax6 in neural stem cells (NSCs) results in not only down-regulation but also increased expression of genes in the developing CNS, suggesting that Pax6 could act as a transcriptional repressor. In addition, it has been suggested that Pax6 preferentially binds to methylated DNA. We have noted that RNA knockdown of Pax6 in embryonic cortical NSCs by shRNA induce premature neuronal differentiation along with a significant cell death and up-regulation of genes associated with neuronal differentiation, such as NeuroD6/Nex and Dlk-1. Here we show by using an inducible/integrated GAL reporter system, that Pax6 can indeed act as a direct transcriptional repressor and that the Pax6-mediated repression is primarily associated with demethylation of trimethylated lysine 4 on histone H3, H3K4me3. H3K4me3 is a positive mark for transcription associated with the recruitment of the transcriptional initiator complex to the promoter, and a demethylation of H3K4me3 is considered to have a strong repressive effect. Mapping of the expression patterns of histone demethylases in NSCs in vivo and in vitro demonstrated that the only H3K4me3 demethylase expressed at significant levels in NSCs is SMCX/KDM5C, a histone demethylase linked to X-linked mental retardation. ChIP-Seq experiments to investigate genome wide occupancy of SMCX in NSCs and statistical comparison with previous reports of Pax6 occupancy, revealed that Pax6 and SMCX co-occupy a subset of genes, including Dlk-1, and that SMCX but not Pax6 is occupying almost all genes associated with the family of Notch receptors and ligands. Preliminary results using siRNA and CRISPR/Cas9 suggest that Pax6 and SMCX are repressing the

expression of the imprinted factor Dlk1 in NSCs, an essential part of the differentiation program in the cortex. These data shed light on the mechanisms underlying Pax6 function, and point to a possible link to X-linked mental retardation.

W-1195

CHARACTERIZATION OF CHROMATIN LANDSCAPES AND SINGLE CELL TRANSCRIPTOMES IN STEM CELLS OF THE EMBRYONIC AND ADULT BRAIN

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The brain consists of billions of neurons and glial cells. The development and maintenance of this complex organ ultimately relies on a population of self-renewing stem cells, which differentiate into post-mitotic progeny during neuro- and gliogenesis. Despite the fact that stem cells are essential for nervous system development and maintenance, as well as carry hope for future innovations in regenerative medicine, their molecular signatures are currently undescribed. The transcription factor Sox2 is expressed in all undifferentiated cells in the brain and it has recently been shown that a reduction in Sox2 expression levels is a key signature in the transition between stem cells and more committed progenitor cells. Here we have utilized a Sox2-GFP mouse line to isolate stem and progenitor cells from the embryonic and adult brain based on their levels of GFP expression. To characterize the potential of these cell populations we have used single cell differentiation and neurosphere forming assays. In parallel we have examined the transcriptomes and chromatin landscapes of these cell populations using single-cell RNA-seq together with ATAC-seq. Together these experiments have revealed important functional and molecular insights into stem and progenitor cells in the embryonic and adult brain.

W-1196

HYPOXIA-INDUCIBLE FACTORS (HIFS) EXPRESSION MAY PLAY AN ESSENTIAL ROLE ON NEURONAL DIFFERENTIATION

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The condition of hypoxia is harmful to affect the neuronal expansion in fetal brain. To express the hypoxia-inducible factor 1alpha (HIF-1a) may assist the differentiation and maturation of neuronal precursor cells (NPC) during transitory hypoxic condition. The biological functions of HIF-1a/DNA complex have been found to promote about hundreds of hypoxia target genes. The possibility of HIF-1a to participate neuronal differentiation in the developing CNS remains to be revealed. The results from our previous studies indicate that the neuronal differentiation occurs among fetal human nestin/CD133 positive brain cells predominantly rather than the cells isolated from adult brain tissue with same markers. Both mRNA and protein levels of HIF-1a expression in the fetal brain cells are higher than that in the adult brain cells. We also notice that the HIF-1a expression is reduced after the neural precursor cells go through the differentiation stage. The level of HIF-1a expression in the differentiated neuronal cells is less than that in neural precursors. Our hypotheses were that HIF-1a expression might be necessary for the neuronal differentiation, and reduction of HIF-1a expression would be lethal for the neural precursor cells. To explore the bio-functions of HIF-1a to regulate neuronal differentiation, HIF-1a deoxynucleotides antisense (DNA) of HIF-1a were used for this study. With HIF-1a DNA treatment, the level of HIF-1a expression is decreased among the NPC. The results were confirmed using RT-PCR, Northern blotting and protein assays. However, inhibition of HIF-1a expression does not affect neuronal differentiation from NPCs under normal culture condition. There is no apoptotic signal to be detected after the cultures treated with DNA. In contrast, under hypoxic condition, the neuronal differentiation is suppressed. In addition, reducing of HIF-1a expression increases glio-differentiation dramatically. The results indicate that the reduction of HIF-a expression significantly inhibits the neuronal differentiation of NPCs only under hypoxia condition. Suppression of HIF-a mRNA, hypoxia becomes lethal to neuronal precursor cells. Taken together, expression of HIF-1a is necessary for the neuronal differentiation, and prevents the gliogenesis in vitro.

W-1197

CLONING FROM POSTMITOTIC NEURONS REVEALS EXTENSIVE GENOME DIVERSITY IN THE ADULT MOUSE BRAIN

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Neurons exhibit remarkable cell type diversity and persist without cell division for the lifetime of the individual. These key features of neurons - their diversity, their longevity and their irreversible post-mitotic status - pose a series of intriguing and unresolved questions regarding neuronal genomes. In particular, given that neurons neither divide nor serve as precursors to other cell types, do neuronal genomes maintain epigenetic plasticity over the lifetime of the organism? Further, given the post-mitotic status and unique physiology of neurons, what is the mutational burden of postmitotic neurons, and how does this burden and the patterns of mutations they acquire compare to those acquired in other somatic lineages? Finally, by analogy to cell type diversification in the immune system, to what extent do programmed genomic changes contribute to the diversification of neurons? To address these questions, we used somatic cell nuclear transfer (SCNT) to reprogram postmitotic neurons from the adult mouse brain. We found that neuronal nuclei developmentally reset by SCNT were able to generate fertile adult mice, indicating that mature neurons retain a similar degree of epigenetic plasticity as other terminally differentiated cell types. Reprogramming neurons by SCNT also provides a unique means of amplifying the genomes of these otherwise non-dividing cells for single cell genomic analyses. Whole genome sequencing

of embryonic stem cells derived from adult neurons by SCNT uncovered extensive genomic diversity among parent neurons. Each neuron harbored ~110 unique somatic mutations including structural variants (0-3), transposable element insertions (0-4), indels (12-34) and single nucleotide variants (SNVs) (62-142). While we did not detect recurrent rearrangements or genomic changes whose structures suggest an obvious role in cell type diversification, most neuronal genomes contained gene-disrupting mutations (0-3). When compared to mutations from other lineages, neuronal SNVs are enriched in genic regions and in neuronally expressed genes. These results predict that somatic mutations could impact neuronal function, particularly if they accumulate in postmitotic neurons during maturation and aging at the high rate suggested by our data.

W-1198

CELL THERAPY APPROACHES FOR SPINAL CORD INJURY SHOULD BE LEVEL DEPENDENT - PRELIMINARY EVIDENCE FOR A UNIQUE SUBACUTE WAVE OF SECONDARY INFLAMMATION IN CERVICAL INJURY

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Vascularity and gray-white matter distribution are just a few of the anatomical differences between the cervical and thoracic segments of the spinal cord. Thus, there may be key pathophysiological differences after spinal cord injury (SCI). Profiling these variations is important for optimizing cellular treatments between the thoracic and cervical injury niches. It was hypothesized that due to the increased vascularity of the cervical region, innate and adaptive immune responses would be more prominent, and the process of neovascularization lengthened compared to thoracic SCI. After laminectomy, female Wistar rats underwent two behaviorally similar injuries, a 1-minute 23gram C6-7 or a 35g T6-7 clip compression injury. Mortality and neurobehavioural data were weekly recorded starting at 3 days post-SCI. Vascularity was measured in vivo using Very High Resolution Ultrasound (VHRUS) and Power Doppler imaging. Spinal cord tissue was collected at 3, 7, 14 or 54 days post-SCI and processed for RNASeq, ELISA array profiling and immunohistochemistry. Preliminary VHRUS Power Doppler results showed an increase in vascularity post-injury from days 3-7 (1.2 ± 0.4%, $p<0.05$, n = 4), but a decrease in vascularity that is exclusive to cervical SCI at day 14 (-1.402 ± 0.4210%, $p<0.05$, n=4). Additionally, cavitation is considerably more prominent at 14 days post-cervical SCI when compared to thoracic SCI (0.4 ± 0.1 mm3, $p<0.05$, n=4). ELISA analyses revealed a biphasic trend in cervical SCI with peaks at 3 and 14 days for pro-inflammatory cytokines IFN-γ, GM-CSF, IL-1β;, IL-17, TNF-α, and RANTES, and chemotaxins CD54, CINC-1, and CCL20. Thoracic RNASeq data for pro-inflammatory cytokines TNF-α and IL-1α showed a single peak at 3 days. These initial results suggest that a second delayed wave of inflammation occurs after cervical, but not thoracic SCI. This subacute inflammatory peak after cervical SCI has important implications for the timing of cell transplantation, as this may result in exacerbation of cell death and impact differentiation in cervical SCI.

W-1199

NOTCH1 STIMULATION INDUCES A VASCULARIZATION SWITCH WITH PERICYTE-LIKE CELL DIFFERENTIATION OF GLIOBLASTOMA STEM CELLS

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Glioblastoma multiforms (GBMs) are highly vascularized brain tumors containing a subpopulation of multipotent cancer stem cells. These cells closely interact with endothelial cells in neurovascular niches. In this study, we have uncovered a close link between the Notch1 pathway and the tumoral vascularization process of GBM stem cells. We observed that although the Notch1 receptor was activated, the typical target proteins (HES5, HEY1, and HEY2) were not or barely expressed in two explored GBM stem cell cultures. Notch1 signaling activation by expression of the intracellular form (NICD) in these cells was found to reduce their growth rate and migration, which was accompanied by the sharp reduction in neural stem cell transcription factor expression (ASCL1, OLIG2, and SOX2), while HEY1/2, KLF9, and SNAI2 transcription factors were upregulated. Expression of OLIG2 and growth were restored after termination of Notch1 stimulation. Remarkably, NICD expression induced the expression of pericyte cell markers (NG2, PDGFRβ, and α-smooth muscle actin [αSMA]) in GBM stem cells. This was paralleled with the induction of several angiogenesis-related factors most notably cytokines (heparin binding epidermal growth factor [HB-EGF], IL8, and PLGF), matrix metalloproteinases (MMP9), and adhesion proteins (vascular cell adhesion molecule 1 [VCAM1], intercellular adhesion molecule 1 [ICAM1], and integrin alpha 9 [ITGA9]). In xenotransplantation experiments, contrasting with the infiltrative and poorly vascularized tumors obtained with control GBM stem cells, Notch1 stimulation resulted in poorly disseminating but highly vascularized grafts containing large vessels with lumen. Notch1-stimulated GBM cells expressed pericyte cell markers and closely associated with endothelial cells. These results reveal an important role for the Notch1 pathway in regulating GBM stem cell plasticity and angiogenic properties.

W-1200

INTRATHECAL TRANSPLANTATION OF SPINAL GABAERGIC NEURONS ATTENUATE NEUROPATHIC PAIN IN SPINAL CORD INJURY RAT MODEL

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Neuropathic pain following spinal cord injury (SCI) is a devastating disease characterizied by spontaneous pain such as hyperalgesia and allodynia. In this study, we investigated a therapeutic potential of ESC-derived spinal GABAergic neurons on the neurophatic pain in SCI rat model. The mouse ES cell-derived neural precursor cells (ES-NPs) were efficiently differentiated into spinal GABAergic neurons in

culture conditions containing 100ng/ml sonic hedgehog (SHH) and 1uM/ml retinoic acid (RA). Low dosage of SHH and RA induced the MGE-like progenitors expressing low levels of DARPP32 and Nkx2.1 genes and high levels of Irx3 and Pax6 genes in RT-PCR analysis, and generated the majority of DARPP32-negative GABAergic nerurons after in vitro differentiation. The spinal ES-NPs were intrathecally tranplanted into the lesion area of spinal cord around T10-T11 at 21 days after SCI. The engrafted spinal GABAergic neurons increased paw withdrawal threshold (PWT) in below level of the lesion and vocalization threshold (VT) in at level of T12, T11 and T10, which indicates attenuation of chronic neuropathic pain by the spinal GABAergic neurons. Our immunostaining analysis demonstrated that the transplanted cells were positive against GABA antibody in injured region, and some cells were migrated to T12, T11 and T13 regions of injured spinal cord and were also survived for more than 7 weeks in L4-L5. The results indicate that ES-NP cell-derived spianl GABAergic neurons attenuated the chronic neuropathic pain following SCI, suggesting that the spinal GABAergic neurons could be used for SCI neuropathic pain treatment as stem cell-based therapies. Supported by the Ministry of Science, ICT and Future planning (201000220349 and 2012M3A9C6050131) and the Ministry of Health and Welfare (A120392120100000300) Grants

W-1201

EFFICIENT CONVERSION OF FUNCTIONAL PARVALBUMIN NEURONS FROM FIBROBLASTS WITH SUPPLEMENTAL CHEMICAL COMPOUND

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Abnormalities of Parvalbumin (PV)-expressing interneurons cause neurodevelopmental disorders such as epilepsy, autism and schizophrenia. Unlike other types of neurons that can be efficiently differentiated from pluripotent stem cells, PV neurons were hardly generated through conventional differentiation strategy. In this study, we developed an adenovirus based transdifferentiation strategy that employs additional chemical compound for efficient generation of induced PV (iPV) neurons. We revealed that a single transcription factor Ascl1 could convert only a few proportion of fibroblasts into neurons. Addition of a chemical compound Forskolin, synergizing with Ascl1 induced approximately 80% of mouse fibroblasts into iPV neurons. The iPV neurons generated through this procedure maturated in 5-7 days post infection, characterized by electrophysiological properties and known neuronal markers such as synapsin and MAP2. Transplantation of these iPV neurons into hippocampus of epileptic mice almost completely relieved the epilepsy and corrected the behavioral deficits. Under improved conditions, human fibroblasts were also converted to iPV neurons. We thus identified an efficient approach to generate high proportion of PV neurons, which are informative to develop yet unavailable large population of human iPV neurons for neurological disease therapy.

W-1202

XENO FREE PURIFICATION OF NEURAL STEM LIKE CELLS FROM HUMAN DENTAL PULP AND THEIR IN VIVO APPLICATION

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Neural stem cells (NSCs) have many advantages for neural regeneration including easy to be differentiated into neural and glial cells and paracrine effects, but ethical and immunological issues from original primary or embryonic stem cells interfere with clinical translation. Recently developed gene-modified NSCs and animal contents-containing culture system may make safety issues. Dental pulp, which is originated from cranial neural crest cells, is known to contain neural stem-like cells and also enable autologous transplantation for neural regeneration in human. The aim of this study is to purify neural stem-like cells from human dental pulp using xeno-free culture system, and whether they survive and migrate well in vivo condition. Human dental pulp tissues were obtained from extracted third molars, and divided into two groups by culture media from initial step of primary culture; 10% fetal bovine serum (FBS) media (FBS group), or xeno-free NSC culture media (NSC group). Cell proliferation rate of cultured cells in NSC group was lower than cultured cells in FBS group. However the population of NSC surface markers representing cells by FACS, and the expression of NSC markers by real-time PCR and immunofluorescence were greater in NSC group than those in FBS group. Cytokine assay also revealed that the concentration of NSC-secreting molecules was also higher in NSC group than in FBS group. Cultured cells in each group were transplanted into neonatal brain of rats, and cell survival and migration were evaluated at 1 week. We found that in vivo survival of cultured cells and NSC marker-positive cells in NSC group within brain was greater than those in FBS group. We concluded that neural stem-like cells are purified more when human dental pulp were cultured in xeno-free NSC media from the initial step, and might be useful for clinical translation of neural regeneration as a source of autologous transplantation.

W-1203

CELLULAR PHENOTYPES ASSOCIATED WITH AUTISM

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The main aim of this project is to study the cellular and molecular phenotypes associated with ASD in humans. We generated four iPS cell lines from two patients with ASD, that have a terminal deletion of chromosome 22 including the genes SHANK3, ACR and RABL2. As controls we used iPSCs from healthy individuals. The structure of a neuron is critical for neuronal function, as the size and shape of the neuron defines its capacity to receive and send signals. Morphological changes such as decreased cell soma area, increased dendritic length and aborization have been associated with Shank3 mutant mice as well as disorders comorbid with Autism. We are investigating the morphological properties of the control and Shank3 neurons. This will enable us to generate an in vitro system to help understand the cellular basis of ASD as well as provide a system for drug screening. Methodology: 1. To show the morphological structure of neurons

using transient transfection of GFP and to manually trace these with the NeuronJ software. 2.To do a high content morphological analysis at a global level using the Cell Insight machine (Cellomics NX11110). 3.To compare the neurite outgrowth in patient and control lines with time-lapse imaging (Nikon Bio station) and to analyze these by developing software in collaboration with Metapmorph.The GFP and Cell Insight revealed morphological differences in the Shank3 neurons at day 30 (immature neurons) and day 45 (young neurons) of neuronal development.The cell soma diameter was lower in the patient neurons while the neurite length and the mean number of neurites per neuron was higher at both the stages.Time Lapse imaging showed significant differences in the rate of primary neurite formation (increased), primary neurite elimination (decreased), neurite extension (increased), retraction (decreased) and cell soma speed (decreased) in the SHANK3 patient neurons. Recent studies have shown that the deficits in dendritic morphology including retractions, formations and eliminations of dendrites contribute to the miscommunication between neurons, which is one of the major underlying causes of ASD. Correspondingly, our patient lines show deficits in neuronal outgrowth, which suggests that SHANK3 may have a role in the normal development of a neuron.

W-1204

MOLECULAR MECHANISMS OF THE ORIENTAL MEDICINE FOR THERAPY PARKINSON'S DISEASE

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Parkinson's disease (PD) is one of the most common neurodegenerative diseases and result primarily from the selective loss of dopaminergic neurons in the substantia nigra of midbrain. Several lines of evidence suggest that treatment of oriental medicine combined with western medicine is one of effective therapies for PD. However, it still remains unclear the underlying molecular mechanisms that mediate therapeutic effects of the oriental medicine for PD. Here, we investigate the therapeutic mechanisms of oriental medicine in the PD patient specific iPS cells as a new clinically relevant disease model. We found that acupuncture treatments and herbal medicine result in the specific changes in gene expressions and histone modifications in the PD patient iPSC models. These studies for the first time will provide the molecular basics for therapeutic effects of the oriental medicine in the PD patient iPSC models. Ultimately, this result shed light on the mechanistic molecular events in the process of neuroprotection of PD and proves valuable in elucidating important understanding of the therapeutic aspects of the PD.

W-1205

COMPARISON OF TWO DIFFERENTIATION METHODS FOR PRODUCTION OF HUMAN PERIPHERAL SENSORY NEU-RONS

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Peripheral somatic sensory neurons (PSNs) are responsible for connecting the central nervous system to the limbs and organs. Nociceptors are specialized peripheral sensory neurons that transmit harmful or noxious stimuli and cause the perception of pain. Nociceptors are an important target for drug development as well as for toxicology screening because for example pain-killers should directly and reliably affect these types of cells. In addition, peripheral diabetic neuropathy is linked to sensory neurons, making these cells important also for the field of disease modeling. Moreover, the development of human peripheral nervous system is not well known on a cellular level. However, two approaches for the differentiation of peripheral sensory neural cells have been published: stromal-derived inducing activity (SDIA) method and small-molecular inhibition (SMI) method. The aim of the study was to produce peripheral sensory neurons from human iPS cells and compare the efficiency and feasibility of the two approaches. The SDIA-method is based on inducing effect from mouse PA6 feeder cells. This method is relatively cheap but has multiple steps and is time consuming. With the SMI-method PSNs are differentiated by inhibiting the other major differentiation pathways with a set of small molecules. This method is considerably more expensive, however, the differentiation time is short and less xenogenic factors are used. PSNs were differentiated from seven iPS lines using both methods. The differentiated cells were studied according to their morphological characteristics and the expression of neuronal markers was assessed at the protein and at the gene expression level. The functionality of the cells was studied with calcium□imaging. The differentiated cells had calcium signaling responses to K+-ions and capsaicin typical for sensory neurons. They also had a correct pseudo-unipolar morphology. Furthermore, the cells expressed specific markers for the peripheral sensory neurons. The differentiation efficiency clearly varied between cell lines, however, each differentiation batch did produce putative sensory neurons. The SMI-method produced more sensory neural marker positive cells but, on the contrary, the SDIA-method produced larger neural network structures and longer individual neural processes.

W-1206

TRANSPLANTATION OF ISOLATED MITOCHONDRIA FROM ADIPOSE-DERIVED STEM CELLS IMPROVES THE LOCOMO-TION OF PARKINSONIAN RATS

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The pathophysiology of Parkinson's disease (PD) is characterized by slowly progressive degeneration of midbrain dopaminergic neurons and accompanied reduction of striatal dopamine release. Traditional therapy using levodopa can ameliorate the bradykinesia but fails to maintain the survival of endangered dopaminergic neurons. Previous studies suggested that the mitochondrial deficits in PD in a main player for triggering the cellular bioenergetic disorder and the neuronal death. Whether providing healthy mitochondria to correct the mitochondria defect can prevent the PD progress is largely unknown. Here, we evaluated the therapeutic efficiency of exogenous mitochondria (1μg and 10μg) from adipose-derived stem cells for the rescue of dopaminergic neurons in the substantia nigra of PD rats. A rat model of PD was established by injection of 6-OHDA into the ascending mesostriatal near the medial forebrain

bundle to remove dopaminergic innervation to the striatum. We demonstrate that transplantation of mitochondria significantly reduced contralateral rotation at 2 and 4 weeks, compared to that of control groups. In addition to the motor functions, histological examinations also revealed that numbers of dopaminergic neurons and the release of dopamine were also significantly recovered. There results suggest that exogenous mitochondria transplantation into PD rats can be a potential therapy for reducing the impairment of locomotion in PD patients.

W-1207

ANGIOGENESIS CONTROLS NEURAL STEM CELL EXPANSION BY REGULATING NICHE OXYGENATION

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The expansion and differentiation of neural stem cells in the developing brain is controlled by their cellular microenvironment, the stem cell niche. Blood vessels are part of this niche, but their functional significance for the regulation of neural stem cell differentiation and the mechanisms involved remain unclear. Here, we report that blood vessel formation coincided in time and space with induction of neural stem cell differentiation in the developing mouse and ferret cerebral cortex. These two processes were functionally linked because selective inhibition of brain angiogenesis in vessel-specific Gpr124 null embryos caused tissue hypoxia and reduced neural stem cell differentiation, favoring their expansion. The hypoxia-inducible factor (HIF)-1α mediated this process, as genetic reduction of HIF-1α was sufficient and required for NSC differentiation. Niche blood vessels regulated NSC differentiation at least in part by providing oxygen, since exposure to increased ambient oxygen levels rescued neural stem cell differentiation in Gpr124 null embryos, lacking functional blood vessels. Our findings establish a novel mechanism of neural stem cell differentiation by blood vessel-mediated niche oxygenation to safeguard proper brain development.

W-1208

NPC-MEDIATED MATURATION OF OPCS FOR THE TREATMENT OF MULTIPLE SCLEROSIS

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Multiple Sclerosis (MS) affects more than 2.7 million people worldwide and is the most common autoimmune disorder affecting the central nervous system (CNS). The symptoms of MS result from damage to myelin sheaths of CNS nerves, interfering with signal transmission. Medications help manage the symptoms of MS but there is currently no cure for this progressive disease. We have reported that transplantation of a specific well-defined population of human ESC- or iPSC-derived neural precursor cells (hNPCs) into the spinal cords of a virally-induced mouse model of MS resulted in a significant improvement in their clinical disease, as well as decreased T-cell and macrophage infiltration and remyelination of nerve fibers. Since the hNPCs were only detectable for 8 days post-transplantation, the majority of improvement occurred after this time point, suggesting that factors secreted by hNPCs may be critical in mediating the long-term improvement of MS-like symptoms observed in this model. We are working toward identification and characterization of the secreted factor(s) that enhance remyelination in order to assess the potential use of such factor(s) in future MS therapies. Our pilot studies have shown enhanced in vitro oligodendrocyte precursor cell (OPC) maturation using either conditioned medium from hNPC cultures or in cultures in which hNPCs are separated from the OPCs by transwells. Gene expression analysis of hNPCs identified transcripts for several secreted proteins as candidate OPC maturation factors, including secreted phosphoprotein 1 (SPP1) and thrombospondin 3 (THBS3). Further experiments will focus on fractionating conditioned medium into several size ranges to identify the fraction(s) that facilitate OPC maturation. This subset will then be subjected to mass spectroscopy to identify the proteins. Identified proteins will tested in vitro to assess their ability to enhance OPC maturation, and delivery parameters such as concentration and timing will be optimized.

W-1209

DIFFERENT MEDIAL GANGLIONIC EMINENCE-GRAFTED CELL PROTOCOLS RESULTED IN DISTINCT PATTERNS OF ANTICONVULSIVE EFFECT AND INTERNEURONAL DIFFERENTIATION

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Neural stem cells from medial ganglionic eminence (MGE) have been tested to control seizure activity in animal models of epilepsy due to their capacity of differentiating in inhibitory neurons. Different methods have been proposed to prepare these cells for transplantation in order to better control their proliferation and differentiation. These cells can be dissected from MGE and immediately transplanted as "fresh cells" or cultivated and transplanted as dissociated neurospheres. Here we compared these two methods to investigate their anticonvulsive potential in vivo. MGE fresh cells or 7 days cultured neurospheres were obtained from GFP rat embryos (E14) and transplanted into hippocampal dentate gyrus (DG) of epileptic rats 8 days after pilocarpine induced-status epilepticus. A total of 5x104 cells as fresh cells (MGE-f, n=8) or dissociated cells from neurospheres (MGE-n, n=12) were injected into the DG. Epileptic rats were observed for 540h to assess the frequency of spontaneous seizures (SRS) and their brains processed for immunofluorescence for GFP, GFAP and interneuronal markers. Our data showed a significant reduction in the frequency of seizures type V ($p<0.05$) when compared MGE-f group with non-graffed group (control, n=10). No difference in SRS type IV was found when MGE-f group and MGE-n group were compared with controls. We detected co-localization of GFP+ cells with interneuronal markers in the hippocampus of both MGE-grafted epileptic animals. We also observed a large number of astrocytes in the hippocampus of MGE-n animals when compared with MGE-f group. Our data suggest

that MGE progenitor cells, when transplanted as fresh cells, were able to attenuate the seizure frequency and preserved their original identity of inhibitory interneurons, when compared to cells cultured as neurospheres. These results might suggest that modifications in cell culture should be further investigated in order to obtain a more protective effect in epilepsy.

W-1210

HIPSC DERIVED NEURONS AND ASTROCYTES FOR STUDIES OF CELLULAR INTERACTIONS RELATED TO ALZHEIMER'S DISEASE

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The progress of neurodegenerative disorders is an intricate interplay between neural cells. The interaction between neurons and astrocytes is being increasingly recognized as significant for the development of for example Alzheimer's disease. To study these interactions in human cells we are deriving patient specific neurons and astrocytes. The cells are studied in conventional co-cultures but also in 3D cell culture systems to increase the in vivo representation of the model, thus making it more biological relevant. There is today no efficient and reproducible differentiation protocol for astrocytes from pluripotent cells. We aim to create an efficient, scalable model that is applicable in screening applications. This demands significant shortening of differentiation timelines, preferably by isolation of cell specific, cryo-preservable progenitors through a highly robust and reproducible protocol. The derivation of neuroepithelial stem (NES) cells from hiPSC has created such a system for neurons. Optimizing the derivation of human astrocytes will lead to the direct application of a co-culture model. Moreover we are applying CRISPR-Cas9 to generate cell type specific reporter lines and KO-models in order to directly observed neuron-astrocyte interaction in screening platforms together with an increased simplicity of evaluating 3D-models. Focusing on Alzheimer's disease, CRISPR-Cas9 will also provide the possibility to introduce and rescue gene specific mutations in AD. Virtually creating perfect control models where one can evaluate diseased neurons with healthy astrocyte derived from the same patient. With this model at hand, we can start asking questions about if and how healthy astrocytes may rescue patient neurons from death?

W-1211

TUMORIGENICITY STUDIES OF IPS CELL-DERIVED DOPAMINERGIC PROGENITORS FOR CLINICAL APPLICATION OF PARKINSON'S DISEASE

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Induced pluripotent stem cells (iPSCs) have gained wide attention in the field of regenerative medicine. Their clinical application for a number of diseases in the subject of research because they can be cultured on a large scale due to their ability for self-renewal and they have the potential for pluripotent differentiation into various somatic cells. However, risks for progression to tumor and/or ectopic tissue formation are associated with retention of undifferentiated iPSCs in the transplant region. Thus, assessment and management of transplanted cells is accordingly considered to be of crucial importance in any cell therapy utilizing iPSCs. We have already established a protocol for the differentiation of iPSC-derived dopamine neurons, which includes cell sorting to purify target cells, with the aim of conducting clinical trials of an iPSC-derived dopamine neuron graft therapy for Parkinson's disease. We have previously reported on transplants of human embryo stem cell (hESC)-derived dopamine neurons into immunodeficient mice and Parkinson's disease model cynomolgus monkeys under the same differentiation protocol and the absence of any subsequent proliferation of transplanted cells. We are conducting a long-term tumorigenicity study using iPSC-derived dopamine neurons, and we will outline this study and report on its results at the annual meeting.

W-1212

P53 MAINTAINS SELF-RENEWAL OF HUMAN NEUROEPITHELIAL STEM CELLS BY CONTROLLING CELL POLARITY, PLOIDY, AND METABOLIC PROCESSES

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Neuroepithelial stem (NES) cells are a robust cell line that can be derived from human induced pluripotent stem cells or ES cells. NES cells maintain their stem cell potential upon long term passaging in vitro and readily differentiate into neurons and glia upon removal of growth factors, they therefore represent a good model for studying both neural stem cell self-renewal processes as well as neural differentiation. The tumor suppressor p53 is the most commonly mutated gene in adult cancers but has also been shown to play a role during murine neurogenesis. However, the potential role of p53 as key regulator for maintaining the stem cell pool in the human brain still remains elusive. Using shRNA to knock-down (KD) p53 in NES cells we found that p53 KD initially leads to an increase in proliferation but this rapidly declines after a few passages due to cell cycle arrest in the G2/M phase and an accumulation of multinucleated and polyploid cells. We observed an amplification of centrosomes in p53 KD cells resulting in an inability to form a proper mitotic spindle during cell division. This demonstrates the importance of p53 in maintaining NES cell self-renewal capacity through controlling the correct ploidy. Normally NES cells organize themselves into neural rosettes with an apical/basal orientation, this is thought to be important for maintaining their self-renewal capacity. Under normal conditions ZO-1, a junction molecule involved in maintaining NES polarity, is expressed in the apical side of NES cells. However, upon p53 down-regulation, ZO-1 is uniformly distributed indicating loss of cell polarity. In addition, the eventual exhaustion of p53 KD NES cells proliferative capacity leads us to question the effect p53 KD has on energy production and consumption in NES cells. Preliminary results show a higher glycolytic capacity in NES cells upon p53 KD. Taken together, we demonstrate that p53 is an important regulator of self-renewal and maintenance of human neural stem cells by controlling cell polarity and ploidy. Furthermore, we show that p53 modulates metabolic pathways that may impede both proliferation and differentiation processes in human neural stem cells.

W-1213

MODELING SUSCEPTIBILITY TO NEURODEGENERATIVE PROCESSES ASSOCIATED WITH AMYOTROPHIC LATERAL SCLEROSIS USING HUMAN PLURIPOTENT STEM CELL-DERIVED NEURONS

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Amyotrophic lateral sclerosis (ALS) is characterized by the degenerative loss of both subcerebral cortical projection neurons (CPNs) and their axonal targets, the spinal cord motor neurons (sMNs). Disease-related functional changes in axonal transport and cell death have been detected in cultured mouse embryonic neurons, whereas the first electrophysiological changes were detected in vivo soon after birth. In addition, glutamate-induced excitotoxicity associated with hyperexcitability can incite neurodegeneration through activation of calcium dependent enzymatic pathways. Studies on mice harboring the mutant SOD1G93A human transgene have shown that cell death in ALS is non-cell autonomous, as the astrocytes and microglia that surround motor neurons contribute to disease onset and progression. This neuroinflammatory response was shown to occur in the motor cortices of mutant SOD1G93A mice and humans harboring mutated SOD1. These studies suggest that human CPNs would be susceptible to the neurotoxic effects of glia harboring mutated SOD1. To determine why particular neurons are susceptible to ALS-associated neurodegeneration, we generated human pluripotent stem cell-derived CPNs and sMNs from healthy individuals and co-cultured them with glia derived from transgenic mice that contain either the wild type (WT) SOD1 gene or mutant (SOD1G93A) SOD1 gene. As cortical interneurons do not appear to degenerate in ALS, we also co-cultured human ES-derived cortical interneurons as our control. Over a defined temporal window, we measured neuronal survival, soma size, neurite length, and the neuroinflammation within each neuronal population. In addition, we performed multi-electrode recordings to measure differences in spontaneous neuronal activity. While the molecular and cellular mechanisms that precede neurodegeneration are still being determined, several aspects including the unfolded protein response, mitochondrial swelling, and enhanced ER stress should be observable upon determining the window of pathological symptom initiation in our in vitro system. Thus, our in vitro model for ALS disease progression will be invaluable for the study neurodegenerative susceptibility in particular neuronal populations.

W-1214

AGE-DEPENDENT IMPAIRMENT OF NUCLEO-CYTOPLASMIC COMPARTMENTALIZATION IN AGE-EQUIVALENT INDUCED NEURONS

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Human aging is the main risk factor for several diseases, including neurodegenerative disorders. To better understand age-related pathogenesis, the in vitro generation of human neurons for modeling is an attractive approach. We generated iPSCs from a broad range of aged donors and found that they erased transcriptomic aging signatures. Alternatively, direct conversion into induced neurons (iNs) preserved aging signatures. Importantly, iNs showed an age-dependent decrease in the nuclear transport receptor RanBP17, which also becomes down-regulated in aging human fibroblasts and brain samples. Using a reporter system for nucleo-cytoplasmic compartmentalization (NCC), we detected an age-dependent loss of NCC in old fibroblasts and neurons; reduction of RanBP17 impaired NCC in young cells and iPSC rejuvenation restored NCC in old cells. Our data demonstrate that directly converted iNs retain important aging signatures, thus allowing for modeling aging in vitro, and we have identified impaired NCC as an important factor involved in human aging.

W-1215

ANALYSIS OF HIPSC DERIVED NEURONS REVEALS A NOVEL CHOLESTEROL TRAFFICKING PATHWAY IN NIEMANN PICK TYPE C1 (NPC1)

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Niemann Pick type C1 (NPC1) is a rare pediatric dementia caused by mutations of the lysosomal cholesterol transporter NPC1. NPC1 has no cure or treatment, and affected patients die in their childhood. Although rare, the disease imposes a burden on patients and families that is disproportionate to its relative infrequency. Furthermore, NPC1 shares clinical and histologic features with Alzheimer's disease, a more prevalent public health problem, suggesting common mechanisms of onset and progression. Our prior work showed that NPC1 knockdown neurons have disrupted autophagy that leads to abnormal mitochondrial turnover. However, the specific mechanism that triggers autophagy activation in NPC1 neurons is not known. To test the hypothesis that autophagy is activated in NPC1 neurons as a backup mechanism to redistribute lysosomal cholesterol, we generated control and NPC1 patient-specific human induced pluripotent stem cell (hIPSC) lines and derived neuronal cultures by a standard differentiation protocol. We found that cholesterol starvation is sufficient to induce autophagy in human neurons, and that autophagy redistributes lysosomal cholesterol, albeit at a lower efficiency, but enough to protect short-term neuronal viability in the absence of NPC1 function. Our data also suggests that NPC1L1, a cholesterol transporter with high homology to NPC1, has a previously unrecognized role in autophagy-mediated lysosomal cholesterol trafficking. NPC1L1 is expressed in human cultured neurons and brain tissue, and its inhibition with the widely used compound ezetimibe aggravates cholesterol accumulation in NPC1 neurons. However, our data also show that persistently activated autophagy induces mitochondrial dysfunction and reactive oxygen stress which are likely to contribute to the slow neuronal failure observed in NPC1. Our in-vitro model agrees with the natural history of NPC1, as patients survive through development and for a few years before suffering slowly progressive neurodegeneration. Our data reveal a novel role for autophagy in intracellular cholesterol trafficking. Furthermore, our approach highlights the importance of generating careful mechanistic data to guide therapeutic development and defines a new drug screening strategy for NPC1 and related neurodegenerative diseases.

W-1216

SUFU-GLI-SOX10 IN ENTERIC NERVOUS SYSTEM DEVELOPMENT AND HIRSCHSPRUNG DISEASE

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Hirschsprung (HSCR) disease is defined by deficit of enteric neurons, which are derived from neural crest cells (NCC). Aberrant glial differentiation of NCCs represents a possible cause of HSCR, but the gene machinery involved remains unclear. Here we identify several mutations in GLI1-3 in HSCR patients that all lead to increased GLI transcriptional activity on Sox10 expression. We show Sufu-Gli expression is dynamically regulated during NCC differentiation and correlates with the segregation into neurons and glia. Importantly, Sufu-Gli and Sox10 form a regulatory loop to control neuronal versus glial lineage differentiation and migration of NCCs. Aberrantly high Gli activity caused by loss of Sufu disturbs the reciprocal balance of Sufu-Gli and Sox10, resulting in disrupted enteric NCC chain migration, defective axonal fasciculation and intestinal hypoganglionosis. Collectively, GLI mutations were identified for the first time in HSCR patients, and perturbed Sufu-Gli-Sox10 regulatory nexus contributes to HSCR pathogenesis in mouse and human.

W-1217

NEURONS DERIVED FROM HESCS OVEREXPRESSING MUTANT PRESENILIN-1 AS ALZHEIMER'S DISEASE MODELS

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Disease models derived from human pluripotent stem cells are highly useful for analysis of disease mechanisms and exploration of new drugs. To create cellular Alzheimer disease (AD) models, we generated hESCs overexpressing mutant Presenilin-1 (mutant PS1-hESCs) by using the site-specific gene integration system we developed previously. The mutant PS1-ESCs normally differentiated to neurons, while the neurons overexpressing mutant PS1 (mutant PS1-neurons) showed AD phenotypes such as increase of Aβ42 production and decrease of excitatory postsynaptic currents. Therefore, our AD models could become useful tools for AD research. In this study, to examine responsiveness of the mutant PS1-neurons to drugs that inhibit Aβ production or secretions, we performed the drug screening using the mutant PS1-neurons with chemical libraries composed of known chemicals. As the result of the screening, 52 chemicals, including all of β- and γ-secretase inhibitors, were detected out of 2,408 chemicals in chemical libraries. Also, the mutant PS1-neurons detected additional chemicals, which have been reported as inhibitors of Aβ production such as HMG-CoA reductase inhibitors and estrogen receptor antagonists. These results suggest that our AD model of the mutant PS1-neurons can be used in drug discovery for AD.

W-1218

A ROLE FOR NOTCH ACTIVITY IN INTERPRETATION OF SONIC HEDGEHOG SIGNALING AND ASSIGNMENT OF NEURAL PROGENITOR FATES

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Throughout the developing nervous system, neural stem and progenitor cells give rise to diverse classes of neurons and glial cells in a spatially and temporally coordinated manner. In the ventral spinal cord, much of this diversity emerges through the morphogen actions of Sonic hedgehog (Shh). Interpretation of the Shh gradient depends on both the amount of ligand and duration of exposure, but the mechanisms permitting prolonged responses to Shh are not well understood. We demonstrate that Notch signaling plays an essential role in this process, enabling neural progenitors to attain sufficiently high levels of Shh pathway activity needed to direct the ventral-most cell fates. Notch activity regulates subcellular localization of the Shh receptor Patched1, gating the translocation of the key effector Smoothened to primary cilia and its downstream signaling activities. These data reveal an unexpected role for Notch shaping the interpretation of the Shh morphogen gradient and influencing cell fate determination.

W-1219

PLEKHG6 REGULATES NEUROPROGENITOR AND POST-MITOTIC NEURONS WITHIN THE DEVELOPING MOUSE BRAIN

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Periventricular heterotopia (PH) is a cortical malformation charaterised by a failure for neurons to populate the outer cortex of the brain, forcing them to adopt ectopic positions along their sites of origin – the lateral ventricles. The cellular and molecular mechanisms involved in the pathogenesis of PH remain, to a large extent, unclear. With advances in next-generation sequencing taking on new dimensions it is now possible to identify rare loss-of-function mutations, or gene-knockouts, within clinical cohorts. Such rare events are overrepresented in autosomal recessive conditions and offer a unique opportunity to scrutinise such loci identified in large clinical cohorts with defined disease states. Here we sequenced all coding regions of the genome (the exome) in 47 unrelated patients with PH, and their unaffected parents. Restricting our analysis to novel biallelic loss-of-function events we identified one gene, PH Domain-Containing Family G Member 6 (PLEKHG6), in a single case. Downregulation of Plekhg6 within the developing mouse

neuroepithelium lead to a transient increase in neural stem cell differentiation and altered post-mitotic neuronal migration, disrupting the distribution of neurons within the developing cortex. Consistent with the reported Rho GTPase function of this gene, the altered neuronal distribution was remedied by introducing a spontaneously activated ("fast-cycling") mutant form of RhoA. PLEKHG6 contains two differentially expressed isoforms. The human brain expresses both forms, while that of the murine only allows for the detection of one. Furthermore, no orthologue of the second isoform is present in any species outside that of the primate lineages analysed. Together, these findings implicate Plekhg6 as an upstream regulator of RhoA for the modulation of both neuroprogenitor and post-mitotic neuronal activity within the developing mouse brain. Studies focusing on the differences between the human and mouse forms of this gene could implicate evolutionary distinct roles for PLEKHG6 within the developing primate brain.

W-1220

USING CHEMICAL COMPOUNDS TO ADVANCE MATURITY OF NEURAL STEM CELL-DERIVED CULTURES

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Human neural stem cells mature at rates similar to human gestation, requiring much longer time in culture than other model systems. This is a serious limitation of human neural stem cells and often results in studying immature human fetal stage cells. We are taking a bioinformatic approach to identify compounds that may speed up maturity. We will test our prediction by adding chemical compounds to neural stem cells to increase neuron growth and maturity. To maximize neurogenesis, we first tested the effects under different culture conditions for neural differentiation. We cultured neural progenitor cells in two different differentiation medias; media A (Neurobasal A, B27, GlutaMAX, BDNF, NT3, Retinoic acid, forskolin, KCl) and media B (Media A +DMEM/F12, +N2, +cAMP). A trend was observed for a higher percentage of neurons generated in media B after 1 week post-differentiation (42% vs 38%; p-value=0.035; n=20 fields each). This demonstrates exogenous culture conditions can have a detectable impact on neurogenesis potential. We then sought to expand this approach by identifying chemical compounds that may increase human neurogenesis. In vivo standard data was used to identify genes with lower expression in the ventricular zone and subventricular zone (progenitors) compared to the inner and outer cortical plate (neurons) during fetal development and models where eigengene are related to maturity. We used Lincscloud, a genome-wide transcriptional expression dataset from cultured human neural stem cells which finds drugs that influence the neural stem cells expression profile similar to our in vivo standard. For consistency, we intersected five query results and found 53 common drugs. Seventeen compounds were chosen to add to the neural progenitor cell culture by identifying drugs within biological categories. The maturity of top neurogenesis-inducing compounds will be judged by measuring gene expression and comparing transcriptomes to in vivo standards using our CoNTExt pipeline. Improving in vitro neural maturation is key to help more effectively model neuropsychiatric diseases.

W-1221

THE ANTI-INFLAMMATORY MICROGLIAL SECRETEOME PROMOTED NEURAL STEM CELL MIGRATION AND REDUCED ASTROGLIOSIS

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The existence of neural stem cells (NSCs) in adult mammalian brain, including humans, offers a possibility for endogenous repair after brain injury. Brain injury is often associated with neuro-inflammation. Microglia are the resident immune cells in the brain, and is key players in neuro-inflammation. Upon activation, microglia can acquire pro-inflammatory or anti-inflammatory properties. Here, we wanted to investigate the effect of microglial subtypes on NSCs. NSCs were grown in vitro in conditioned media collected form either non-stimulated microglia, or microglia stimulated with lipopolysaccharide (LPS; pro-inflammatory) or with interleukin-4 (IL-4 anti-inflammatory). The survival, proliferation, migration, and differentiation of the NSCs were subsequently analyzed using biochemical assays and immunofluorescence. We found that NSCs kept in conditioned medium from the anti-inflammatory subtype had the highest survival rate, increased migration, and lower tendency toward astrocytic differentiation. No alterations in proliferation, neuronal or oligodendrocytic differentiation were seen when NSCs were exposed to the different conditioned media. Our results show that microglial subtypes regulate NSCs differently. Hence, spatial and temporal activation of different microglial subtypes after injury require consideration, for example when timing NSCs engraftment.

W-1222

FINE-TUNING OF WNT/BETA-CATENIN SIGNALING IN MDDA NEURON DEVELOPMENT AND ITS POTENTIAL IMPORTANCE FOR CELL REPLACEMENT STRATEGIES

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In Parkinson's Disease (PD), meso-diencephalic dopaminergic (mdDA) neurons are degenerating and so far there is no cure. One approach for a successful treatment could be cell replacement therapies. It is therefore of importance to understand the underlying molecular mechanisms during embryonic development of mdDA neurons. WNT/β-catenin signaling is crucial for the induction of mdDA precursors and can both promote and inhibit their differentiation depending on cell context and the level of signaling. Therefore, our hypothesis was that a fine tuning of WNT/β-catenin signaling is required for the correct differentiation of mdDA neurons. Here we show the expression of R-spondin 2 (Rspo2), a WNT agonist, to be confined to the ventral midline of the developing mouse midbrain. This region gives rise to mdDA progenitors suggesting a role of Rspo2 in mdDA neuron development. Treatment of primary cells with increasing concentrations of RSPO2 did not affect their proliferation but inhibited the differentiation of mdDA progenitors into mature Tyrosine hydroxylase (TH) and Paired-like homeodomain transcription factor 3 (PITX3) double positive mdDA neurons. Downstream of WNT signaling the transcription factor Pitx3, important for terminal differentiation of mdDA neurons, is

activated by the WNT target LIM homeobox transcription factor 1 alpha (LMX1A). Here we show an additional direct regulation of Pitx3 by WNT/β-catenin signaling. Interestingly, high levels of WNT signaling had an inhibiting effect on the activation of Pitx3 by LMX1A, whereas a direct activation of the Pitx3 promoter was observed at low levels of WNT/β-catenin signaling. In summary, these results suggest that there is a switch in the embryonic development of mdDA neurons requiring higher levels of WNT/β-catenin signaling for the induction of mdDA neurons and lower levels of this signaling pathway for the terminal differentiation. Modulation of WNT/β-catenin signaling could therefore be a promising tool for cell replacement therapies where strong signaling supports the generation of a mdDA progenitor pool and a reduction of WNT/β-catenin signaling allows terminal differentiation.

W-1223

GAP JUNCTION COUPLING AND CONNEXIN EXPRESSION IN NEURAL PRECURSOR CELLS

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Gap junctions formed by connexins (Cxs) enable direct cytoplasmic communication between neighboring cells. We have previously shown that transplantation of adult neural precursor cells (NPCs) rescued the ataxic phenotype in a spinocerebellar ataxia type 1 mouse model. The rescue was contact dependent and associated with Cx43 expression between grafted NPCs and rescued Purkinje cells. Here we investigated the mRNA and protein expression of different Cxs (Cx26, 36, 43 and 45) in NPCs derived from the Sub Ventricular Zone of adult FVB/N mice. Differentiation of the NPCs increased expression of in particular Cx43. Dye transfer demonstrated the presence of functional gap junctions in both differentiated and undifferentiated cultures. NPCs formed Cx43 gap junctions to ex vivo cerebellar Purkinje cells within 5 days after engraftment. Live time-lapse recordings, using the calcium sensitive dye Fluo-4 AM, revealed spontaneous calcium oscillations in both undifferentiated and differentiated individual NPCs. Cross-correlation signal analysis and graph theory was applied to the recordings. This revealed the presence of intercellular Ca2+ activity and confirmed that individual NPCs are part of a network of interconnected cells. The connectivity and the consolidation of a scale-free and small world network increased during differentiation of NPCs. Addition of the gap junction blockers Carbenoxolone or 18-α-glycyrrhetinic acid inhibited connectivity but did not abolish spontaneous calcium oscillations in all of the NPCs. We thus show that the gap junctions are essential for the establishment of functional networks. Somewhat unexpected, neither the rate of proliferation of the cultured NPCs, nor their ability to differentiate into an early neuronal phenotype (Tuj-1), was affected by the presence of gap junction blockers during the first week. However, the formation of gap junction dependent cellular networks increased the NPCs survival by reducing apoptosis. Intercellular communication between grafted NPCs and host Purkinje cells via a natural Cx43 mediated pathway thus enable them to rescue endangered host cells.

W-1224

CHARACTERIZATION OF NRXN1 MEDIATED SYNAPTIC DEVELOPMENT IN PATIENT DERIVED NEURAL STEM CELLS

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Induced pluripotent stem (iPS) cells, derived from autism patients and healthy individual, provide a powerful tool to investigate pathological mechanisms of Autism Spectrum Disorders (ASD) and bring a new perspective on the understanding of this neurodevelopmental disorder. Previously, heterozygous partial deletions in the neurexin 1 gene (NRXN1, chromosome region 2p16.3) have been observed in ASD patients. The NRXN1 are presynaptic adhesion transmembrane proteins that bridge between pre-and postsynaptic neurons, modulate synaptic functions which ultimately involve in neuronal networks and communications. NRXN1 play an important role in synapse formation, maintenance, and synaptic differentiation. ASD pathogenesis is believed to result from dysfunctions in the balance between synapse transmissions caused by mutation in the NRXN1 genes but little is known about the precise role of NRX1 in synapse formations and neurite development. In order to characterize NRX1 function we performed these studies in human context by using iPS cell derived from autism patients and healthy individuals (iPS model established by our group). Using this model system, we explored in more detail the mechanism of regulation of NRXN1 and interacting partners linked to ASD by evaluating the effect of NRXN1 mutation on synaptogenesis and neurite outgrowths. Genome-wide expression analysis was employed to compare the transcriptome of autism patients and healthy individuals. Immunofluorescence data provided the evidence that NRX1 expressed less in neurons from autism patients, differentiated (D28) neurons were confirmed by beta-tubulin III and doublecortin staining. Impaired neurite outgrowth and loss of NRX1 alpha and neuroligin (NLG) gene expressions were shown in autism patients neurons compared to those from healthy individuals. These results strongly suggest the possibility that loss of expression levels of NRX1 genes might be involved in the pathophysiology of ASD.

W-1225

THERAPEUTIC UTILIZATION OF STEM CELLS, HYDROGELS AND SMALL MOLECULES FOR SPINAL CORD INJURY

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In the United States, an estimated 6 million individuals live with paralysis, 23% of these incidents are due to spinal cord injury. Despite many attempts, a technique to repair spinal cord injury still remains elusive. The main obstacle being that axons fail to regenerate when transected, due to molecular inhibition and barriers produced during the injury response. Recent studies indicate that engraftment of stem cells may be a potential therapy for spinal cord injury. However, there are many limitations to stem cell based therapeutics,

such as ensuring cell survival and proper differentiation into the desired mature cell types. To address these shortcomings, we are collaborating with the Deming lab and utilizing a bioengineered amphiphilic diblock copolypeptide hydrogel (DCH) to deliver stem cells into the spinal cord injury site. It has been previously shown that DCH has rheological properties within the spectrum of central nervous system (CNS) tissue and induces minimal inflammation. An advantage of DCH is the composition can be adjusted to facilitate a sustained delivery of small molecules, thereby allowing us to pharmacologically manipulate the engrafted cells in vivo. X5050 is a small molecule that inhibits the RE1 silencing transcription factor and drives drive the expression of neuronal genes. We hypothesize that DCH along with X5050 will aid in cell engraftment and neuronal differentiation, thereby developing a strategy that can be used to promote repair to spinal cord injury. This study is supported by the CIRM UCLA/CSUN Bridges grant TB1-01183.

W-1226

IND-ENABLING STUDIES DEMONSTRATE SAFETY AND THERAPEUTIC POTENTIAL OF HUMAN PARTHENOGENETIC DERIVED NEURAL STEM CELLS IN PARKINSON'S DISEASE

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Cell based therapies hold great promise in Parkinson's disease (PD) because clinical studies have shown that grafted fetal neural tissue can achieve considerable biochemical and motor improvements decades after implantation. Unfortunately the source of fetal tissue is limited and clinically impractical. Human parthenogenetic stem cells offer a good alternative because they can be expanded in vitro to generate an unlimited supply of neural tissue. Preliminary data has shown that human parthenogenetic stem cell derived neural stem cells (hpNSCs) are safe and effective in treating PD in animal models. Here we present IND-enabling data of four preclinical studies testing the safety and therapeutic potential of hpNSCs in rodent and non-human primates. hpNSCs were manufactured under cGMP conditions and tested in pharmacology, toxicology, biodistribution, tumorigenicity and efficacy studies. A battery of assays including clinical observation, behavioral testing, hematology, clinical chemistries, necropsy, histopathology, biodistribution by qPCR, immunohistochemistry, and dopamine analysis were performed. Results of these animal studies demonstrate that nigrostriatal administration of hpNSCs is safe and well tolerated. hpNSCs do not induce dyskinesia, systemic toxicity, or host immune rejection. Tissue analysis showed no signs of ectopic tissue, tumors, hyperproliferation, or biodistribution of cells outside the central nervous system. hpNSCs may have provided neuroprotection and neurotrophic support to the host nigrostriatal system as manifested by increased dopamine levels and improved motor function. These results are supportive of an IND application with the FDA of human parthenogenetic stem cell derived neural stem cells for the treatment of Parkinson's disease.

W-1227

DYSTROGLYCAN REGULATES NEURAL STEM CELL ORGANIZATION, PROLIFERATION, AND DIFFERENTIATION IN THE DEVELOPING SUBVENTRICULAR ZONE

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The subventricular zones (SVZ) of the lateral ventricles are the largest germinal niches in the adult mammalian brain, generating much of the cortical plate during embryogenesis, the majority of forebrain glia during postnatal development, and continuing to produce both neurons and glia into adulthood. Perinatally, SVZ neural stem cells (NSCs) termed radial glia transform into either adult NSCs or multicilliated epithelial-like ependymal cells. While the extracellular matrix (ECM) is a critical regulator of stem cell niche organization and function throughout the body, its role in the development of the SVZ niche remains virtually unknown. We now report that during postnatal development the ECM of the VZ/SVZ becomes reorganized into laminin-rich ECM "hubs" at NSC/ependymal cell junctions near the ventricle surface, a process that appears to help establish adult niche cellular spatial configuration. We furthermore found that the ECM receptor dystroglycan is critical for this regulated ECM reorganization during SVZ niche development. Dystroglycan loss leads to ECM disruptions that persist into young adulthood, such that dystroglycan-deficient mice have many fewer VZ/SVZ ECM hubs. In addition to its role in organizing niche ECM, we found that neural lineage-specific dystroglycan loss leads to distinct deficits in SVZ niche maturation and function. Here, dystroglycan loss dysregulates SVZ NSC proliferation and production of oligodendrocyte precursor cells during early postnatal development, as well as the ability of NSCs to mature into niche ependymal cells during the establishment of the adult niche configuration. Preliminary experiments indicate that dystroglycan may exert its effects on SVZ stem cells, at least in part, through the regulation of Notch signaling, thereby suppressing oligodendroglial fates and promoting NSC maturation into ependymal cells. Finally, we found that ependymal cells substantially upregulate dystroglycan levels as they mature, and that dystroglycan-deficient ependymal cells have profound alterations in planar cell polarity, including defective basal body patch positioning and size, suggesting that dystroglycan is a key regulator of ependymal cell maturation.

W-1228

HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURAL STEM CELL GRAFTING EARLY AFTER HIPPOCAMPUS INJURY EASES MEMORY AND MOOD IMPAIRMENTS

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Hippocampus, important for functions such as memory and mood, is prone for acquiring dysfunction after brain insults. Even a partial injury to the hippocampus can lead to memory and mood impairments. We ascertained the usefulness of human induced pluripotent stem cell (hiPSC)-derived neural stem cell (NSC) grafting for preventing deficiencies in memory and mood function triggered by a partial unilateral hippocampal injury. A partial injury to the

right hippocampus was induced in young adult F344 rats through kainic acid (KA; 0.25 μg in 1 μl of saline) administration into the right lateral ventricle. Seven days later, a group of rats received grafts of hiPSC-derived NSCs into 3 sites of the injured hippocampus (~100,000 live cells/site) and a second group of rats received sham-grafting surgery. Animals receiving grafting or sham-grafting surgery were also treated daily with cyclosporine. Three-four months later, animals were tested for memory and mood function using several behavioral tests. Characterization through a water maze test (WMT), a novel object recognition test (NORT) and a forced swim test (FST) revealed impairments in spatial and recognition memories and increased depressive-like behavior in animals that received sham-grating surgery after hippocampus injury. In contrast, animals that received hiPSC-derived NSC grafts into the hippocampus after injury displayed ability for spatial memory retrieval in WMT and normal recognition memory function in NORT. In addition, animals receiving NSC grafts after hippocampus injury displayed decreased immobility time in FST. Analyses of the grafted hippocampus using human specific nuclear antigen revealed excellent spread and survival of cells derived from grafts. Dual immunofluorescence analyses revealed differentiation of a larger fraction (>80%) of graft-derived cells into NeuN+/TuJ-1+neurons. Smaller fractions graft-derived cells expressed GABA (a marker of inhibitor neurons), S-100 beta (a marker of mature astrocytes) and NG2 (a marker of oligodendrocyte progenitors). Analyses with Ki-67 revealed proliferative activity in only a minority of graft-derived cells (<1%). The results underscore that, hiPSC-derived NSC grafting into the hippocampus after injury is efficacious for preventing injury-induced memory and mood dysfunction.

W-1229

NURR1 COOPERATES WITH TFII-I TO REGULATE TYROSINE HYDROXYLASE GENE TRANSCRIPTION DURING DOPAMINERGIC NEUROGENESIS

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Nurr1 plays an important role in midbrain dopaminergic (DA) neurogenesis. Our previous study demonstrated that Nurr1 preferentially binds to NBRE-A site in promoter to modulate transcription of human tyrosine hydroxylase (TH) genes. However, Nurr1 actively represses hTH transcription in DA precursor cells, while it activates hTH expression in DA neuronal cells. To investigate the molecular mechanisms by which these cofactors are involved in transcriptional regulation, we performed DNA pull-down assay and mass spectrometry and identified one Nurr1 interacting protein, Williams-Beuren syndrome transcription factor TFII-I. Interestingly, TFII-I binding sites, Inr and E-box, are localized in proximity to NBRE-A site. The mutation of these sites modulated the TH activity. Moreover, Nurr1 and TFII-I interaction and the subcellular localization of TFII-I were shown to regulate the TH gene expression. Our findings suggest that Nurr1 and TFII-I complicated complex significantly play an important role in TH regulation during DA neuronal differentiation.

W-1230

SURVIVALL ANALYSIS OF HUMAN IN VITRO-DERIVED NEURONS USING NEW LIVE CELL EXTENDED TIME-LAPSE IMAGING TECHNOLOGY

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Long-term live cell imaging of in vitro derived neurons from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) has the potential to be a novel platform for drug discovery in neurodegenerative disease. Time-lapse analysis of neurons during differentiation, survival, stress response and/or death has several advantages, including the ability to identify early morphological changes that accompany disease-associated processes. Further, single cell tracking can compensate for the morphological heterogeneity inherent in stem cell-derived cell cultures. We have conducted detailed long-term analysis of individual stem cell-derived motor neurons (MNs) from early times after their differentiation until their death. Experiments were carried out using a Nikon BioStation CT, whose robust cell tracking capabilities were utilized to follow individual MNs. Imaging analysis algorithms accurately tracked key attributes such as cell body size, neurite number and neurite length. We used these imaging tools to study MNs subjected to two different stressors that mimic disease-like circumstances: neurotrophic factor (TF) withdrawal and treatment with the proteasome inhibitor MG132. By analyzing many MNs, we have defined a new, morphological predictor for cell death that involves careful measurement of neuritic changes. This analysis algorithm determines the "healthy time (HT)" for each MN, and this measurement reveals the kinetics of survival responses that are not apparent with endpoint analysis. Quantifying disrupted neurites as an index of disease onset is advantageous since these changes occur early in the death process and appear to provide greater sensitivity than that seen by counting surviving cells. We are currently expanding these studies to diseased MNs derived from Spinal Muscular Atrophy and Amyotrophic Lateral Sclerosis (ALS) patient samples. We are also initiating studies to examine other disease-relevant neuron populations, such as cortical neurons (affected in autism and Alzheimer's Disease) and dopaminergic neurons (affected in Parkinson'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.

W-1231

ANTIDEPRESSANT TOXICITY EVALUATED IN NEURONAL CELLS

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A number of in vitro toxicity screening tests are now routinely performed at early stage of drug discovery to identify potential organ toxicity of pharmacologically active molecules. Some of

them have been accepted as useful tools to eliminate compounds with potential toxicity while others are still under development. Neurotoxicity is adverse effect on the structure or function of the central and/or peripheral nervous systems, and represents one of the troublesome side effects of drugs or drug candidate molecules. We have selected a series of tricyclic antidepressant drugs to assess the correlation between in vitro toxicity tests and their adverse effects reported in clinics. Various neuronal cell lines such as HB1. F3, (immortalized human neural stem cells), cholinergic-SN56, dopaminergic-SH-SY5Y cells (differentiated and undifferentiated), have been used to compare their susceptibility to antidepressants and elucidate underlying mechanisms of neurotoxicity. Cell viability was measured using CCK-8 after treatment with varying concentrations of antidepressants. All investigated drugs showed varying degrees of neurotoxic effects compared to the reference control. Differentiated SH-SY5Y was more sensitive to amitriptyline than undifferentiated SH-SY5Y. Further study is in progress to elucidate the molecular mechanisms for cellular toxicity or defense against toxicity. Both SN56 and SH-SY5Y showed remarkable expression of LC3B at amitriptyline concentration lower than where cell viability was significantly affected. These data have been compared with those from iPSC derived neuronal cells and fetal midbrain derived neuronal precursor cells, a primary cell culture representing in vitro system more relevant to in vivo. In conclusion, a battery of in vitro system is useful as preliminary tool to investigate neuronal toxicity although no single cell type alone can be satisfactory to predict neuronal adverse effect in clinics.

W-1232

SELF RENEWAL OF MOTONEURON PRECURSORS BY COCULTURING OLFACTORY ENSHEATHING CELLS

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The significant progress in stem cell biology brings potential therapeutic approaches for the treatment of spinal cord injury (SCI). Recently, a first-in-human clinical trial has shown the safety and tolerability of intraspinal transplantation of neural stem cells in patients with amyotrophic lateral sclerosis (ALS). Functional improvement in SCI patients by cell transplantation is the next challenge and needs sufficient motor neurons and its long-term survival in vivo. However, how to sustaining the stemness status of motor neuron precursors is scarcely unknown. We discovered that coculturing with olfactory ensheathing cells (OEC) sustained the replicative status of mouse embryonic stem cells (mESC)-derived motor neurons (HB9::GFP+ cells) and also prevented their terminal differentiation. Expanded HB9::GFP+ cells can be purified by FACS sorting and be steered to differentiate into mature motor neurons. Especially, this OEC coculture promotes the neuroprotection and maintenance of stemness for motor neurons in the engrafted spinal cord of rats with SCI. This combined OEC/motoneuron cell therapy advances the improvement of the motor function than the outcome of individual cells transplantation in SCI rodents.

W-1233

SYSTEMATIC PHYSIOLOGICAL CHARACTERIZATION OF HUMAN SPINAL MUSCULAR ATROPHY PATIENT IPSC-DERIVED MOTOR NEURONS

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Spinal muscular atrophy (SMA) affects approximately 1 in 6,000-10,000 live births, and there is currently no effective cure. SMA is a monogenic neurodegenerative disorder that is caused by the reduced expression of survival motor neuron (SMN) protein. While SMN is ubiquitously expressed in most cell types, spinal cord motor neurons are most susceptible to low SMN levels and degenerate in SMA. To understand the functional consequences of low SMN in motor neurons, we performed systematic physiological characterization of motor neurons from SMA patients. Induced pluripotent stem cells (iPSCs) were reprogrammed from fibroblasts from SMA patients with mild to severe forms of disease and then differentiated into motor neurons. As revealed by patch-clamp recordings, these motor neurons were able to fire action potentials, respond to GABA and AMPA application, and display spontaneous synaptic transmission. GCaMP-based calcium imaging was also employed to examine spontaneous and stimulated calcium transients at high throughput. As an additional control, cortical neurons were induced by expressing neurogenin-2 in control and patient iPSCs with the goal of identifying motor neuron-specific disease phenotypes. Our study aims to identify functional defects in motor neurons from SMA patients that are manifest well before cell death, thus enabling improved drug screening for new SMA drug candidates.

W-1234

IN VITRO ELECTROPHYSIOLOGICAL DRUG TESTING USING HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONS

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Human induced pluripotent stem cell (hiPSC)-derived neurons may be effectively used for drug discovery and cell-based therapy. We here used a multi-electrode array (MEA) system to investigate the functional characteristics of hiPSC-derived neurons on their long-term spontaneous activity and drug responsiveness. We demonstrated that hiPSC-derived neurons allowed the culture to be maintained over 6 months with long-term spontaneous activity. After 70 days of culture, we observed synchronous burst firing activity due to synapse transmission within neuronal networks. Addition of the synapse agonist and antagonists kainic acid, L-gulutamate, bicuculline, CNQX and AP5 induced significant changes of the firing rate in spontaneous firings and electrical evoked responses. Furthermore, we demonstrated that epilepsy phenomenon was evoked by administration of pentylentetrazole (PTZ) and was inhibited by anti-epilepsy drug phenytoin and sodium valproate (VPA). High frequency synchronized bursts were evoked over PTZ 100 µM. These bursts were gradually decreased with the increasing the dose of anti-epilepsy drug, and disappeared over phenytoin 100µM

or VPA 1 mM respectively. These results suggested that long-term electrophysiological measurements in hiPSC-derived neurons using a MEA system may be beneficial for drug screening applications.

REPROGRAMMING

W-1236

SUCCESSFUL GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELL (HIPSC) LINES FROM BLOOD SAMPLES HELD AT ROOM TEMPERATURE FOR UP TO 48 HOURS

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Using hIPSCs generated from patients with rare diseases will enable research into cellular models of such diseases. Clinics for these patients may be some distance away from the research laboratories. Thus it is pertinent to know the viability of starting material during transportation and storage as this will determine where samples can be collected from. The effects of the stability of blood-derived cells on their ability to reprogram into IPSCs have not yet been addressed. We have investigated the stability of blood at room temperature between 5 and 48 hours post-draw for the ability to generate hIPSCs. We collected blood samples from 6 donors and kept them at room temperature for three time-points (5, 24, and 48 hours). Subsequently, peripheral mononuclear cells were isolated from each sample and grown in media favouring the expansion of erythroid lineage for 9 days, and analysed for erythroid marker expression by flow cytometry. Cells obtained from blood at 24- or 48-hour time-points had significantly less erythroid marker expression than samples processed 5 hours post-draw. We transduced derived erythroblasts with Sendai virus vectors expressing Oct3/4, Sox2, Klf4, and c-Myc, and successfully obtained colonies from each time-point after 21 days. Colonies displaying the best morphology (n = 3) were selected for quality control, including the capacity for self-renewal, differentiation and the genomic integrity by Multiplex-FISH karyotyping. The reprogramming efficiency of samples held at room temperature for 5-hour post-draw was about 3- and 10-fold higher than those kept for 24 and 48 hours, respectively. Increasing input cell numbers and virus transduction volumes improved the reprogramming potential of samples kept at room temperature for 24 and 48 hours. Immunostaining for stem cell markers confirmed the pluripotency of each line derived from the three time-points and their capacity to undergo differentiation into three germ layers. Karyotype analysis revealed no marked differences in rates of chromosomal aberrations in iPSCs derived from the three stability time points. Taken together, our study suggests that high quality IPSCs can be derived from blood samples kept at room temperature for up to 48 hours, and thus enabling sample collections from trial sites distance away from the research laboratories.

W-1237

DIRECT REPROGRAMMING OF FIBROBLASTS TO NEURONS REVEALS A FAMILY OF LONG NONCODING RNAS RECURRENTLY MUTATED IN AUTISM

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Long non-coding RNAs (lncRNAs) are critical contributors to the establishment and maintenance of cellular identity and have increasingly been found to be involved in disease progression. Here we show that direct lineage reprogramming of fibroblasts to induced neuronal (iN) cells upregulates a subset of lncRNAs which are conserved in mouse and human brain development. Overexpression of some of the lncRNA candidates in mouse embryonic fibroblasts is sufficient to promote neuronal reprogramming and contribute to induced neuronal maturation. Overlapping the candidates we obtained from the overexpression screen with the database of copy number variation (CNV) morbidity map of patients with neurodevelopmental delay shows a recurrent focal genomic mutations affecting one of lncRNA candidates (blincRNA-E). Finally, we located a triad family where blincRNA-E is the only transcript disrupted in a chromosomal translocation event where only the father and the son inherited the translocated chromosome display neurocognitive deficits. Summing up, this shows how integration of candidates obtained from sufficiency screen done on the induced neuron platform with human genomic data provides a novel way and an exquisite insight into the effect of lncRNA in human neurogenesis and brain development.

W-1238

GENOMIC INSTABILITY LIMITS REPROGRAMMING FOLLOWING NUCLEAR TRANSFER

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Somatic nuclear transfer (SCNT) enables the rapid conversion of a somatic cell type to an embryonic one within a single cell cycle. Using SCNT, we demonstrate that DNA damage is a limiting factor for successful reprogramming. Transcriptome sequencing reveals that genes involved in DNA damage, such as Gadd45a and Rad51, are among the most significantly upregulated transcripts in SCNT embryos. Here we show that γH2AX+RPA+ foci, a marker of DNA damage, become apparent in SCNT embryos following progression through S-phase in the 2nd cell cycle, coinciding with the major wave of zygotic gene activation. Notably, SCNT embryos exhibit elevated frequencies of γH2AX+RPA+ foci relative to parthenotes suggesting that aberrant epigenetic status of the incompletely reprogrammed somatic nuclei may be the cause of DNA damage. Brca1, RPA32 and Rad51 co-localize with γH2AX+ foci, an indication of stalled replication forks. SCNT embryos derived from Brca1 null oocytes exhibit significant increase in the number of γH2AX+RPA+ foci. The frequency of DNA damage is dependent on donor cell-type - transferred embryonic nuclei exhibit lower frequency of mitotic segregation errors relative to differentiated cell-types. Our studies show that genetic instability is a major impediment to cellular reprogramming after nuclear transfer, as has previously been observed in iPS generation. Unlike after iPS reprogramming, we can exclude that abnormal transcriptional reprogramming or the limited

proliferation potential are cause for DNA damage. These results suggest that genomic stability is intimately tied to cellular identity, and that cellular states outside of a normal physiologically relevant state compromise the integrity of the genome, which is detected by genome surveillance proteins. Therefore, we propose that tumor suppressors (such as BRCA1) not only safeguard genomic stability, but are also 'gatekeepers of cell identity', suppressing the proliferation of abnormally reprogrammed cells, and perhaps of tumor cells.

W-1239

DELINEATING THE ROLE OF THE 'YAMANAKA' FACTORS IN SHAPING THE EPIGENETIC LANDSCAPE TOWARDS A PLURIPOTENT GENOME

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Transcription factor-induced reprogramming of somatic cells to iPSCs is a process by which a differentiated cell is re-wired to an ESC-like epigenomic state. Our focus lies on deciphering the mechanisms by which Oct4, Sox2, Klf4 and cMyc induce this transition. First, we have mapped OSKM binding at different stages of reprogramming in relation to epigenetic states. To this end, we generated comprehensive maps of TF binding, histone marks, transcription patterns, and DNA methylation at early reprogramming stages, in a late intermediate, and the final pluripotent state. We generated chromatin state models that allow the annotation and discovery of cis-regulatory elements important for reprogramming, and the kinetics of engagement by the reprogramming factors. We find that reprogramming is associated with a step-wise reorganization of the reprogramming factors, mirrored by the global rewiring of the enhancer landscape. Early OSK binding has two key characteristics: when initially expressed in fibroblasts, the reprogramming factors, particularly OSK, strongly associate with active, fibroblast-specific enhancers and promoters, which eventually become silenced and inaccessible. The decommissioning of the somatic program starts early on, and is associated with the quick loss of somatic transcription factors from their binding sites. Secondly, many enhancer regions of genes critical for stem cell self-renewal and pluripotency are engaged within first 48hrs by OSK, and stay constitutively bound by the reprogramming factors throughout the process. A number of these sites start off in fibroblasts with no or low histone mark signal, and gain enhancer marks gradually, as other pluripotency transcription factors become expressed and co-bind. Mechanistically, all three factors are necessary to co-bind to allow engagement of these locations early in reprogramming. Taken together, our study illuminates regulatory mechanisms underlying reprogramming to pluripotency, and indicates that in the mouse system, Oct4, Sox2, and Klf4 are uniquely suited for reprogramming due to their ability to engage many pluripotency-binding sites in a synergistic manner early in reprogramming. Moreover, the output of the reprogramming factors is influenced by the presence of additional stage-specific transcription factors.

W-1240

THE USE OF MODIFIED MRNA TO ENHANCE THE GENERATION OF INDUCED NEURAL PRECURSOR CELLS FROM ADULT HUMAN FIBROBLASTS

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Reprogramming technology has recently provided the capability to directly generate specific neuronal lineages, such as dopaminergic neurons or motor neurons. However, the ability to only generate a specific neuronal population may be advantageous or limiting, depending on the research goals. Recently we developed an efficient system for directly generating neural stem/precursors from adult human fibroblasts. We have shown that transient ectopic expression of neural-promoting transcription factors, SOX2 and PAX6, in human fibroblast cells was sufficient to reprogram them to neural stem/precursor cells, which could then be further differentiated to region-specific neuronal populations. Characterization of the induced neural stem/precursor cells (iNPs) showed they express a range of neural positional markers and their progeny included glutamatergic, GABAergic and dopaminergic neurons. The methodology initially used to generate iNPs involved transfection of fibroblasts with SOX2 and PAX6 cDNA plasmids. Whilst this approach is desirable in that it reduces potential genomic integration of ectopic factors, plasmid transfection efficiency is relatively poor compared to traditional viral transduction. To advance this technology, we have optimized a novel modified mRNA gene delivery system for direct iNP reprogramming. Modified mRNA has the benefit of being extremely stable and non-immunogenic, allowing us to co-transfect adult human fibroblasts with our reprograming factors SOX2 and PAX6 with an efficiency of >80%, significantly higher than the 10-20% transfection efficiency obtained with plasmids. Cell survival was >85% post-transfection, also significantly greater than 20-40% survival with plasmid transfection. Most importantly, co-transfection with SOX2 and PAX6 mRNA increased the rate of iNP reprogramming to ~14-21 days compared with ~45-65 days required using plasmid transfection. Expression of neural positional genes was observed through qPCR, and differentiation of mRNA-derived iNPs generated TuJ1-positive cells co-expressing phenotypic markers including GAD65/67, vGlut and tyrosine hydroxylase. These results represent the first time an mRNA approach has been used to directly reprogram adult human fibroblasts to neural precursor cells.

W-1241

FUNCTIONAL RETINAL PIGMENT EPITHELIUM LIKE CELLS FROM HUMAN FIBROBLASTS

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The retinal pigment epithelium (RPE) provides vital support to photoreceptor cells and its dysfunction is associated with the onset and progression of age-related macular degeneration (AMD). Surgical provision of RPE cells may ameliorate AMD and thus it may

be valuable to develop sources of patient-matched RPE cells via reprogramming. We used a computational approach to generate an atlas of candidate master transcriptional regulators for a broad spectrum of human cells and then used candidate RPE regulators to guide investigation of the transcriptional regulatory circuitry of RPE cells and to reprogram human fibroblasts into RPE-like cells. The RPE-like cells share key features with RPE cells derived from healthy individuals, including morphology, gene expression and function. The approach described here should be useful for systematically discovering regulatory circuitries and reprogramming cells for additional clinically important cell types.

W-1242

DEVELOPING A PROTOCOL FOR EFFICIENT DOPAMINERGIC NEURONAL CONVERSION OF PATIENT DERIVED FIBRO-BLAST

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Direct reprogramming of somatic cells into specific neuronal subtypes has opened up the possibility not only to study disease processes in patient-specific neurons, but also to use these as a source of cells for brain repair therapies. Cell-based replacement therapy for Parkinson's disease using dopaminergic neurons has proven to be an effective experimental therapy, although the current source of cell used - that is fetal ventral midbrain derived cells - still holds ethical and scalability issues. Given that induced neurons (iNs) do not go through a pluripotent stage they, might constitute a safer source of cells and could allow autologous grafting. We and others have successfully reprogrammed human fibroblasts into functional dopaminergic neurons, although conversion efficiency using adult human fibroblasts remains low. Here, using previously identified factors for dopaminergic conversion (Ascl1, Lmx1a, Lmx1b, FoxA2 and Otx2), we are comparing our current best protocol (delayed transgene activation in combination with inhibition of SMAD signaling and activation of canonical WNT signaling) with three new approaches: the use of 1) a new combination of small molecules, 2) microRNAs miR9/9* and miR124 and 3) a specific midbrain chromatin remodeler. This study will help improve dopaminergic conversion efficiency of directly reprogrammed adult fibroblasts, which will be a step forward towards using these cells for cell-based replacement therapy as well as disease modeling of Parkinson's disease using patient-specific skin cells.

W-1243

SMALL MOLECULES TOWARDS SOMATIC CELL REPROGRAMMING

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Recent work showed that a combination of small molecules targeting epigenetic modifiers or signaling pathways enhance the reprogramming efficiency and eliminate the use of reprogramming factors in mouse cells and replace two, and in some cases three of the four Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc, also abbreviated OSKM) in adult human cells. Through the use of small molecules targeting chromatin remodeling enzymes, we aim to develop a method to increase the efficiency of iPSC generation and reduce the dependency on ectopic expression of reprogramming factors. The importance of the chromatin modifier histone H3K9 methyl-transferase Suv39H1 in reprogramming was previously shown via its shRNA-mediated suppression. In this study, in parallel to shRNA-mediated suppression, we used the small molecule Chaetocin to suppress the activity of Suv39H1 in human fibroblasts during their reprogramming with the Yamanaka factors. The effect of Chaetocin-mediated suppression of Suv39H1 on reprogramming was observed by Tra-1-60 staining of the cell colonies after 21 days. Chaetocin-mediated suppression of Suv39H1 and shRNA-mediated knock-down of Suv39H1 both generated significantly more iPSC colonies than control cells, indicating that suppression of Suv39H1 increases reprogramming efficiency. Overexpression of Suv39H1 had the opposite effect on iPSC generation, thereby confirming the specificity of Chaetocin. In order to expand our insight on chromatin dynamics during reprogramming, we employed numerous small molecules and found that, combination of small molecules and OSKM induction increased iPSC colony formation by 15-fold compared to OSKM induction only. Moreover, two of the Yamanaka factors, Klf-4 and c-Myc, were successfully replaced when cells were induced with the reprogramming factors Oct-4 and Sox2 together with the selected small molecules. The results of this study will contribute to the general understanding of the chromatin-based epigenetic mechanisms of reprogramming, improve efficiency of iPSCs generation, and reduce the dependency on ectopic expression of reprogramming factors. This research supported by TUBITAK with 212T095 project number.

W-1244

REPROGRAMMING TO PLURIPOTENCY OF CELLS FROM A LONG-LIVED AND CANCER RESISTANT BLIND MOLE-RAT, SPALAX

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The subterranean blind mole-rat, Spalax, is a long-lived (>20 years) hypoxia-tolerant rodent showing no signs of aging, and with an extraordinary resistance to cancer. No spontaneous tumors have ever been observed in thousands of individuals studied for more than 40 years. Even more, standard protocols of chemical carcinogenesis that resulted in tumor development in 100% of mouse or rats, failed to promote tumor growth in Spalax with the exception of only 2 cases of fibrosarcomas observed after 3-Methylcholanthrene (3MCA) treatment out of a total of 12 treated animals. Cellular reprogramming to iPSCs is a powerful in vitro system to uncover basic mechanisms governing stem cell biology but has also been used to reveal a previously unsuspected link between tumor suppressors and pluripotency factors.

These studies have served to highlight the similarities between reprogramming to iPSC and oncogenic transformation to cancer cell. In addition, the process of aging has also been shown to represent a barrier for reprogramming. This is probably due to an increase in the activity of tumor suppressor genes that normally accumulate during aging. Given the extraordinary biology of Spalax, we think that cells derived from these animals offer an excellent model to study the relationship between aging, cancer and reprogramming. To this end, we have compared the reprogramming of fibroblasts derived from Spalax or mouse neonatal skin. We used different delivery systems and compared the expression of the reprogramming factors and the potential generation of iPSC colonies. In addition, we have used an established cell line derived from a 3MCA-induced fibrosarcoma generated in Spalax. These tumor cells were subjected to the reprogramming protocol in parallel with similarly obtained fibrosarcoma cell lines from mice. In summary, this system offers the possibility of identifying key regulatory mechanisms underlying aging and cancer.

W-1245

DIRECT REPROGRAMMING TO ATRIAL / VENTRICULAR CARDIOMYOCYTES BY DEFINED FACTORS

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Heart failure is a major cause of morbidity and mortality in the world. Cardiomyocytes consist of several types of cardiomyocytes, including atrial myocytes, ventricular myocytes, pacemaker cells and conduction cells, which are terminal differentiated cells with limited regenerative capacity in adult mammalian heart. Therefore, new therapies that can regenerate damaged tissue and improve heart function are needed. In recent year, it had been reported that overexpression of three cardiac transcription factors (GMT; Gata4/ Mef2c/ Tbx5) reprogrammed fibroblasts directly into differentiated cardiomyocyte-like cells. Direct reprogramming of fibroblasts into functional cardiomyocytes will be a useful therapeutic approach to regenerate the heart. However, the authors concluded that these induced cardiomyocytes were composed of the various types of immature cardiomyocytes. Immature cardiomyocyte may trigger arrhythmias in the injury heart. Thus, it will be important to promote cardiac reprogramming toward the desired cardiac cell types such as atria myocytes, ventricular myocytes or conduction cells. The specific induction of atrial or ventricular myocytes has not been established yet. Especially, ventricular myocytes will be expected as source for heart therapies, because myocardial infarction mainly occurs in ventricle. We demonstrated that a combination between GMT and ventricular candidate factors reprogrammed embryonic fibroblasts directly into differentiated ventricular myocyte-like cells with induction of ventricular specific gene in mammalian model.

W-1246

INVESTIGATING THE ROLE OF REPROGRAMMING MONOCYTE/MACROPHAGES IN OSTEOARTHRITIS

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Osteoarthritis (OA) is a degenerative joint disorder associated with chronic inflammation. Mesenchymal stromal cells (MSCs) are being clinically investigated in OA both for their immunomodulatory and regenerative properties. We hypothesize that clinical effects in OA may in part be due to MSC-reprogramming of monocytes/ macrophages to a more homeostatic M2 phenotype. We investigated the MSC-mediated monocyte reprogramming (4 days), and its stability upon susbsequent inflammatory challenge of both human peripheral blood monocytes, and GM-CSF-differentiated monocyte-derived macrophages (MDMs). This is important therapeutically, as any ex-vivo reprogrammed monocytes/ macrophages would need to remain phenotypically and functionally stable upon encountering an inflammatory environment, as present in an OA joint. Coculture with MSCs increased the frequency of CD14+CD206+CD163+ M2 monocytes (M2-Mφ) (47.0% vs 0.2%); an effect that was partly reproduced in MSC transwell cocultures (5.5% vs. 0.2%), suggestive of a role for soluble factors. The increased M2-Mφ frequency was accompanied by increased secretion of IL10: 165.6 pg/ml in cocultures, 109.9 pg/ml in transwell cocultures and 0.2 pg/ml without MSCs. Importantly, upon removal of MSCs, the M2-Mφ frequency remained relatively stable (8.0%), even with the addition of inflammatory challenges, IFNγ and LPS (10.5 %); relatively stable levels of IL10 secretion were also maintained (19.3 pg/ml with IFNγ vs. 25.8 pg/ml without IFNγ). The frequency of M2 MDMs increased when cocultured with MSCs (52.4% vs. 17.3% MDMs alone). Importantly, MDMs promoted T cell proliferation (32% vs. 22% with no MDMs), especially IFNγ stimulated MDMs (91% vs. 22% with no MDMs). Reprogramming with MSCs, both with and without IFNγ abrogated the MDM-Th cell activation (T cell proliferation in MSC-MDMs vs. MDMs alone at 12% vs. 32%, without IFNγ, and 29% vs 91% with IFNγ), confirming a MSC-mediated functional switch of the MDMs. Taken together, our data suggests that MSC-mediated reprogramming of monocytes or macrophages induces a relatively stable functional and phenotypic switch even in pro-inflammatory environments. Further in vitro and in vivo investigation is ongoing to demonstrate that monocyte reprogramming in itself can provide a possible therapeutic option for OA.

W-1247

A NOVEL FOUR TRANSFECTION PROTOCOL FOR REPROGRAMMING HUMAN BLOOD-DERIVED EPCS AND ADULT HUMAN DERMAL FIBROBLASTS USING NON-MODIFIED REPROGRAMMING AND IMMUNE EVASION MRNAS

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Many groups have demonstrated that the repeated transfection of fibroblasts with a cocktail of reprogramming associated mRNAs

results in the derivation of integration-free human induced pluripotent stem (iPS) cells. While many advancements have been made to refine this process on fibroblasts (incorporation of ECMs, miRNAs, and more efficient transfection reagents), to date no group has been able to demonstrate efficient, RNA-based reprogramming of a blood derived cell type. This is due to the inability to efficiently and repeatedly deliver mRNA to blood derived cell types without inducing cytotoxicity. Peripheral blood provides easy access to adult human cell types for reprogramming purposes. Notably, endothelial progenitor cells (EPCs) are a rare, circulating population of cells that can be clonally isolated from both fresh and frozen human peripheral blood and cord blood samples. The EPCs adherent nature and high proliferative capacity while maintaining their cell identity makes them highly desirable for repeated transfection with RNA when compared to hematopoietic suspension cell types commonly isolated from peripheral blood which typically have limited expansion potential and exhibit maturation in culture. Lastly, the ability to generate clinical grade iPS cells from EPCs using RNA reprogramming technologies presents a unique therapeutic opportunity to treat myeloproliferative disorders in which the disease-causing somatic mutations are restricted to cells in the hematopoietic lineage. Here we present an RNA-based reprogramming method that utilizes a novel cocktail of synthetic, non-modified reprogramming and immune evasion mRNAs and reprogramming enhancing miRNAs for the generation of clinical grade iPS cell lines from human blood-derived EPCs. This novel, four transfection protocol results in the highly efficient generation of stable, fully pluripotent iPS cell lines from both blood-derived EPCs (0.25%) and HUVECs (0.6%) as well as human adult dermal fibroblasts (1.6%), all within 11 days.

W-1249

NON-CODING Y-RNA1 IS ESSENTIAL FOR IPS CELL REPROGRAMMING

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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 cell 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. To elucidate the changing of non-coding RNAs during iPS cell reprogramming, human fetal fibroblasts TIG-1 were infected with OCT4, SOX2, KLF4, and c-MYC (OSKM), which were constructed in Sendai virus. Total RNAs from OSKM-infected TIG-1 at every three days were analyzed for the non-cording RNAs expression by Lnc Profiler qPCR Array Kit. To evaluate the effect of the selected non-coding RNA, we assessed the efficacy of iPSCs reprogramming by the knockdown and overexpression experiments for the candidate gene. The iPS cell reprogramming was examined at each day after the gene transduction by qRT-PCR, FACS (TRA-1-60 and SSEA4), Alkaline Phosphatase (ALP) staining, the number of iPS cell colonies, and microarray methods. All data were analyzed by one-way ANOVA, or t-test using MeV software. Non-cording RNA, Y-RNA1 was selected for further study based on stage specific expression profiles. Y-RNA1 is conserved in all vertebrates, in which they have been studied. Expression of Y-RNA1 transiently and sharply increased during early stage (Day 0 to 9 after the transduction), and the localization was cellular cytoplasm. The siRNA for Y-RNA1 was introduced to TIG-1, where Y-RNA1 expression lessens to less than 15%, compared with conventional iPS cell reprogramming. The knockdown of Y-RNA1 gave rise to the rare generation of iPS cell colinies, and the inhibitory phenomenon was verified by FACS analysis and ALP staining. At day 3, siRNA mediated TIG-1 demonstrated that cell cycle related genes increased, and apoptosis and RIG-I like receptor signaling pathway related genes decreased by microarray analysis. On the other hand, there were not significant different between Y-RNA1 over-expressed TIG-1 and control TIG-1. Our data demonstrate that Y-RNA1 is essential role for iPS cell reprogramming, and works at the early stage of the process.

W-1250

ISOXAZOLE INDUCES ENDOCRINE DIFERENTIATION IN THE PANC-1 HUMAN DUCTAL CELL LINE

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Transdifferentiation of pancreatic cells to β cells is an increasingly desirable target for treatment of diabetes mellitus in order to increase total β-cell mass and insulin output. Insulin production in pancreatic β cells can be increased using a small molecule, 3,5-disubstituted isoxazole, N-cyclopropyl-t-(thiophen-2-yl) isoxazole-3-carboxamide (Isx) but its effect on other pancreatic cell types has not been reported. Here, we investigated transdifferentiation of PANC1 pancreatic ductal cells to insulin producing cells by exposing them to Isx. Gene expression was performed using RT-PCR and qRT-PCR for ~30 genes critical to β cell development and function, and quantitative proteomic profiling was performed using LC/MS, monitoring protein abundance as PANC1 cells differentiated into islet-like cells compared to time-matched controls. After 48 hours of Isx treatment, PANC1 cells aggregated into islet-like clusters and gene expression analysis revealed induction of important developmental β cell markers including NGN3 (>200-fold increase; n=3), NEUROD1 (>200-fold increase; n=3) and insulin (60-fold increase; n=3). In addition, β cell specific cell surface markers were also upregulated such as TSPAN7, CD200, FXYD2 and the mature β cell markers GPR50, GLUT2 and SLC30A8. Using LC/MS a catalogue of approximately 2800 identified proteins was generated; 142 proteins were upregulated and 54 proteins downregulated at $p<0.05$. Amongst the proteins upregulated were the β cell surface markers SPINT1 and LAMP1, and two glucose transporters, SLC2A1 (GLUT1) and SLC2A3 (GLUT3). The expression of the majority of these proteins has not been previously reported or studied in the context of β cell differentiation. Functional analysis of the relative protein changes was determined using Ingenuity Pathway Analysis (IPA) software, which revealed the regulation of several cellular canonical pathways including protein transport, metabolic pathways, remodelling of epithelial adherens junctions and actin cytoskeleton signalling. To our knowledge, this is the first large-scale proteomic and mRNA profiling of PANC1 transdifferentiation towards endocrine cells and shows that Isx treatment is a useful tool with which to explore this process.

W-1252

ENHANCING PLURIPOTENT ABILITY OF HUMAN ADIPO-SE-DERIVED STEM CELLS BY ACIDIC SOLUTION AND SMALL MOLECULES TREATMENTS

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Pluripotent stem cells (PSCs), such as embryonic stem cells (ESCs) and induced-pluripotent stem cells (iPSCs), have known its potential to form three germ layers and differentiated into any types of somatic cells. This provides the potential to generate patient-specific cells and apply in clinical use. However, there exists several risks for applying human iPSCs in clinical such as ethical problems by using ESCs, gene-integrated problems by using iPSCs, and xeno-contamination for PSC cultivation. Recently, several publications show mouse somatic cells could be reprogrammed into pluripotent-like cells.1 Hou et al. has demonstrated mouse somatic cells could generate iPSCs by small molecules treatment.2 These results are showing the potential of strategies to generate iPSCs without gene-integration and safer way for clinical applications. However, those cell reprogramming methods has not yet applied on human iPSC generation successfully. In this study, we investigated whether it is possible to enhance the pluripotent ability of human somatic cells by acidic solution treatment and/or additional small chemical compounds. Furthermore, we found the hADSCs in permeation solution and recovery solution by membrane filtration method have higher pluripotency than those in stromal vascular fractions. Here, we tried to compare different sources of hADSCs reprogramed into pluripotent stem cells by acidic solution treatment and/or additional small chemical compounds. Our results showed permeation and recovery cells through the membrane filtration retained higher potential to be reprogramed into pluripotent stem cells. Our method which is solely using physical and chemical stress without using genetic materials to generate hiPSCs should be a great impact and a promising strategy for clinical application of hiPSCs.

W-1253

ENDOGENOUS FACTORS SELF-REGULATE HIGH-EFFICIENCY HUMAN CELL REPROGRAMMING THROUGH MICROFLUIDIC TECHNOLOGY

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Human induced pluripotent stem cells (hiPS) are derived from adult somatic ones by reprogramming. This process is performed by actively forcing the establishment of the pluripotency gene regulatory network by ectopic expression of few transcription factors. Many studies have focused on understanding reprogramming and discovered epigenetic and transcriptional barriers, while only few addressed the role of signaling pathways, mainly during the initial stages. Nonetheless, current understanding is still limited and only a minority of cells are reprogrammed to a status of pluripotency (up to 3%). By analyzing microarray data of sequential steps during the reprogramming of human somatic cells, we found that important cell secreted soluble factors are finely up- and down-regulated along the process. Thus, we asked whether endogenous signaling pathways could play an important role in reprogramming progression. In order to explore this hypothesis, we downscaled reprogramming into a microfluidic system and developed optimal conditions for the study: no feeder layer, defined medium, and precise control of transcription factor expression by non-integrating mRNAs. We found both mRNA transfections and, more importantly, cell reprogramming to occur with high efficiency in microfluidics. After considerable optimization, we consistently obtained ~150% reprogramming efficiency (TRA-1-60+/NANOG+ hiPS colonies/seeded cells). We thus explored why the efficiency is so high in such a confined culture environment. First, we transcriptionally analyzed colonies freshly obtained in microfluidics and in conventional wells. We found that differentially expressed genes significantly fell into the GO category extracellular space, and, among them, multiple up-regulated TGF-β pathway components. This strongly supported our initial hypothesis. Then, we verified if TGF-β pathway was effectively active, by conditioned media experiments. We found that microfluidics-conditioned medium strongly promoted the pathway in a temporally regulated way. Exogenous TGF-β completely abolished hiPS formation, and its inhibition at different stages had little effect on the outcome. In conclusion, we found an important cell self-regulation during reprogramming.

W-1254

MOLECULAR MECHANISMS OF ASTROGLIA-TO-NEURON REPROGRAMMING BY FORCED EXPRESSION OF PRONEU-RAL GENES

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Direct reprogramming of differentiated cells into neurons is a powerful tool to study the molecular mechanisms underlying cell specification and differentiation. Our group has previously shown that astroglia cells can be efficiently converted into very different functional neuronal subtypes upon the over-expression of Neurog2, generating glutamatergic projection neurons and Ascl1, generating GABAergic interneurons. To investigate the molecular mechanisms of neuronal reprogramming and address the similarities between the neurogenic programs towards very different neuronal subtypes, we generated a tamoxifen inducible system from the 2 TFs. Indeed, both Neurog2ERT2 and Ascl1ERT2 generated neurons from astrocytes upon tamoxifen induction, while no neurons were observed in controls. Through an unbiased transcriptome profile on Neurog2-(or Ascl1)-ERT2 expressing astrocytes induced for 4, 24 and 48 hours, we found a fast and very dynamic change in the transcriptome upon the activation of each transcription factor. Furthermore, we observed a small overlap between the genes induced by Neurog2ERT2 and Ascl1ERT2 at any time point, suggesting that the TFs activate rather different neurogenic programs within the exact same cellular background already at a very early stage of reprogramming. As common targets might contribute to the execution of a generic neuronal program, we knocked some of them down through microRNAs and found their functional requirement for TF-induced neuronal conversion. Among common targets, NeuroD4 plays an

instructive role, as its expression either alone or together with other downstream targets induced the generation of functional neurons. Interestingly, REST regulates NeuroD4 expression, and REST cKO astrocytes convert more efficiently into neurons than their wild type counterpart. The reprogramming ability of the identified downstream targets is not restricted to murine astrocytes, but also MEFs and human astrocytes could be reprogrammed into neurons. These data provide a first insight into the early stages of direct reprogramming towards neurogenesis, highlighting the early activation of a neurogenic program, and identifying the distinct neurogenic programs directing neurons to different neuronal subtypes within the same cellular and transcriptional background.

W-1255

AN IMPORTANT ROLE FOR STRESS KINASES DURING RE-PROGRAMMIING OF HUMAN FIBROBLASTS TO INDUCED PLURIPOTENT STEM CELLS

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Somatic cells are reprogrammed to pluripotent stem cells (iPSC) state through forced expression of key transcription factors. Understanding the mechanisms that drive this process is crucial for increasing its efficiency and safety profile. Several publications to date have indicated that at the cellular level, activation of signalling network occurs during various phases of reprogramming. The MAP kinases (MAPKs) are a family of signalling proteins implicated in numerous cellular responses ranging from stress to apoptosis, proliferation and survival. In this study, we have investigated the role of stress-activated SAPK/JNK and p38 signalling cascade as well as their upstream kinases MKK4 and MKK7 during reprogramming of both neonatal and adult human fibroblasts. Application of single or combined specific inhibitors for SAPK/JNK (SP600125), p38ą and p38β (SB202190) at different time points during the induction protocol led to disaggregation of iPSC colonies during the second window of reprogramming process, implicating an important role for these kinases in the reprogramming process. Similar results were obtained when these kinases were downregulated in neonatal and adult human fibroblasts by RNA interference. Furthermore, we observed that adult fibroblasts were more sensitive to application of these inhibitors when compared to neonatal fibroblasts and current work is focused on understanding of differences between these two cell types with regard to expression and role of stress kinase signalling.

W-1256

DIRECT REPROGRAMMING OF HUMAN BONE MARROW STROMAL CELLS INTO FUNCTIONAL RENAL CELLS USING CELL-FREE EXTRACTS

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Human bone marrow stromal cells (BMSCs), also known as bone marrow-derived mesenchymal cells, are gaining recognition in regenerative medicine applications. We inquired whether BMSCs could be directly reprogrammed to acquire additional desired properties of somatic cells. Here, we show that human BMSCs can be reprogrammed into renal proximal tubular-like epithelial cells using cell-free extracts. Streptolysin-O-permeabilized BMSCs exposed to HK2-cell extracts underwent morphological changes: formation of "domes" and tubule-like structures, and acquired epithelial functional properties as transepithelial resistance, albumin binding and uptake and specific markers, E-cadherin and Aquaporin1. Transmission electron microscopy revealed the presence of brush border microvilli and tight intercellular contacts. RNA sequencing showed tubular epithelial transcript abundance and revealed the upregulation of components of the EGFR pathway. Reprogrammed BMSCs integrated into self-forming kidney tissue and formed tubular structures. Infusion of reprogrammed BMSCs in immunodeficient mice with cisplatin-induced acute kidney injury engrafted into proximal tubuli, reduced renal injury and improved function. Thus, reprogrammed BMSCs consist a promising cell resource for future cell therapy.

W-1257

ENHANCING DIRECT CONVERSION OF MOUSE FIBROBLASTS TO INDUCED HEPATOCYTES

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Transdifferentiation is the conversion of a differentiated cell to another differentiated cell. Compared to directed differentiation of pluripotent stem cells, it is deemed to happen faster and be potentially safer for future clinical applications. To understand changes in chromatin modifications during this process, we analyzed the conversion of mouse fibroblasts to induced hepatocytes. We expressed the two transcription factors necessary for the conversion in fibroblasts, and treated the cells with small molecule inhibitors of various chromatin modifiers. We then tested both the efficiency and the quality of lineage conversion at the gene expression level. We observed that removal of activating H3K79me2 mark through inhibition of DOT1L, and suppression of repressive H3K27me3 mark through the inhibition of EZH2 are facilitators of this direct lineage conversion. Inhibition of these two modifications led to the generation of induced hepatocytes that are more similar to mouse hepatocytes than untreated controls are in terms of hepatocyte marker expression. Furthermore, we found that chemical treatment of cells subsequent to transcription factor expression is more effective than pretreatment. However, none of the chemicals tested are able to fully replace the two transcription factors. These preliminary findings provide insight into the chromatin modifications that are important in establishing the identity of engineered hepatocytes.

W-1258

LINEAGE CONVERSION OF HUMAN CELLS TO AN ADRENOCORTICAL PHENOTYPE: A NEW TECHNOLOGY TO STUDY THE ADRENAL GLAND

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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 cause by a number of adrenal disorders, and lifelong management of these patients with exogenous steroids can be challenging. No drug suitably mimics the diurnal pattern of cortisol noted in healthy individuals, and objective variables to measure replacement quality are lacking. 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. The potential to produce and expand disease- and patient-specific cells may also revolutionize our understanding of the underlying pathophysiology of adrenal disorders, paving the way for the identification of novel therapeutic targets. 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 phenotype/lineage; its absolute requirement for steroidogenesis has been recently demonstrated in vivo. Also, the capacity to impose an SF1-dependent steroidogenic-like gene expression program in a variety of murine cells (embryonic fibroblasts, adipose stromal fraction, bone marrow stroma, ES cells, IPSCs) has been verified by several groups. By forcing the expression of SF1, but not of other transcription factors involved in adrenal development, we demonstrate the ability of human fibroblasts, blood-derived late outgrowth endothelial progenitor cells (L-EPCs) and urine-derived stem cells (USCs) to lineage convert to steroidogenic-like cells, as assessed by changes in cell morphology, gene expression, activation of adrenal-specific signaling pathways and and hormonal output.

W-1259

THE MATISSE™ EPISOMAL REPROGRAMMING SYSTEM: REPROGRAMMING HUMAN SOMATIC CELLS VIA DISCRETE IDENTICAL EXPRESSION CONTROL ELEMENTS INTEGRATED INTO A SINGLE PLASMID VECTOR

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Despite developments in multiple reprogramming methods, most strategies have significant limitations. Progenitor Life Science's Matisse™ Reprogramming Technology overcomes impediments of traditional iPSC methods through an all-in-one episomal vector that delivers OKSM reprogramming factors (OCT3/4, KLF4, SOX2, and c-MYC) under the independent control of factor paired attenuated CMV promoters. The Matisse™ System provides coordinated expression bypassing multistep screening procedures required to ensure homogeneous gene transfer associated with other systems. The Matisse™ Reprogramming System increases efficiency and reduces the time and workload required during the reprogramming process. Electroporation or liposomal transfection of the Matisse™ reprogramming vector induced efficient hIPSC formation in EBV-immortalized B-lymphocytes (LB cells) and BJ human skin fibroblasts in feeder cell-free conditions. LB and BJ-derived iPSCs exhibited ES cell morphology, expressed characteristic pluripotency genes, surface markers, efficiently formed embryoid bodies, and differentiation to several terminal cell types.

W-1260

AAV VECTOR-MEDIATED IN VIVO REPROGRAMMING OF VARIOUS CELL TYPES IN ADULT MICE

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The 2006/7 discovery that somatic cells of different tissue origins can be converted into induced pluripotent stem cells (iPSC) by mere introduction of a cocktail of "reprogramming" factors, such as Oct4, Klf4, Sox2 and c-Myc (OKSM), has revolutionized the field of regenerative medicine. Here, we specifically engineered Adeno-associated viral (AAV) vectors for OKSM delivery, based on their superior safety profile and their typical persistence as episomal DNA that is lost during cell division. A third advantage is that AAV vectors can be pseudotyped with natural or synthetic viral capsids exhibiting high specificities and efficiencies in a given cell. Indeed, we reproducibly found that OKSM expression from four AAV vectors - each encoding a single factor under the strong and ubiquitous SFFV promoter, and pseudotyped with the potent chimeric AAV-DJ capsid - resulted in reprogramming of murine embryonic fibroblasts. Notably, the up to 0.2% reprogramming efficiency obtained with our AAV vectors is comparable to the best lentiviral system. Curiously, and defying our original prediction, we observed by PCR that the AAV vector DNA persisted in the reprogrammed cells even after extended cultivation for up to 21 passages. Using a combination of LAM-PCR and SureSelect technologies, we were then able to identify a variety of OKSM vector integration sites in the mouse genome, including a previously reported hot spot for AAV vector insertion. We next asked, based on the robust ex vivo efficiency, whether our OKSM vectors could also mediate reprogramming in vivo, and therefore applied different doses of AAV8-pseudotyped variants to adult mice via tail vein injection. Strikingly, four to eight weeks later, we found various small and large teratomas in their livers that we identified through histological analysis. Moreover, by week eight, one mouse showed signs of extra-hepatic reprogramming in the pancreas and the thoracic cavity, congruent with the in vivo tropism of AAV serotype 8. These features make our novel AAV vector-mediated in vivo reprogramming strategy comparable to our previously published OKSM-transgenic mice, with the added benefits that AAV vectors can be applied in any mouse strain or other animals, and can be re-targeted to specific tissues, which opens up entirely novel avenues for stem cell research.

W-1261

AMELIORATION OF TYPE I DIABETES IN MICE TRANSPLANTED WITH IMMUNE-TOLERABLE PANCREATIC BETA CELLS DERIVED FROM AUTOLOGOUS HEPATOCYTE REPROGRAMMING

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Islet transplantation has been recognized as an efficient approach for treating for type I diabetes patients. However, shortages of donor-supply results in most type I diabetic patients that are unable to receive the transplantation treatment. We and others had previously demonstrated that hepatocytes can be transdifferentiated into insulin-producing beta cells after introducing certain pancreatic transcriptional factors. However, it is unclear whether or not these transdifferentiated beta cells are glucose responsive and expandable. Moreover, there is no evidence demonstrating transdifferentiated beta cells derive from hepatocytes are immune-tolerable in autoimmune diabetes. In the current work, we carried out a direct conversion approach by introducing three transcriptional factors to primary hepatocytes isolated from Non-obese diabetic (NOD) mice which spontaneously develop autoimmune diabetes. We demonstrated that simultaneously expressing Pdx1, Ngn3 and PDGFRα could induce transdifferentiation of hepatocyte to insulin-producing cells displaying characteristics of pancreatic beta cells including expression of MafA, Nkx2.2, Rfx6, Kir6.2, Glut2 and proprotein convertase 1/3 and possessing the capability to secrete insulin in response to stimulatory levels of glucose. Addition of PDGF-AA could activate PDGF pathway, induce RB phosphorylation and trigger proliferation of transdifferentiated beta-cells. Importantly, the transdifferentiated beta cells were found to display reduced levels of MHC class I molecules and GAD65 autoantigens. Therefore, transplantation of the expandable transdifferentiated beta-cells to diabetic NOD mice could significantly improve hyperglycemic situation without needs of tolerogenic treatments. These findings support the possibility of developing cell therapeutic strategies for type I diabetic patients via autologous hepatocyte reprogramming.

W-1262

HUMAN AMNIOCYTES CHEMICALLY REPROGRAMMED TO FUNCTIONAL PLURIPOTENCY VIA DOWNREGULATION OF P53

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Pluripotent stem cells (PSC) favor glycolysis over oxidative phosphorylation (OXPHOS), and have a metabolism that matches their catabolic and anabolic needs. High glycolytic flux generates adenosine triphosphate (ATP) and precursors for biosynthetic reactions, which are essential to sustain the rapidly dividing nature of PSC. In contrast, differentiated cells shift their energy production to the mitochondrion via OXPHOS. We isolated human amniocytes from mid-gestation amniocentesis. We derived translatable integration-free, viral-free induced pluripotent stem (iPS) cells without ectopic expression of Yamanaka factors or other RNA or proteins, by culturing hAFSC in ES/iPS medium supplemented with valproic acid (VPA), a small molecule HDAC inhibitor. Reprogrammed cells regained the capacity to differentiate into lineages of the three germ layers, as evidenced by embryoid body formation in vitro and teratoma formation in vivo. Yet reprogrammed cells share only 90% transcriptional homology with ES cells, and express Oct4, Nanog, KLF4, c-MYC, PODXL, Lin28, ZFP42, BMI1, DNMT3B and TDGF1 among others. Here, we investigate whether the restoration of functional pluripotency in hAFSC correlates with metabolic reprogramming towards glycolysis, assaying cell cycle stage, reactive oxygen species (ROS) generation, metabolite production, gene expression of metabolic and oxidative stress pathways and mitochondrial network. We found that chemically reprogrammed hAFSC are less dependent on ATP production via OXPHOS for their growth and proliferation, and produce key metabolites of glycolysis. Contrary to the parental population, the majority of the reprogrammed AFSC are in S phase of the cell cycle as are embryonic stem cells. Gene expression reveals a metabolic profile that favors glycolysis over OXPHOS, via down-regulation of the p53 pathway. In conclusion, chemical functional reprogramming of hAFSC promotes the acquisition of a more glycolytic phenotype, typical of true pluripotent stem cells.

W-1263

EFFICIENT GENERATION OF FUNCTIONALLY MATURE SUBTYPE-SPECIFIC NEURONS

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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 distinct neuronal subtypes may limit the translational utility of this approach. We utilized a single-cell approach to provide clearer resolution of induced neuron heterogeneity, particularly as it relates to distinct, disease-specific subtypes. Our single-cell transcriptional profiling reveals distinct neuronal subpopulations activate the motor neuron-specific Hb9 reporter. Despite heterogeneity of both morphological and gene expression properties, we do produce neuronal cells with the proper gene regulatory network. We further identified heterogeneity within the neuronal population. Additionally, to enable more efficient generation of homogeneous motor neurons, we then explored a regulatory role for TP53 during direct conversion to induced motor neurons. Production of bona fide neuronal populations from adult human fibroblasts is highly difficult, but p53 inhibition allows more than 40% conversion of adult human fibroblasts into iMNs. Deeper

analyses reveal p53-inhibited iMNs acquire more complex, multipolar motor neuron morphology and more mature electrophysiological properties than counterparts generated in the presence of p53. Notably, it appears p53 inhibition acts upon cell division to promote successful conversion, validated through various chemical and genetic methods. Furthermore, we observe that increased cell division augments conversion by increasing reprogramming factor binding to target sites. 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.

W-1264

DIRECT CONVERSION OF RESIDENT OLIGODENDROCYTE PROGENITOR CELLS INTO MATURE NEURONS WITH MULTIPLE NEURONAL SUBTYPE SPECIFICATION

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Direct conversion of resident glia to neurons has emerged recently as a potential strategy for repair in the adult CNS. Oligodendrocyte Progenitor Cells (OPCs) are the most abundant proliferating resident neural cell population in the adult CNS. OPCs have not yet adopted a mature phenotype and represent a suitable cell population to recruit for neuronal repair by direct in vivo conversion. Furthermore, there is evidence that OPCs maintain population homeostasis, suggesting the functional population of resident OPCs would not be depleted following lineage respecification to neurons. Adult rat cortical OPCs were isolated using magnetic activated cell sorting for O4 antigen selection and maintained as a primary culture for screening combinations of putative neurogenic transcription factors including neurogenin2 (ngn2), ascl1, dlx2, sox2, neuroD1, pax6, and VP16Olig2. Beta-III-tubulin-expressing cells were observed by 10 days post transduction (dpt) of OPCs with retroviral supernatants of ngn2, ascl1, ascl1/dlx2, or neuroD1, with ngn2 exhibiting the most robust response. Ngn2-transduced cell expressed the mature neuronal markers, MAP2 and NeuN, and the post-mitotic projection neuron marker, tbr1, at 7dpt, suggesting successful neuronal subtype specification. Ngn2-transduced cells generated action potentials upon stimulation and displayed spontaneous activity and received synaptophysin-labeled connections from co-cultured postnatal primary neurons. OPCs transduced with the combination of ascl1 and dlx2 expressed inhibitory neuron marker, GAD67, demonstrating the generation of multiple neuronal subtypes from delivery of specific transcription factors. Direct in vivo delivery of retroviral neurogenin2 to the adult rat cortex demonstrated co-expression of immature neuronal marker, doublecortin, in transduced cells one week following gene delivery. Transduced cells expressed NeuN at three weeks and exhibited mature neuronal morphology with extensive processes and dendritic spines. Direct in vivo conversion of resident OPCs may provide an alternative to cell transplantation for neuronal replacement or modulation of dysfunctional circuitry.

IPS CELLS

W-1266

SYSTEM-DEPENDENT REPROGRAMMING ROADBLOCKS: THE PARADIGM OF NANOG

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After the first generation of iPSCs, dozens of labs started investigating how cells regain pluripotency using a variety of reprogramming systems. However, the system of choice largely affects the reprogramming efficiency, suggesting different underlying molecular obstacles. For example, reprogramming with existing polycistronic cassettes containing all 4 Yamanaka factors in different orders led to distinct reprogramming efficiencies and proportions of partially reprogrammed cells. The most striking differences were observed between MKOS and OKMS (2A-peptide-linked, c-Myc-Klf4-Oct4-Sox2 = MKOS or Oct4-Klf4-c-Myc-Sox2 = OKMS); almost all colonies generated with the MKOS cassette became Nanog-GFP+ by day 15, while >95% of OKMS colonies remained Nanog-GFP-. Detailed analysis of their reprogramming processes by monitoring E-CADHERIN, CD44, ICAM1 and Nanog-GFP expression changes revealed that reprogramming with the MKOS and OKMS cassettes gave rise to distinct intermediate populations. Over 90% of cells had become E-CADHERIN+ by day 5 in MKOS reprogramming, while over 70% remained E-CADHERIN- in OKMS reprogramming. Even post-MET (Mesenchymal-Epithelial Transition) intermediates with the same CD44, ICAM1 expression pattern had distinct potential to form iPSC colonies and gene expression profiles. On the contrary, Nanog-GFP+ cells derived with either cassette had very similar gene expression and were pluripotent. These data highlight the existence of distinct roadblocks in different reprogramming systems. Similarly, we found that the significance of endogenous Nanog for successful reprogramming was also system-dependent. When optimal reprogramming cassettes were used, Nanog-/- MEFs could become iPSCs with comparable efficiency, routes and kinetics to wild type MEFs, rebutting previous studies that Nanog is critical for iPSC generation. Overall, we demonstrate that some reprogramming roadblocks like MET or Nanog absence are system-dependent highlighting the need to pursue mechanistic studies with close attention to the systems to better understand reprogramming.

W-1267

USING SENDAI REPROGRAMMING AS A VERSATILE TOOL ACROSS CELL TYPES AND SPECIES

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One of the challenges of iPSC core facilities is the diversity of cells they are asked to reprogram. This could be at the cell type level, with samples commonly originating from skin biopsies, blood samples or urine samples. Additionally, some labs are interested in other species, such as rodents or domestic species. In this poster we will present some insights regarding the sequential acquisition of markers of pluripotency during Sendai reprogramming of human fibroblasts. We will also present the summary of our Sendai reprogramming tryouts across species and cell types.

W-1268

GRANULOSA CELL-DERIVED IPSC EXHIBIT PRO TROPHOBLASTIC DIFFERENTIATION POTENTIAL

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Human induced pluripotent stem cells (hiPSCs) have been derived from various somatic cell types. Granulosa cells, a group of cells which surround oocytes and are obtained from the (normally discarded) retrieved egg follicles of women undergoing infertility treatment, are a possible cell source for induced pluripotent stem cell (iPSC) generation. Here, we explored the possibility of using human granulosa cells as a donor cell type for iPSC reprogramming, and compared granulosa cell-derived iPSCs (iGRAs) with those derived from other cell sources, to determine the potential suitability of iGRAs for the study of female reproductive disease.

W-1269

AN IPSC BASED BASED FOR ALZHEIMER S DISEASE MODELING

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Alzheimer's disease 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 Alzheimer's disease. Therefore, we first established a robust and scalable differentiation protocol into neural precursor cells. Secondly, we undertook a comprehensive study of the differentiation of these precursor cells into neurons. We analyzed the transcriptome of cells differentiated with 4 alternative protocols over the course of 5 weeks. Amongst other trends, we identified a strong direct correlation ($R=0.971$, $p<0.0001$) between the expression of APP and neuronal markers, reinforcing the concept that optimized differentiation is necessary for accurate modeling. We then proceeded to use patient-derived iPSCs to investigate the production of amyloid peptides (Aβ42 and Aβ40) and other pathological hallmarks of Alzheimer's disease, such as phosphorylated Tau. While the relevance of β-amyloid production in the context of neuronal death still needs to be elucidated, our model provides a robust platform to investigate this disease and to test therapeutically relevant compounds in vitro.

W-1270

GENERATION OF A COHORT OF HUMAN IPSC AND IPSC-DERIVED CARDIOMYOCYTES FROM 222 INDIVIDUALS TO STUDY THE ROLE OF HUMAN VARIANTS IN CARDIAC PHENOTYPES

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Genome-wide association studies have identified hundreds of DNA variants associated with cardiac diseases, but few are understood on a functional level. To investigate how genetic variation influences molecular phenotypes in cardiac human cells, we have successfully generated over 1,000 human induced pluripotent stem cell (hiPSC) lines from 222 distinct individuals using a high-throughput protocol. This resource will enable functional genetic studies aimed at understanding the role of human variants in cardiac phenotypes. Our cohort comprises 82 singletons, 140 individuals from 41 families including 8 monozygotic and 2 dizygotic twin pairs, where 46 individuals have cardiac traits of interest and the remainders serve as healthy controls. Whole-genome sequencing (30X) was performed on DNA from blood samples of all individuals. Fibroblasts from each individual were transformed into hiPSC using Sendai virus and on average three clones were frozen at passage 3, of which one was further expanded and frozen at passage 12. To track sample identity and identify karyotype alterations in the hiPSC lines, we hybridized DNA from the hiPSCs and matched germline to the Illumina HumanCoreExome arrays. We observed high sample fidelity with only two hiPSC lines that were inconsistent with the germline DNA and therefore removed from the study. To identify high-resolution copy number variants (CNVs), we optimized SNP array methods for large-scale digital karyotyping and determined that more than 50% of the hiPSC lines had no detectable CNVs and only 9% showed large (>1Mbp) CNVs, which is lower than previous estimates for established hiPSC lines. To study how cardiac disease-associated variants function in relevant human cells, we are deriving cardiomyocytes from these hiPSCs through a highly standardized pipeline that we have developed. We freeze the hiPSC-derived cardiomyocytes at day 15 and then unfreeze in batches for molecular and physiology functional assays. These cardiomyocytes are 80-90% pure as verified by cTNT, cTNI and MLC2A staining and manifest synchronous beating. This resource of high quality, low passage hiPSC lines will allow for interrogation of common and rare genetic variation across a number of disorders and will be made publicly available as part of the NHGRI sponsored NextGen Consortium through WiCell.

W-1271

ASSESSMENT OF HUMAN PLURIPOTENCY: A COMPARISON OF TERATOMAS AND IN VITRO ASSAYS

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The teratoma assay is considered as the gold standard to test pluripotency of human pluripotent stem cells (PSCs) in vivo. Nevertheless the assay is animal-dependent, time-consuming, expensive, difficult to quantify and experimental procedures are not standardized. Recently in vitro assays for the assessment of pluripotency have been developed: whereas PluriTest analyses the gene expression profile of undifferentiated PSCs the Scorecard test evaluates their short-term differentiation potential into the three germ layers. Whether teratomas can be replaced by the in vitro assays is currently a matter of debate. Here we tested a validated human embryonic stem cell line (H9), an induced PSC line with reactivated transgenes (RT-hiPSCs) and a differentiation-defective embryonal carcinoma (EC) line in the various assays. H9 and RT-hiPS cells were cultured on vitronectin in TESR-E8 media. For PluriTest total RNA was isolated from undifferentiated cells with more than 90% of the cells expressing TRA-1-81 and SSEA4 as determined by FACS. Both H9 and RT-hiPSCs had comparable pluripotency scores indicating a similar pluripotency status. The EC cells scored less but were still considered pluripotent. For the teratoma assay 1x 106 cells were injected with matrigel subcutaneously. Tumors were harvested when reaching a diameter of 1cm. As expected EC-derived teratomas consisted mainly of undifferentiated cells expressing OCT3/4 and Nanog as shown by immunofluorescent (IF) staining. Interestingly the RT-hiPSC teratomas also contained significant amounts of undifferentiated cells whereas those cells were absent in H9 teratomas. IF staining of markers representing ectoderm (βIII-tubulin), mesoderm (smooth muscle actin (SMA)) and endoderm (α-fetoprotein (AFP)) revealed derivatives of the three germ layers in H9 and RT-hiPSC teratomas. By contrast EC-teratomas only contained SMA-expressing cells. For quantification of gene expression teratomas are currently being analysed by microarray. In addition all cell lines are being tested in in vitro differentiation assays for their capacity to generate ectoderm, mesoderm and endoderm. By comparing the results we will evaluate the potential of the in vitro assays to replace the commonly used in vivo method.

W-1272

TWEAKING MRNA REPROGRAMMING: MORE EFFICIENT, MORE VERSATILE

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Somatic cell reprogramming has become a key tool for disease modelling, cell fate regulation studies and drug screening. The next step will be to translate reprogramming in clinical grade conditions. To do so, we need fast, efficient and robust reprogramming protocols. mRNA reprogramming is the fastest and most faithful reprogramming method. However, it was originally restricted to fibroblasts. Another issue was the complexity of the protocol. In this poster we will present our enhanced mRNA reprogramming protocols, and the various cell types we managed to reprogram using those protocols.

W-1273

DEFINING DYNAMIC CHANGE IN DEVELOPMENTALLY EQUIVALENT HUMAN PLURIPOTENT CELLS

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Human pluripotent stem cells provide an ideal substrate for phenotypic analysis of variation between human genomes. To define the constant and variable features of renewal and early differentiation we developed precise methods for cell culture and spatial analysis of unconstrained colony formation. Time-lapse recording and live-cell immunocytochemistry revealed that after passage, a reproducible set of developmental domains is established across four days in monolayer iPSC culture. The establishment of monolayer colonies involves first mesenchyme aggregation into cell clusters that establish stable boundaries. Next, an epithelial core emerges on the internal surface with neuroectodermal properties of tight cell packing and expression of transcription factors SOX2/SOX21/OTX2. RNAseq analysis of these states through time using non-negative matrix factor analysis reveals patterns of dynamic gene expression that identify multiple cellular sub-populations and correctly predict differences in differentiation potential in iPSC lines from different donors. Similar to neural progenitor cells, iPSCs from any developmental domain are transiently reset to a more primitive state through standard cell passage after which they again regenerate developmental domains. Thus, although evidence of lineage priming in iPSCs predicts later developmental events, this priming program is routinely reset at every passage, suggesting that these cells are a developmental equivalence group during this early developmental period. Using this analytical platform we demonstrate quantitative differences in the kinetics of domain production in iPS cells from multiple donors. Powerful image segmentation and analysis approaches make this system amenable to chemical library screening. These results show at the level of single-cell resolution, models of self-renewal and differentiation must consider the critical domains of space, time, and donor genotype.

W-1274

PHOSPHATIDIC ACID IMPROVES REPROGRAMMING WITHOUT COMPROMISING THE QUALITY OF IPS CELLS

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Phosphatidic acid (PA) is the biosynthetic precursor of cellular acylglycerol lipids that has diverse functions such as influencing membrane curvature or recruiting cytosolic proteins to appropriate membranes. As an important component of cellular membrane, it is not clear whether PA will affect the cell reprogramming and iPS cell generation. Here, using cell reprogramming with Yamanaka factors as a model system, we found that inhibiting the production of PA dramatically inhibited the iPS cell induction. Supplement of PA in culture significantly increased the cellular PA in mouse embryonic fibroblasts (MEF) and almost tripled the efficacy of iPS cell generation. The iPS cells that were generated in the presence of PA expressed the pluripotent markers such as Oct4 and Nanog, differentiated into cells of the three germ layers in vitro and contributed to form chimera mice when they were injected into blastocyst. In tetraploid

complementation assays, iPS cells that were generated with or without PA produced vital mice at a similar rate, indicating that PA treatment did not affect the grade of pluripotency. Although PA did reduce cell apoptosis at late stage of reprogramming, the improvement of iPS cell generation was unlikely due to the selective amplification, or decreased cell death of the PA induced iPS cells, as PA was effective in improving the iPS cell induction only when it was used during the early to mid- stage of reprogramming. Among possible effectors that might act downstream of PA treatment, we identified that Zeb1/Zeb2 and E-cadherin exhibited a sequential change during the 12 days of reprogramming when PA was administrated. Zeb1 and Zeb2 were both up-regulated by PA during the first 5 days. In the following 5 days, PA treatment repressed the expression of Zeb2 but did not affect that of Zeb1 significantly. PA treatment sustained E-Cadherin at low level until the reprogramming was about to accomplish at day 12. We thus identified a novel type of culture supplement that is cellular component in nature, can improve the efficiency of reprogramming by initiating a sequential change from pro epithelial-to-mesenchymal transition (EMT) to pro mesenchymal-to-epithelial transition (MET) yet without affecting the quality of iPS cells, which would be valuable to generate high quality of iPS cells for further application.

W-1275

TOWARDS GENERATION OF CLINICALLY SAFE HUMAN IPS CELLS FROM VARIOUS CELL TYPES

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Human pluripotent stem cells (hPSCs), which include human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPS cells), hold great promise for treating incurable neurological diseases. Therefore, efficient generation of safe (xeno- and integration-free) iPSCs is absolutely required for their therapeutic use. In this study, we reported an efficient culture method to generate clinically compliant iPS cells using small molecules. This culture system is of great use to propagate iPS cells and hESCs without differentiation. Furthermore, it provides a very efficient platform to generate xeno-free and chromosome integration-free iPS cells for clinical purposes. In addition, we examined various human somatic cells for their reprogramming propensity. Taken together, our method for clinically safe iPSC generation will provide an important platform for the generation of iPS cells from any type of human somatic cells for use in cell therapies. This work was supported by grants from the Stem Cell Research Program (2010-0020347) and 2012M3A9C7050130 from the MSIP, and A120254-1201-0000200 from the Ministry of Health and Welfare, Korea

W-1276

TUMORIGENICITY ASSAYS OF CARDIOMYOCYTES DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Although transplantation of cardiomyocytes derived from induced pluripotent stem cells (iPSCs) into the heart is a promising approach for heart disease, presence of residual undifferentiated iPSCs may limit safety of this treatment. Transplantation of human iPSCs into nude rat leads to the formation of differentiated tumors comprising all three germ layers, resembling spontaneous human teratomas. Teratoma assays are considered the standard for demonstrating differentiation potential of pluripotent human iPSCs and hold promise as a standard for assessing safety among human iPSCs-derived cell cardiomyocytes (hiPSCs-CM) intended for therapeutic applications. We examined three in vitro tumorigenicity assays (soft agar colony formation assay, flow cytometry assay and quantitative real-time polymerase chain reaction [qRT-PCR] assay) and in vivo assay to detect residual undifferentiated cells. Soft agar colony formation assay was unable to detect hiPSCs and hiPSCs-CM. Next, we tried to detect residual undifferentiated cells by flow cytometry using six antibodies which recognize stem cell marker antigens, (Oct3/4, Nanog, SSEA-3, SSEA-4, TRA1-60, and TRA1-81). The anti-TRA1-60 antibody clearly distinguished hiPSCs from cardiomyocytes; it detected 0.1% undifferentiated hiPSCs that were spiked in primary cardiomyocytes. Furthermore, to identify highly selective markers for undifferentiated hiPSCs, we compared mRNA levels of Oct3/4, Nanog, Sox2, Lin28 and Rex1 in undifferentiated hiPSCs and primary cardiomyocytes by qRT-PCR. Lin28 mRNA was not detected in primary cardiomyocytes, whereas the qRT-PCR assay detected 0.01% undifferentiated hiPSCs that were spiked in primary cardiomyocytes. In vivo study, the potency of teratoma formation was tested in nude rat. Among hiPSC-derived cardiomyocytes, cells that contain more than 0.33% positive fraction formed tumor. However, cell population that contained a Lin28 fraction less than 0.1% did not form tumors. We established parameters for detecting residual tumor-forming cells. Our results provide highly sensitive and quantitative assays essential for facilitating safety studies of hiPSC-derived cardiomyocytes for future therapeutic application.

W-1277

GENERATION AND MUSCULAR THERAPEUTIC POTENTIALS OF EQUINE INDUCED PLURIPOTENT STEM (IPS) CELLS

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As the horse related industries and sports filed develop, health of horse has become one of major concerns to the horse industry. The importance of healthy muscle of horse in the performance of exercise, as well as the activities of daily living, has never been questioned. In this study, we report generation and examination of the muscle therapeutic potentials of equine iPS cells. We reprogrammed equine adipose-derived stem cells into iPS cells using polycistronic lentiviral vector (combination of four transcription factors, Oct4, Sox2, Klf4, and c-Myc)) and checked pluripotent characteristics. And then, the established E-iPS cells transplanted into muscle-injured rag/mdx mice. In microscopic findings, E-iPS cells transplanted mice showed enhanced muscle regeneration compared to the mice were not. In additions, myofibers induced from equine iPS cells were observed in injured muscles, although undifferentated cells also existed. In conclusion, we consider that equine iPS cells pave the way for more understanding equine iPS cells technology and development of muscle regeneration therapy in veterinary medicine,while there remain challenges of assuring safety.

W-1278

SIRT2 IS NECESSARY FOR EFFICIENT REPROGRAMMING TO PLURIPOTENCY

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Somatic cells can be stochastically reprogrammed to pluripotent stem cells. Thus, there have been lots of studies to decipher the reprogramming mechanism and to find new factors for enhancing the reprogramming efficiency. Among the numerous candidate factors to promote reprogramming efficiency, we have focused on sirtuin (Sir2), a mammalian homolog of yeast silent information regulator 2. Based on previous studies, Sirt1 and Sirt6 assists reprogramming to pluripotency, but little is known about the role of other sirtuins (Sirt2, Sirt3, Sirt4, Sirt5, Sirt7) in the reprogramming. Because Sirt1 and Sirt2 showed a reverse expression pattern during embryonic stem cell (ESC) differentiation, we hypothesized that Sirt2 may impede the reprogramming to pluripotency.Employing Sirt2-knockout (KO) mice in breeding, we got three types of mouse embryonic fibroblasts (MEFs); Sirt2-WT (+/+), Sirt2-HT (+/-), Sirt2-KO (-/-). Subsequent reprogramming was conducted using those MEFs and Yamanaka factors. It was intriguing that the result was opposite to our initial hypothesis. From the repetitive tests, Sirt2-KO MEFs seldom made ESC-like colonies. Also in the case of Sirt2-HT MEFs, numerous colonies were made from them but the real induced pluripotent stem cell (iPSC) colonies were less than those from the Sirt2-WT MEFs. Because Sirt2 plays a key role in cell cycle regulation, its deficiency may hamper the robust proliferation of cells, which is known as an essential condition for successful reprogramming to pluripotency. Although the reason of the low expression level of Sirt2 in pluripotent cells remains elusive, Sirt2 is necessary to reprogram the somatic cells into iPSC as similar to other sirtuins known for enhancing reprogramming to pluripotency.

W-1279

EXOGENOUS FGF2 MODULATES EXPRESSION OF ACTIVIN A FROM HUMAN FORESKIN FIBROBLAST TO SUPPORT HIPSC PLURIPOTENCY

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The development of an in vitro model of disease based on human induced pluripotent stem cell (hiPSC) requires a precise control of cell culture microenvironment to keep hiPSC in an undifferentiated state. Traditionally, the feeder cells layer, which are originated from mouse or human fibroblasts, and exogenous FGF2 have been used to prevent differentiation of hiPSCs during prolonged cell culture. However, the ability of exogenous FGF2 to control the stemness of hiPSCs on human foreskin fibroblast (HFF)-derived feeder cells is not well known. In this study, we found that the efficiency to maintain undifferentiated hiPSCs using HFF depend upon the method used to inactivate HFF. Human iPSCs obtained from reprogrammed lymphocytes by Sendai virus transfection with OCT3/4, KLF4, SOX2, c-MYC were cultured on mitomycin C-inactivated HFF (M/C HFF) or irradiated HFF (iHFF). Human iPSC rapidly differentiated without exogenous FGF2; however, hiPSCs on iHFF maintained less than 50% undifferentiated states even without exogenous FGF2. Undifferentiated hiPSCs were increased depending on 0, 4 and 40 ng/µl concentrations of FGF2, relatively in both M/C HFF and iHFF. Analyses of mRNA expression from HFF revealed significant differences between the methods of inactivation. Irradiated HFF expressed lower levels of FGF2, Activin A, TGFß-1, BMP2, and BMP4 when they were compared to M/C HFF. Moreover, interestingly, Activin A, which is a key regulator of stemness of hiPSCs, was highly increased by exogenous FGF2 in M/C HFF even at 4 ng/µl concentration. On the other hand, there was a small change in the expression level of Activin A in iHFF at 40 ng/µl FGF2. These results suggest that exogenous FGF2 is crucial to maintain undifferentiated states of hiPSCs on HFF for long term culture, by modulating the expression of Activin A, specifically in M/C HFF. Human foreskin fibroblasts revealed critical expression differences of trophic factors, according to the method of inactivation. Our results may be expanded to develop the clinical grade of hiPSCs grown in optimized xeno and feeder-free conditions for regenerative medicine.

W-1280

ANEUPLOIDY IN IPS CELLS DERIVED FROM ELDERLY ALS PATIENT

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Induced pluripotent stem cells (iPSCs) offer an unlimited source of mature cells and tissues for autologous cell replacement therapies to treat neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS). However, iPSC lines harbor various genomic, epigenomic and transcriptomic abnormalities compared to their alternative counterparts derived by somatic cell nuclear transfer (nuclear transfer-embryonic stem cells; NT-ESCs). Here, we investigated karyotype stability in multiple iPSCs and NT-ESCs derived from a 72-year-old patient with ALS. A total of 23 iPSC lines were generated from skin fibroblasts using non-integrating Sendai viral vectors. In addition, 2 isogenic NT-ESC lines were produced using our previously reported SCNT approach. Cytogenetic analysis was performed by G-banding on six randomly selected iPSC lines and two NT-ESC lines at an early passage (under 7) and at later passages (15-17). Several chromosome aberrations including gain, loss or structural changes (translocations, insertions, additions or deletions) were found in some cell populations (10-30%) in 4 out of 6 examined iPSC lines at early passage. However, the percentage of cells carrying these aberrations increased significantly in later passages of iPSCs reaching 50-100%. In contrast, no cytogenetic abnormalities were seen in NT-ESCs and they remained stable during extended culture. In summary, we demonstrate that iPSCs derived from an elderly ALS patient are prone to progressive accumulation of cytogenetic abnormalities with culture and passaging. In contrast, alternative pluripotent cells derived by SCNT maintain a stable karyotype.

W-1281

FDG-PET IS THE SHARPEST IMAGING TOOL IN DIAGNOSING TERATOCARCINOMA FORMATION FOLLOWING TRANS-PLANTATION OF INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIAC TISSUE CONSTRUCTS IN MURINE.

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Tumorigenic potential is the major limitation in realizing transplantation therapy of induced pluripotent stem cell (iPSC)-derivatives for treating cardiac disease. As well as elimination of tumorigenicity prior to the transplantation, early accurate diagnosis of the tumor formation after the transplantation is important in warranting safety of the treatment. We herein imaged tumor formation after transplantation of iPSC-derived cardiac construct in mice, by using the clinically latest imaging tools for tumor-diagnosis, such as computed tomography (CT), enhanced magnetic resonance imaging (MRI), ultrasonography (US) and fluorodeoxyglucose (FDG)-positron emission tomography (PET) study. iPSC-derived cardiac constructs was generated from iPSCs of C57BL/6N mouse origin, showing 5-10% positivity of SSEA-1 and 60-70% positivity of cardiac troponin T. This cardiac constructs that formed cell-sheets under thermoresponsive dish were transplanted over the cardiac surface of the C57BL/6N mice as a syngeneic tissue transplant model (n=12). Histological study showed neoplastic growth from the transplanted tissue constructs at day 3 and then formation of teratocarcinoma at day 7, while Ki67 index, assessed by immunohistolabelling, was significantly greater at day 7 or day 10 than that at day 3 or day 5. Serial FDG-PET studies detected a high FDG uptake in the cardiac surface at day 7, with the standardized uptake value (SUV) max being significantly higher at day 7 (8.20 ± 0.66) or day 10 (8.87 ± 0.87) than day 3 (3.93 ± 0.31) or day 5 (3.20 ± 0.70, $p<0.05$). The SUV max was significantly correlated with the Ki67 index ($R2=0.76$). There was no false positive uptake of FDG. In contrast, tumor formation was detected by enhanced MRI at day 10, and not detected by CT or US studies by day 10 in the same mice studied by FDG-PET. FDG-PET study achieved the earliest diagnosis of teratocarcinoma formation following cardiac-transplantation of iPSC-derived cardiac constructs amongst the clinically available imaging tools in murine, indicating usefulness of this imaging tool to guarantee safety of the clinical study using iPSC-derived cardiac constructs.

W-1282

REVERSION OF THE HUMAN IPS CELLS TO NAIVE PLURIPOTENT STATE BY SHORT-TERM EXPRESSION OF REPROGRAMMING FACTORS

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Human pluripotent stem cells, including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, show different characteristics compared with mouse ES cells derived from inner cell mass (ICM) of blastocyst stage embryos. It is possible that the difference represents a different developmental stage because human ES/iPS cells share many characteristics with mouse epiblast stem cells (EpiSCs) derived from postimplantation embryos. The pluripotent state of mouse EpiSCs is termed primed state to distinguish from naïve state pluripotency of mouse ES cells, and conventional human pluripotent stem cells also have been regarded as primed state pluripotent stem cells. Using human iPS cells, we have carried out the conversion from primed state to naïve state pluripotency. Doxycycline (Dox) -inducible reprogramming factors transgenes were introduced into human iPS cells by piggyBac transposon system, and the cells were cultured in a medium containing LIF, Dox and cocktail of small molecules. As a result, the cells formed mouse ES cell-like dome-shaped colonies and elevated expression of naïve markers. Furthermore, TGF-beta/activin inhibitors did not suppress expression of pluripotency markers, unlike conventional human pluripotent stem cells. Moreover, removal of Dox also did not suppress expression of pluripotency markers, meaning that these converted cells could be maintained without continuous expression of transgenes. The properties of the human iPS cells were similar to those of mouse ES cells, suggesting that the cells acquired naïve state pluripotency.

W-1283

INDUCTION, FUNCTIONAL CHARACTERIZATION AND DIFFERENTIATION OF HUMAN MAMMARY EPITHELIAL AND STROMAL DERIVED PLURIPOTENT STEM CELL LINES FROM NORMAL AND PRECANCEROUS BRCA1/2 MUTANT BREAST TISSUE.

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Breast cancer is the most common cancer to affect women worldwide. The majority of breast cancer cases result from spontaneous mutations however rare germ-line mutations, such as those in BRCA1 and BRCA2 tumor suppressor genes, predispose carriers to developing breast cancer. The precise molecular and cellular mechanisms that lead to an increase in lifetime risk remain to be elucidated. Interestingly, an expanded population of aberrant luminal progenitor cells was identified in BRCA1 preneoplastic tissue, suggesting that these cells serve as the likely cell of origin for basal like breast cancer. We have derived a series of human induced pluripotent stem cell (hiPSC) lines from breast epithelial and stromal cells isolated from BRCA1 and BRCA2 mutation carriers and from non-carriers who undergo prophylactic mastectomy or reduction mammoplasty. These cell lines have been extensively characterized to validate their pluripotent potential, to establish their immunophenotype, confirm that they retain a normal karyotype, and verify their BRCA1/2 mutation status. Using directed differentiation, we are currently performing functional assays to promote commitment to a mammary epithelial cell fate. This study should provide a renewable source of cells harboring BRCA1 or BRCA2 mutations and help define master regulators of mammary epithelial fate to enable the elucidation of mechanisms underlying breast tumor development in mutation carriers.

W-1284

PLANT EXTRACTS STIMULATE THE REPROGRAMMING OF SOMATIC CELLS TO PLURIPOTENCY

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Natural plant extracts have been an abundant and valuable source of bioactive small molecules for drug discovery. Natural products themselves or direct derivatives of them have continued to provide small molecules that have entered clinical trials, such as anti-cancer and -microbial drugs. Especially, small molecules modulating pluripotent stem cell fate and property can allow to be widely applied in clinical uses for regenerative medicine. Here, we tested 3700 extracts from native plants to examine whether they can improve induced pluripotent stem cell (iPSC) generation using genetically homogeneous secondary mouse embryonic fibroblasts (MEFs) harboring the doxycycline (dox)-inducible OSKM transgenes. Among tested, extracts from duramen and stalk of Camellia japonica could enhance iPSC generation. Camellia japonica is one of the best known species of the genus Camellia that belongs to the Theaceae family. Our findings identified, for the first time, nature product from plant extracts as a novel regulator capable of enhancing cellular reprogramming and will provide useful tool in the development of a more efficient and safer way to produce clinical-grade of iPSCs.

W-1285

GENERATION OF INDUCED PLURIPOTENT STEM CELLS WITHOUT GENETIC DEFECTS BY SMALL MOLECULES

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The generation of induced pluripotent stem cells (iPSCs) often causes genetic and epigenetic defects, which may limit their clinical applications. Here, we show that reprogramming in the presence of small molecules preserved the genomic stability of iPSCs by inhibiting DNA double-strand breaks (DSBs) and activating Zscan4 gene. Surprisingly, the small molecules protected normal karyotype by facilitating repair of the DSBs that occurred during the early reprogramming process and long-term culture of iPSCs. The stemness and cell growth of iPSCs(+) were normally sustained with high expression of pluripotency genes compared that of iPSCs(-). Moreover, small molecules maintained the differentiation potential of iPSCs(+) for the three germ layers, whereas it was lost in iPSCs(-). Our results demonstrate that the defined small molecules are potent factors for generation of high quality iPSCs with preservation of genomic integrity by facilitating the reprogramming process.
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W-1286

THE INHIBITION OF PGC-1ALPHA EXPRESSION DON´T INHIBIT IPSC PLURIPOTENCY

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PGC-1alpha is an important transcription coactivator that plays a central role in the regulation of cellular metabolism. It stimulates mitochondrial biogenesis in human cells and is related with many disorders, including cardiomyopathies. Embryonic stem cells can generate any type of human body cell but they can´t be ethically used. So, induced pluripotent stem cells (iPSC) are an efficient model to study cardiomyocytes physiology and heart disorders. Our goal was to investigate if PGC-1alpha could interfere with the pluripotency and its role in cardiomyocytes differentiation. We reprogramed fibroblasts into iPSC with the Stemcca lentivirus, and we used the conventional methods to characterize this cells (morphology, immunofluorescence, PCR, embryonic body, differentiation, karyotype, alkaline phosphatase). We inhibit the PGC-1alpha expression in iPSC with a shRNA lentivirus and we compared the expression of pluripotency factors by Real Time PCR. The cardiomyocytes differentiation was performed by the inhibition/activation of GSK3 and Wnt pathways. Fibroblasts have a very low expression of PGC-1alpha due to their low metabolic demand. Embryonic stem cells present higher expression of PGC-1alpha than iPSC maybe because this last cells are derived from fibroblasts. iPSCs inhibited with PGC-1alpha shRNA expresses the same pluripotency factors than normal iPSC. Cardiomyocytes derived from ESC present more PGC-1alpha expression than cardiomyocytes derived from iPSC and both cells have a markedly increased of the PGC-1alpha expression comparing with the pluripotent stem cells. Variations of PGC-1alpha expression don´t affect the pluripotency of hiPSC and cardiomyocytes express this protein at high levels due to their high energy demand.

W-1287

A NOVEL NON-CODING RNA INDUCES STEMNESS IN MOUSE AND HUMAN CELLS

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The genetic program of complex organisms including human are shown to be transacted by non-coding RNAs that rival that of proteins. Rather, the extent of control exerted by non-protein-coding RNAs increases with increasing complexity of the organisms, perhaps exceeding that of proteins in well-developed organisms, reaching an estimated value of ~ 98.8% in humans. Non-coding RNAs with a size of >200 bases categorized as long non-coding RNAs are dynamically transcribed, with many showing specific expression patterns and sub-cellular localizations, but their exact functions are not well defined. The emerging evidence indicates, however, that several of these long nc-RNAs control the epigenetic states that govern development and that many of them are dys-regulated in cancer and other complex diseases. Moreover, it appears that plasticity in these ncRNA functions might exist, especially in the brain. We have identified in our lab a pair of long non-coding RNAs (Ginir and Giniras) that is enriched in the brain and interestingly is conserved across evolution. These pair of over-lapping sense and anti-sense

transcripts is localized to mouse X- chromosome and are located on chromosome 6 in human cells. Our studies demonstrate role of one of these transcripts -Ginir in inducing stemness wherein its over-expression in human fibroblasts induces pluri-potency generating Induced pluripotent stem cell phenotype (hiPSC). Interestingly, the Ginir non-coding RNA transcript possesses the potential to induce de-differentiation when over-expressed in transformed neuro-epithelial cell-lines like SK-N-MC and generate cancer stem cell phenotype. By, using approaches of RNA-IP and RNA pull-down assays we have identified unique protein interacting partners of this pair of long non-coding RNAs and further shown the significance of these interactions in stemess and transformation. We provide evidence for genomic instability as driver for these processes and show role of chromatin remodeling and specific protein interacting partners for our RNA in these processes. We show that the processes of stemness and transformation are governed by common key regulatory mechanisms and un-ravelling role of non-coding RNAs in these processes may hold keys to delineation of these important biological processes in development and cancer.

W-1288

IMPACT OF CELL TYPE OF ORIGIN AND REPROGRAMMING METHOD ON THE CAPACITY OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO DIFFERENTIATE TOWARDS THE PANCREATIC LINEAGE

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Human IPSCs have the unique ability to self renew in vitro while maintaining their capacity to differentiate into derivatives of the three germ layers. Thus, they represent a valuable system for disease modelling, drug screening and ultimately cell-based therapy. The success of human IPSC cell-based therapies will depend on the development of efficient and robust methods to differentiate human IPSC into clinically relevant and safe population of cells. However, systematic studies are currently lacking to evaluate the quality and safety of human IPSC lines generated with different reprogramming methods and from different cell types. Here, we perform extensive genome wide analyses to define the impact of these different aspects on pancreatic cell production from human IPSCs. We generated human IPSC lines from both blood and fibroblasts of 5 donors using Sendai virus and episomal plasmids (n=45). In collaboration with the HipSci initiative, the resulting lines were characterised using extensive genome wide assays including RNA-seq, ChiP-seq, exome sequencing, 450K-methylation array as well as M-FISH karyotyping. Furthermore, human IPSC lines were subsequently differentiated into pancreatic beta-like cells and subjected to RNA-seq and functional characterisation. These analyses reveal the impact of several confounding factors that could affect pancreatic cells production from human IPSCs including genetic variability, impact of reprogramming and somatic cell memory. Together, these results provide an approach to characterise at a deeper molecular level human IPSCs and their differentiated derivatives thereby providing new standards for their use in the clinic.

W-1289

REPROGRAMMING OF HUMAN DERMAL FIBROBLASTS BY RECOMBINANT PROTEINS

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Induced pluripotent stem cells (iPSCs) are novel candidates for disease modeling, drug screening, regenerative medicine and cell therapy. But integration of the transcription factors genes limits the utility of iPSCs because of the risk of mutations being inserted into the target cell's genome. We avoid the use of viral or DNA-based expression vectors within target cells using a novel method utilizing an inactivated viral particle to deliver four isolated Yamanaka transcription factor proteins (Sox2, Oct4, Klf4 and c-Myc), resulting in reprograming of human fibroblasts. Recombinant purified proteins entered the cytoplasm, translocated into the nucleus and were degraded within 72 hrs. The reprogrammed fibroblasts show high expression levels of pluripotency markers, and successfully differentiated into three embryonic germ layers, namely osteoblast, neuron and pancreatic islet cells, after induction in vitro. Adipose and bone tissue can also be constructed in vivo from these cells with 3D scaffolds. Furthermore, specific combinations of transcription factors directly differentiated fibroblasts into cell types representing three embryonic germ layers, namely adipocyte, neuron and hepatocyte. Thus, protein-based iPSC reprogramming potentially offers a novel high efficiency technology for regenerative medicine using patient specific cells as a source for therapeutic applications.

W-1290

DEVELOPING ROBUST MANAGEMENT PROCEDURES FOR HUMAN INDUCED PLURIPOTENT STEM CELL LINE ACQUISITION FROM A PROFESSIONAL IPSC RESOURCE CENTRE: EXPERIENCE OF THE EUROPEAN BANK FOR INDUCED STEM CELLS (EBISC)

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The EBiSC iPSC cell bank is being established under joint support from the Innovative Medicines Initiative (IMI; http://www.imi.europa.eu/) (€24M) and in kind contributions from a consortium of European Federation of Pharmaceutical Industries and Associations

(EFPIA) members (AstraZeneca AB, H Lundbeck A/S, Janssen Pharmaceutica AB, Novonordisk A/S, Pfizer Ltd, UCB Biopharma SPRL). It will provide access to 1000 iPSC lines from disease affected and unaffectedindividuals (www.ebisc.org/). Implementation of robust procedures for cell line acquisition is vital to assure they are fit for purpose for disease research and development of assays for industry. This requires detailed scrutiny of ethical provenance, safety screening, scientific characteristics and intellectual property issues. We have established acceptability criteria and a management process to approve new lines, and assure consistency at an early phase of accession. These procedures are captured in a Quality Manual designed to assure consistent delivery of high quality cell lines to users. A fast track process has been established with project partners at the Universities of Bonn, Cologne, Hubrecht, Newcastle, Instituto de Salud Carlos III, Bioneer and Roslin Cells, to provide early release of established iPSC lines in a "Hot-Start" phase. Data characterising these lines supplied by the depositors will be available via the hESCreg database (www.hescreg.eu/). Cell lines will be stored at central and mirror banking facilities at Roslin Cells, Edinburgh UK, and Fraunhofer IBMT, Sulzbach Germany, and distributed via the European Collection of Cell Cultures, Public Health England, UK) with certificates of analysis for each lot of cells. A large number of Hot-Start lines have either been deposited or derived, the first cell lines supplied to customers in December 2014. It is possible that additional cell line supply partners include other IMI funded projects (e.g. StemBANCC, EU-AIMS) and the UK MRC and Wellcome Trust funded HiPSCi project. Further cell lines of value to the research community are currently being commissioned. Centralised banking and testing at larger scale will be carried out at a new Roslin Cells facility at Babraham, UK. This poster will present the procedures for the acquisition for a large scale and responsive iPSC supply.

W-1291

MYBL2 AS A GATEKEEPER OF SOMATIC CELL REPROGRAMMING

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Mice generated without Sox2 or Oct4 fail to develop past the blastocyst stage due to impaired formation of the inner cell mass. This phenotype is mirrored in mice lacking the transcription factor MYBL2 (B-Myb), highlighting the importance of MYBL2 during the early stages of embryonic development. In fact, MYBL2 transcripts are 1000-10000 times more abundant in embryonic stem cells (ESC) compared to somatic cells. Our previous work demonstrated that MYBL2 does not affect the expression of Oct4 and Sox2 levels, but MYBL2 controls replication dynamics necessary to maintain genome stability, possibly through the action of key multifunctional proteins such as c-Myc. In the non-physiological process of cellular reprogramming, induced pluripotent stem cells (iPSC) are successfully generated upon exogenous expression of a combination of four factors, namely Oct4, Sox2, Klf4 and c-Myc (OSKM). In agreement with high expression of MYBL2 in pluripotent cells, several groups have shown that its upregulation takes place during reprogramming at the same time as other pluripotency factors such as Nanog and Lin 28. In order to evaluate the relative importance of *MYBL2* during reprogramming we performed loss-of-function and gain-of-function assays similar to the ones already described for *Nanog* and *Lin28*. By using a polycistronic lentiviral vector encoding the transcription factors OSKM we have shown that *mybl2*Δ/Δ MEFs are unable to be reprogrammed. Unexpectedly, and contrary to the gain-of-function assays described for Nanog or Lin28, overexpression of MYBL2 also inhibits somatic reprogramming in both primary and secondary reprogramming systems. We have shown that MEFs infected with an OSK-Bmyb polycistronic lentiviral vector do not senescence or die and that this lack of reprogramming is independent of the p53 pathway. Our preliminary results show that MYBL2 seems to be regulating the expression of genes required for the proper transition of the reprograming phases. These results show that MYBL2 has an important role in reprogramming and are investigated further. Understanding MYBL2 mediated inhibition will lead to insights into the molecular mechanisms of reprogramming process.

W-1292

DIFFERENTIATION OF FUNCTIONAL ISLETS FROM HUMAN IPS CELLS IN VITRO

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Pancreatic islets, also known as islets of Langerhans, are clusters of endocrine cells, including insulin-producing β cells, glucagon-producing α cells, somatostatin-producing δ cells, pancreatic polypeptide producing PP cells, and ghrelin-producing 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 in vitro from human ES/iPS cells, it has been difficult to produce islets with proper functions and structure. We previously showed that functional islets with a 3-dimensional structure consisting of multiple endocrine cells were formed in vitro from mouse fetal pancreatic cells. And by adopting this culture system to the differentiation protocol of mouse ES/iPS cells to pancreatic cells, we generated islets with proper structure and function of adult mouse pancreatic islets. By further modifying this culture system to human iPS cells, we were able to generate human pancreatic islet-like cell clusters. In this differentiation system, cell clusters of endocrine cells were formed on adherent cells derived from human iPS cells. Moreover, the cell clusters including α, β, and δ cells, and exhibited a three-dimensional structure similar to human islets. Those islet-like cell clusters secreted human c-peptide in response to a high glucose concentration 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. These results indicate that the functional islets can be generated from human iPS cells. We are currently trying to scale up the culture system for production of a large quantity of islets.

W-1293

NOVEL METHOD FOR THE GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM HUMAN PERIPHERAL BLOOD

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In terms of 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-available 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 show a novel population of peripheral blood-derived stem cells, which can be easily reprogrammed to iPS cells. We isolated peripheral mononuclear cells (PBMC) from human peripheral blood and cultured them on the fibronectin-coated or non-coated plate. 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. Furthermore, we have also confirmed that these cells can be differentiated into adipogenic, osteogenic, 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 than 65 iPS cell lines from PBMC of patients with diverse diseases and normal volunteers. Our study 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 approach could be one of ideal methods for clinical application of iPS cells in the near future.

W-1294

A NON INTEGRATIVE STRATEGY DECREASES CHROMOSOME INSTABILITY AND IMPROVE REPROGRAMMING OF PORCINE INDUCED PLURIPOTENT STEM CELLS.

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In the present study, we compare the effect of integrative and non integrative strategies on chromosome stability during pig somatic cells reprogramming to pluripotency. We overexpressed Oct4, Sox2, Klf4 and c-Myc in pig fibroblasts by retrovirus or lentivirus (integrative, I) or Sendaï (non-integrative, NI) virus transduction in different genetic backgrounds. The induced Pluripotent Stem (iPS) cell lines were characterized for pluripotency, cell cycle and differentiation potential by conventional methods. Genomic and chromosome stability was analyzed by G-banding karyotype, Comparative Genomic Hybridization and FISH. The porcine iPS cell lines produced with integrative strategies (I-iPSCs) harbored characteristics of ground and naïve pluripotency when cultured in specific media. They expressed several pluripotency genes and harbored an ES-like cell cycle. Nevertheless, contrary to mouse and human iPS, they did not silence the integrated exogenes, leading to a poor differentiation potential. Moreover, cytogenetic analysis revealed a high genomic instability upon passaging which suggest the development of population with an increased selective advantage. We characterized the selected duplications and compared them to those previously described in other species. In contrast, non-integrative reprogramming system gives us promising results regarding differentiation potential and genomic stability. Pig NI-iPSCs exhibit a normal karyotype after more than 12 months in culture and maintain the expression of endogenous pluripotency markers at a higher level than I-iPSCs. Pig NI-iPSCs differentiate in vitro in structures that mimick the blastocyst morphology with compact clumps of Sox2 positive cells surrounded by large epithelial Sox2 negative cells. We propose that these NI-iPSCs can be used as a model to bring new insights into the molecular factors controlling and maintaining pluripotency in the pig species.

IPS CELLS: DIRECTED DIFFERENTIATION

W-1296

DIFFERENTIATION OF HUMAN MULTIPOTENT AND PLURIPOTENT STEM CELLS TO DEVELOP A HUMAN 3D IN VITRO BLOOD BRAIN BARRIER MODEL

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Human induced pluripotent stem cells (hiPSCs), as well as human multipotent fetal stem cells, pose reliable and effective cell sources to generate differentiated functional brain cells and have the advantage of being independent of postnatal brain tissue biopsies, including their variations and limitations during in vitro culture. The aim of this study is the direct differentiation of hiPSCs into human brain astrocytes and endothelial cells. In particular, we want to develop a 3D in vitro co-culture model, which closely simulates the in vivo situation and can be used as a tool in preclinical research such as in drug transport or infection studies. We are able to maintain undifferentiated hiPSCs under feeder free conditions shown by positive FACS analyses for the pluripotency markers Nanog, Oct 3/4, Sox-2 and alkaline phosphatase assay. Furthermore, differentiation protocols to generate brain endothelial cells as well as human astrocytes from iPSCs and neural stem cells (NSC) mimicking the in vivo embryogenesis are performed as described recently. The NSCs and differentiated astrocytes were characterized by positive immunohistological stainings against Sox-1, Sox-2, Pax-6, S100-β and GFAP. BBB endothelial cells were positively-stained for CD31, ZO-1 and specific transporter molecules like glucose transporter 1 as well as tight junction proteins like claudin 5. Furthermore,

the relevant gens could be analyzed by PCR. Finally, different co-culture setups were performed and BBB integrity was tested by measurement of transepithelial electrical resistance (TEER) as well as permeability of 4 kDa FITC-Dextran, which results in tight barrier properties comparable to in vivo-like TEER values higher than 1000 Ω*cm^2. The results of our study demonstrate that we are able to differentiate human stem cells into BBB relevant cells. Co-culture of these cells can provide tight BBB models necessary for use in pre-clinical research. In order to closely mimic the microenvironment of the BBB in vivo we use 3D scaffolds consisting of collagen and basal membrane proteins and cultivation in dynamic flow reactor systems to simulate the bloodstream. To further improve BBB model characterization, we are using non-invasive methods like RAMAN spectroscopy and impedance measurement.

W-1297

DIRECTED DIFFERENTIATION OF HUMAN IPS CELLS IN A STIRRED VESSEL VIA DEFINITIVE ENDODERM INDUCED THE PAX8 AND TTF-1 POSITIVE EXPRESSING EBS

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Current therapy for the patients suffering from hypothyroidism depends on oral administration to supplement thyroid hormone. We have reported the tissue engineered thyroid sheets, utilizing the temperature-responsive culture dishes, could recover the thyroid function of the hypothyroidism rat models post-total thyroidectomy. Though regenerated thyroid tissue would be a novel alternative therapy for hypothyroidism, the problem relating to the cell source remains necessarily. In this study, we have tried to establish the beneficial culture protocol of human induced pluripotent stem cells (hiPSCs) into thyrocytes via definitive endoderm (DE) by evaluation Sox17 and FOXA2 as DE markers, and Pax8 and TTF-1 as the specific thyrocyte markers. hiPSCs were maintained on feeder layer cells and passaged properly. All procedures of differentiation were done in a stirred vessel for 15-30 days. At first, to form the embryoid bodies (EBs), the cells were collected by dissociation solution and the cell suspension was seeded in a stirred vessel. Secondary, EBs were then harvested in the medium including Activin A to DE for 3 days. As a final step, differentiation of EBs to thyrocytes was performed in the medium added thyroid-stimulating hormone (TSH) for 10-15 days. EBs were collected for analyses at every stages of undifferentiation, DE differentiation and thyrocyte differentiation. RT-qPCR analysis for *FOXA2* and *Sox17* presented the expression increasing significantly in EBs after DE differentiation compared with undifferentiated hiPSCs. Accordingly, 50-60% of cells in almost all of EBs were positive for FOXA2 and Sox17, indicating the successful endodermal differentiation. Moreover, when these endodermal differentiated EBs were further treated with TSH for 10-15 days, the mRNA expressions of Pax8 and TTF-1 in EBs were increased, and Pax8 and TTF-1 positive cells were clear observed in these EBs. In conclusion, hiPSc can differentiate into Pax8/TTF-1 positive thyrocyte progenitor cells via DE differentiation in a stirred vessel, and further optimization of culture conditions will collect us matured thyrocytes for regenerative medicine.

W-1298

DIRECT AND EFFICIENT DERIVATION OF SKELETAL MUSCLE CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS).

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Our ability to generate human induced pluripotent stem cells (hiPSCs) from healthy and patients-specific somatic cells provides us with a powerful tool for disease modeling or drug screening but also with a source of biological material for cell replacement therapies. However, so far modeling of muscular and neuromuscular disorders has been hampered by the lack of efficient differentiation protocols generating high yields and functional muscle cells. Here, we present a new protocol to generate isolated contractile multinucleated skeletal muscle cells and a protocol for co-culture of skeletal muscle and neuronal cells. Fully characterized hiPSCs derived from healthy human control fibroblast cells using the four Yamanaka's factors Oct4; Klf4; Sox2; C-Myc (OKSM) were maintained in feeder free m1TeSR medium. Induction of the differentiation process is performed in a serum-free chemically-defined medium, skeletal muscle progenitors are visible in 10 to 15 days. At this step progenitors (80-90% CD73/CD105 positive and CD56 low) can be frozen and thawed without loss of differentiation capacity. For final differentiation, skeletal muscle progenitors are grown to confluency in a specific medium. At confluency, cell morphology changes and fusiform cells start to orientate and fuse to form mature multinucleated skeletal muscle cells as seen after 24 hours time-laps recording. Generated skeletal muscle cells are expressing myogenic markers such as MyoD (58-70%), MYH2, MYH3, MYH7, MYH8, MGN and DESMIN. Alternatively, skeletal muscle cells are grown in presence of human neurons also generated from human induced pluripotent stem. Under these conditions, multinucleated skeletal muscle cells become striated and form contractile myotubes as shown by video recording. Using this efficient feeder and serum free procedure we observed skeletal muscle differentiation from hiPSCs in 40 to 50 days after induction. Finally we show that this protocol can be applied to investigate muscular dystrophies.

W-1299

ENHANCED NEURONAL DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN 3D CULTURE USING ELECTROCONDUCTIVE HYDROGEL

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Human induced pluripotent stem cells (hiPSCs) are reprogrammed cells from human somatic cells by delivering several defined factors. The patient-specific hiPSCs not only provide autologous cell therapy, but also provide biomedical platforms for disease modeling and drug screening for personalized medicine. Recently, neuronal differentiation of hiPSCs has been studied for neurodegenerative diseases. Diverse

biomedical methodologies have been applied to promote neuronal differentiation from hiPSCs for disease modeling and cell therapy. In this study, we report electroconductive 3D hydrogel for enhancing neuronal differentiation of hiPSC-derived neuronal progenitor cells (NPCs). Hyaluronic acid (HA) hydrogel with incorporation of carbon nanotubes was found to exhibit electroconductivity and mechanical properties for stem cell activation. The hiPSC-derived NPCs were highly viable in electroconductive HA hydrogel. 3D culture using the HA hydrogel led to the increased expression of neuronal markers in hiPSC-derived NPCs. Our results demonstrate that electroconductive 3D microenvironments can provide stem cell niche for enhancing neuronal differentiation of hiPSCs.

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W-1300

ELUCIDATE THE SIGNALLING PATHWAYS WHICH REGULATE THE QKI MRNA SPLICING ISOFORMS DURING VASCULAR CELL DIFFERENTIATION AND TEST THEIR FUNCTION IN CARDIOVASCULAR THERAPY

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Our objective was to investigate the mechanisms and regulation of mRNA binding protein QKI and its splicing isoforms during EC differentiation from iPSc for use in Regenerative Medicine. CVD is the leading cause of death worldwide and is characterised by EC dysfunction. Replacing damaged ECs has been a potential therapeutic option but identification and availability of appropriate cell types has been a major limitation. The underlying mechanisms which are implicated in the differentiation process to generate pure and functional populations of ECs are generally unknown and mRNA splicing adds an additional layer of complexity. QKI: QKI belongs to the family of highly conserved RNA binding proteins and is a pre-transcription regulator. QKI has been shown to have a key involvement in embryonic blood vessel formation and remodelling which is observed clearly in vivo where qki null mice were embryonic lethal. We have identified that QKI is a key player in EC differentiation derived from pluripotent stem cells. QKI has been shown to be upregulated in parallel with EC markers during vascular EC differentiation from iPS and ESCs. Our data supports that a unique pattern of its splicing events are regulated in a precise manner during EC differentiation. Overexpression of specific mRNA splicing isoforms of QKI induced the expression of EC markers, while their knockdown by shRNA resulted in their suppression. Notch signalling has been shown to be associated in embryonic vascular development. We now provide evidence that Notch signalling is upregulated when QKI is overexpressed during EC differentiation from pluripotent stem cells. Angiogenesis assays have revealed that QKI induced the vascular tube formation in vitro and vessel formation in vivo. In this study we have shown that functional ECs can be produced through defined culture conditions from pluripotent stem cells and that QKI has an essential role in the process. We provide strong evidence that QKI plays a critical role in EC differentiation derived from pluripotent stem cells, acting as a key regulator of EC-related signaling in vitro and in vivo. This knowledge will provide the tools and potential targets to establish highly efficient protocols to reprogramme functional ECs to be used in vascular therapy and personalised medicine.

W-1301

FUNCTIONAL HUMAN GENOMICS TO MAP LINEAGE PRIMING ONTO DEVELOPMENT AND COMMON COMPLEX DISEASE

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Pluripotent stem cells are at the center of innovation in biomedicine. To explore the diversity of human biology with these systems, it is critical to understand how they relate to in vivo development and how they map onto individual risk for complex human disease. We use novel computational approaches involving a sparse, non-negative matrix factorization (CoGAPS) to deconstruct genome-wide data in diverse biological systems. CoGAPS decomposes overall expression patterns across all genes into component patterns that, much like multiple regulatory elements, act in combination to construct the full dynamic expression pattern of each individual gene. This methodology enables the highly sensitive identification of differentiation dynamics in deep RNA-seq data from human ES and iPS cell lines as they self-renew and initiate differentiation. In addition, this analysis defines stable transcriptional signatures of individual genomes in self-renewal that bias their differentiation potential. We use this informatic dissection to map the dynamics of in vitro morphogenesis onto transcriptional and epigenetic data from distinct cells of the early embryo and from the developing and functioning brain throughout the lifespan. Transcriptional dynamics in pluripotent stem cells can be clearly identified in vivo. Signatures of individual genomes observed in pluripotent cells are stable throughout life and represent a cellular phenptype proximal to genome-wide variation in humans. These observations suggest that these approaches will powerfully define the unique biology of individual human genomes and guide the modeling of complex disease risk.

W-1302

HUMAN INDUCED PLURIPOTENT STEM CELL-BASED KERATINOCYTES FOR PATIENT SPECIFIC SKIN MODELS OF CONGENITAL KERATINIZATION DISEASES

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The generation of induced pluripotent stem cells (iPSC) from adult somatic cells opened an intriguing field of research with a focus

on the possible use of iPSC for therapeutic applications. Because iPSC carry the same genetic information as the adult cells used for reprogramming, these cells are a highly valuable tool for the study of genetic disease mechanisms as well as for patient-specific disease models. Autosomal recessive congenital ichthyosis (ARCI) is a rare skin disorder mainly characterized by impaired function of the skin barrier, leading to generalized scaling of the skin and erythema, and phenotypically and genetically heterogeneous. It is a severe condition, life-threatening at birth, with only symptomatic treatment options available, therefore effective and possibly causal therapies are urgently needed. Hence, the goal of this project is to establish an iPSC-based in vitro skin model system in order to develop and test new therapies for ARCI and other congenital skin diseases. We have generated several iPSC lines using the excisable STEMCCA lentiviral system; these cells were originated from skin fibroblasts of distinct ARCI patients with different causal mutations, in order to obtain models covering the mutation spectrum of this disease. As expected, the resulting iPSC share common features with ESC, like morphology, increased expression of pluripotency markers and the ability to differentiate into cells from the three germ layers. Also, Pluritest™ analysis of whole genome expression data pointed to a clear pluripotent profile of all iPSC lines. Cells obtained from ARCI-iPSC early in the ectodermal differentiation process (day 7 to 15) express the ectodermal marker TP63 and a marker for simple epithelia KRT18. Later in differentiation (day 30-40), basal keratinocytes-specific markers KRT5 and KRT14 are also expressed, showing a definitive commitment to the epidermal fate. At this stage, clear similarities in the morphology can also be visible between the iPSC-derived keratinocytes and human primary keratinocytes. These keratinocyte-like cells are now being used for generation of 3D full skin models in order to mimic the ARCI skin phenotype. Our approach thus promises personalized cell models for the study of ARCI as well as for development of advanced therapeutic interventions.

W-1303

A CLINICAL STUDY OF AUTOLOGOUS TRANSPLANTATION USING IPSCS-DERIVED DOPAMINERGIC PROGENITORS TO PARKINSON'S DISEASE PATIENTS

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We are going to start a clinical study in Japan within a couple of years, which is an autologous transplantation of iPSCs-derived dopaminergic (DA) progenitors to sporadic Parkinson's disease patients. In the clinical study, patient-derived iPSCs are established from peripheral blood, and differentiated to DA progenitors in a cell processing center in the Center for iPS cell Research and Application (CiRA). We induce neural progenitors by a dual-SMAD inhibition method and sort DA progenitors by using antibodies for a floor plate marker, Corin. After sorting, the sorted cells are cultured to make aggregate spheres, and then collected for transplantation. We set three checkpoints of cell products, the first one is quality of iPSCs, the second is purity of sorted cells, and the third is quality of final products. About 15 clones are established from one patient, and we select the best two or three clones by the first checkpoint, quality of iPSCs. The selected clones are differentiated to DA progenitors and if they pass the second and third checkpoint, we can transplant them to the patient. We are going to transplant about 5x106 cells into bilateral putamen by stereotactic neurosurgery. Safety of the grafted iPSC-derived cells will be mainly observed for 2 years in a small number of patients (less than 6 cases) by imaging examinations (MRI and FLT-PET) every 3 to 6 month, and efficacy of the cells will also be observed by F-DOPA PET study and by neurological scoring (UPDRS; Unified Parkinson's Disease Rating Scale). In this poster, I will introduce an outline of the clinical study and quality of "cell products" preparing in FiT.

W-1304

TWO STEP DIFFERENTIATION OF IPSC TO ALVEOLAR EPITHELIAL CELLS

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Induced pluripotent stem cells (iPSc) offer a promising opportunity to generate patient specific cell types that can be used for disease modelling, drug screening and even as cell therapy options. Alveolar epithelium (AEC) plays a vital role in pathogenesis of various lung diseases; in the current study we present a simplified two step protocol for generation of AEC using human iPS cells. iPS cells were generated from human fibroblast cell lines using conventional method. iPS cells were trypsinized and plated as monolayer, followed by treatment with definitive endoderm media for 5 days. 5 days later the cells were grown in defined alveolar epithelial media, after 2 weeks of culture the cells were analyzed for AEC differentiation. Five days after treatment definitive endoderm cells were derived, further culture over a period of 2 weeks the cells were finally differentiated into alveolar epithelial cells. These results were confirmed by gene expression of surfactant protein C, aquaporin 5, CC10, T1alpha, all markers of lung epithelial cells. Microscopy revealed a distinct cobble shaped morphology, furthermore by electron microscopy presence of lamellar bodies and microvilli in the differentiated AEC were observed. We demonstrate a shorter protocol for AEC differentiation. iPSc derived AEC can serve as a platform for patient specific disease model for dissecting yet unknown pathomechanisms of lung disease.

W-1305

ISOLATION ENDOTHELIAL PROGENITOR CELLS FROM INDUCED PLURIPOTENT STEM CELLS

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Diabetic retinopathy is a common blinding disease characterized by progressive degeneration of retinal vessels, which in the long term causes vision loss. It has been shown that endothelial progenitor cells (EPCs) are a promising source for therapeutic revascularization by regenerating damaged blood vessels in the retinal vasculature. EPCs can be differentiated from different sources of human pluripotent stem cells (hPSC) such as human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs). In order to better characterize and optimize the regenerative potential of these cells we need to isolate them as a pure and large population. Therefore, this study compared different methods to enrich EPCs and to enhance their production from hiPSCs. CD34 is the most commonly used marker for these cells and is therefore also used in this study.

hiPSCs were grown to confluency on Matrigel coated plates in mTeSR-1 medium. Then, they were exposed to different conditions, growth factors and bioactive small molecules. The medium was changed according to the experimental plan and cells were fixed on different days from day 5 to 8 and finally immunohistochemistry for CD34 was used to identify the cells of interest. Fluorescent activated cell sorting (MACS) was used to isolate CD34 positive cells and to reseed them into Fibronectin coated plates. We found that administration of BMP4, Activin A and BIO (WNT signalling activator) at an early phase and then VEGF-165 and SB431542 (TGFβ-receptor type one inhibitor) during the later phase of treatment led to efficient differentiation of CD34+ cells from hiPSCs within five days. MACSing the CD34 positive cells and plating them in EGM-2 medium + 25% serum for 4 days differentiate them into endothelial cells expressing VE-Cadherin. Therefore, this study presents an efficient approach to generate hiPSC-derived EPCs by modulating four signalling pathways. This can be useful in developing novel therapeutic avenues for cellular regeneration in diabetic retinopathy. We developed defined culture conditions to enrich CD34+ EPCs which will enables us to isolate these cells in sufficient quantities and characterize these cells in more details.

W-1306

DIFFERENTIATION OF MIDBRAIN FLOOR PLATE PROGENITORS AND DOPAMINERGIC NEURONS FROM HUMAN PLURIPOTENT STEM CELLS

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Midbrain dopaminergic (DA) neurons derived from human pluripotent stem cells (hPSCs) provide an excellent alternative to primary human neurons for disease modeling and drug screening for Parkinson's disease. During brain development, dopaminergic neurons are derived from a distinct population of cells termed midbrain floor plate cells, which are formed during 21-28 days of gestation and located along the ventral midline of developing neural tube. Recent reports have focused on identifying the appropriate in vitro conditions to differentiate hPSCs to properly regionalized floor plate precursors, rather than a more general neural stem cell population, in order to create authentic DA neurons. However, published protocols are quite lengthy and complicated leading to increased variability in differentiation efficiencies. Our objective was to develop a culture media system designed to simplify and standardize this process while compressing timelines and adding increased flexibility in this complex differentiation workflow. Here we describe our results which have broken the process down into 3 distinct steps: (1) specification of hPSC to midbrain floor plate (mFP) cells, (2) expansion and potential for cryopreservation of mFP, and (3) maturation to DA neurons. Characterization of floor plate cells and mature DA neurons was performed by immunostaining for the presence of specific markers including Lmx1, Otx2, FoxA2 and TH, additional qPCR analysis included an expanded lists of genes to help define these cell populations. Electrophysiological activity of these DA neurons was assessed by Multi-electrode array evaluation. In comparison to published protocols, our new system has several advantages including ease of use, significant expansion and preservation of progenitors in relatively short culture duration. This efficient system will benefit researchers with increased scale and flexibility in targeted studies.

W-1307

DYSTROPHIN, AN EMBRYONIC PROTEIN

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The reprograming techniques introduced by Yamanaka have opened new avenues in the understanding of early steps of human development in normal and pathological contexts. We used these approaches for Duchenne Muscular Dystrophy (DMD), a recessive X-linked devastating genetic myopathy. We thus identified a new long dystrophin transcript after a single BMP4 treatment on 2 human embryonic and 7 induced pluripotent stem cells lines (hESCs, hIPSCs). As soon as 72h after BMP4 addition, the expression level of this new dystrophin transcript is similar to Dp427m in adult normal skeletal muscle. This transcript is characterized by a new sequenced exon 1 only conserved in a sub-group of anthropoids including human and is not expressed in any human fetal and adult tissues tested. Unlike DMD hIPSCs, the corresponding dystrophin protein is well detected in BMP4-induced hIPSCs from healthy individuals and characterized by a truncated N-terminal actin-binding domain. The function of this new embryonic dystrophin isoform during development remains to be defined and could allow a better understanding of DMD onset and pathophysiology. This fast, easy and robust cell system which provides a large amount of dystrophin could be a powerful cells platform for the validation of therapeutic strategies such as reading frame restoration, gene editing and gene addition.

W-1308

ENRICHMENT OF DOPAMINERGIC NEURONAL PROGENITORS DIFFERENTIATED FROM PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

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Although there is no FDA approved cell therapy for Parkinson's disease (PD), there is hope that human pluripotent stem cells (PSC) could be used to treat PD by replacing the specific midbrain dopaminergic (DA) neurons that are lost in the progression of the disease. Recent publications use a floor plate progenitor intermediate to produce dopaminergic neurons that appear to be superior to neurons produced by previously reported methods. The use of autologolous cells, such as induced pluripotent stem cells (iPSCs) may result in better outcomes than allogenic cells, due to enhanced engraftment and decreased immune activation. Differences in differentiation efficiency among patient-derived iPSC lines remains a challenge and there is a need for normalization among patient cell lines before patient-specific cell therapies can advance to the clinic. The objective of this study was to purify the differentiating cells at

the floor plate stage, allowing for standardization of cultures among lines. Using SmartFlare technology, we sorted differentiated DA neurons based on mRNA expression of floor plate markers at an early time point of differentiation (day 11) and then compared them to methods that sort based on traditional surface marker expression such as NCAM/CD29 at a later time point (day 30). Sorted and unsorted cells were further differentiated into mature DA neurons and analyzed for expression of DA markers as well as non-DA neuronal markers. Sorting of various iPSC lines derived from PD patients enabled production of more homogeneous DA neurons which were more similar across cell lines compared to unsorted cultures. In adhering to the strict protocols that we have found to be most beneficial, we hope to optimize the culturing of DA neurons and minimize the risks of tumorigenicity and patient variability. These steps will help facilitate personalized medicine for the millions of patients that are currently struggling with this debilitating disease.

W-1309

AUTOLOGOUS OR HLA MATCHED CELL TRANSPLANTATION FOR PARKINSON'S DISEASE WITH INDUCED PLURIPOTENT STEM CELLS

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One of the advantages of induced pluripotent stem cells (iPSCs) for regenerative medicine is the possibility of autologous cell transplantation. We are investigating this potential for the treatment of Parkinson's disease. We established several iPSC lines from non-human primates. Dopamine neurons were differentiated from these cells using a differentiation protocol that included the dual SMAD inhibition strategy. The differentiated dopamine neurons were transplanted back to the brain of the original animals (autologous transplantation). PET scanning and histological observations were performed after the transplantation. We also established iPSCs from donor animals with MHC homozygotes. These donor cells were transplanted to recipient monkeys that had the same MHC haplotype (MHC-matched allogeneic transplantation). The autografts showed less inflammatory response and better survival of the grafted dopamine neurons than the control MHC-mismatched grafts. Histological analysis revealed only minimum lymphocyte infiltration in the autologous grafts, but more noteworthy accumulation of lymphocytes and activated microglia in the graft of some MHC-mismatched 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 show this response is less in autologous transplantation compared with MHC-mismatched transplantation. We are preparing the application of autologous transplantation for Parkinson's disease in a small-scale clinical study. This strategy, however, need high cost and long time to prepare the donor cells for each patients. HLA-matched allogeneic transplantation would be more practical in the clinical situation. For this purpose, HLA-homo iPSCs from healthy volunteers will be stocked in the CiRA iPSC stock project. To confirm the efficacy of this strategy, we are currently investigating the monkeys that received the MHC-matched allogeneic cell grafts differentiated from the MHC-homo donor iPSCs.

W-1310

EXPANSION OF PLATELETS FROM IMMORTALIZED MEGAKARYOCYTE PROGENITOR CELLS (IMMKCLS) DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS) USING A NOVEL NONPEPTIDYL TPO RECEPTOR AGONIST

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Blood platelets can be obtained only by blood donation, which often causes a shortage of platelets in clinical use. We have recently established a self-renewing immortalized megakaryocyte progenitor cell lines (imMKCLs) from hiPSC, displaying long-term expansion capability with yields of functional platelets. While imMKCLs can be promising source toward platelet transfusion, cell growth efficiency is more required for industrialization. Thrombopoietin (TPO) is the primary regulator of human megakaryocyte differentiation and platelet production, as well as expanding platelets from imMKCLs. Several nonpeptidyl small-molecule compounds such as Eltrombopag has been developed, which activates the TPO receptor, c-MPL. Chemically synthesized c-MPL agonists reveal the advantage in terms of biological safety, low-immunogenicity or low-cost manufacturing as compared to peptide-based ligands. To obtain a novel c-MPL agonist that expands imMKCLs more efficiently and cost effectively, we screened small-molecular c-MPL agonists and finally identified TA-316, as the most potent compound that increases platelet productivity. imMKCLs were cultured for 15 days in the presence of either recombinant TPO, personally synthesized Eltrombopag or TA-316. On day 11, the number of imMKCLs cultured with TA-316 was increased >1.5-fold compared with that of TPO. After another 4-day of culture, matured megakaryocytes and platelets were collected. Total number of CD41+CD42b+ platelets with TA-316 was increased >2-fold compared with that of TPO, whereas Eltrombopag had little effect on platelet production. imMKCLs cultured with TA-316 contained a lot of large multinucleated cells as well as those cultured with TPO, contributing to yield of functional platelets. Mechanistic analyses revealed the upregulated major TPO signaling pathways, JAK/STAT, MAPK, and PI3K/AKT. In addition, data suggested anti-apoptotic action at the levels of hematopoietic progenitors by TA-316. These results indicated the c-MPL agonist TA-316 promotes the production of functional platelets from imMKCLs more efficiently than TPO or Eltrombopag, and this could be applicable as an indispensable tool for efficient ex vivo production platelets using a self-renewing imMKCL system.

W-1311

MANIPULATION OF CELLULAR FUNCTION BY GENOME EDITING AND CELL FATE DECISSION BY SMALL MOLECULE-DRIVEN DIFFERENTIATION

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Human patient-derived induced pluripotent stem cells (iPS cells) are of great interest for pharmaceutical research, as they can be differentiated into somatic cells of different lineages enabling the study of disease pathophysiology in vitro. To circumvent variable genetic backgrounds between patients we generated human isogenic cell lines with identical genetic background by introducing mutations responsible for monogenetic disease. This has been possible by genome editing technologies based on engineered nucleases such as transcription activator-like effector nuclease (TALENs) and zinc-finger nucleases (ZFNs). The use of human pluripotent stem cells (PSCs) for in vitro disease-modeling is limited by the ability to rapidly produce pure populations of various cell types in sufficient quantities. Herein, we report a scalable monolayer protocol to induce vascular cells in defined conditions utilizing a new GSK3beta inhibitor. Within six days we generated large cell populations that are highly enriched for endothelial cells (VE-Cadherin+ >=85%). We isolated VE-cadherin+ cells by magnetic activated cell sorting (MACS) to ensure pure and homogenous endothelial cell cultures (>=98%). Time-resolved whole-genome expression and selective qRT-PCR analysis revealed a gene expression pattern closely resembling early embryonic vasculogenesis. Overall the purified VE-Cadherin+ cells present an endothelial-specific expression pattern. The endothelial cell population maintained their cellular identity over the period of cultivation. Further characterizations of VE-Cadherin+ cells, confirm a functional endothelial phenotype. Stem cell-derived endothelial cells give rise to continuous endothelium with dynamic barrier function properties, form vascular network-like structures in angiogenesis assays, and convert into activated endothelium after treatment with pro-inflammatory cytokines. Activated endothelium facilitated the recruitment of co-cultured leukocytes and secreted several mediators associated with impaired endothelial function/ inflammation such as pro-inflammatory cytokines, Endothelin1, Plasminogen activator inhibitor-1, soluble Cellular Adhesion Molecules and Selectins.

W-1312

THE EFFECT OF SECRETOME HARVESTED FROM FRESH ISOLATED MSCS ON CO-CULTURE OF IPS+MSC FOR PANCREATIC BETA-CELL DIFFERENTIATION

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Discovery of novel therapies for diabetes is currently impaired by a scarcity of human beta-cells for drug screening. Beta cells comprise the main population of pancreatic islet cell clusters and are responsible for insulin regulation of glucose. The generation of insulin-producing beta cells from stem cells in vitro would provide an unparalleled cell source for drug discovery in diabetes. Current differentiation protocols produce cells that lack the full functional characteristics of primary human beta cells. In diabetic models co-transplantation of mesenchymal stem cells (MSC) and pancreatic islets into the pancreas, improves both function and survival of islets and aids their revascularization. With this in mind, we have adopted a synergetic co-culture strategy aiming to understand the microenvironmental effects of MSCs on induced pluripotent stem cell (iPS) pancreatic differentiation. MSCs generate a gelatinous media in the first week post isolation. To understand the content and effect of this MSC secretome, it was used as a coating for iPS only and iPS+MSC differentiation to beta cells. On day 18 expression of pancreatic markers were assessed by ICC; expression of endocrine markers such as NKX6.1 were higher in iPS+MSC cultures compared with iPS cultures. Pancreactic factor 1 (PDX1), a determinant protein marker for pancreatic lineage, was found to be expressed in both iPS and iPS+MSC cultures, although characteristic nuclear localization was not observed in either. In comparison to iPS only cultures, iPS+MSC cultures showed higher expression of the typical beta cell protein, C-peptide, and a reduction in pancreatic hormones indicative of differentiation towards alpha or gamma cells. The effect of pre-coating with the MSC secretome on iPS cultures was augmentation of the expression of both NKX6.1 and PDX1. Furthermore, a mixture of matrigel + secretome coating resulted in even higher expression of pancreatic markers but no major differences in C-peptide production. In conclusion, the synergistic effect of the iPS+MSC co-culture promoted an enhancement in beta cell phenotype which is in-part mediated by secretagogues released within the first week of MSC isolation. Characterization of the MSC secretome is ongoing to discriminate the key factors in enhancement of iPS differentiation to beta cells.

W-1313

DIRECT CONVERSION OF HUMAN FIBROBLASTS WITH PAX6 OVEREXPRESSION

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The generation of induced pluripotent stem cells (iPSCs) is potential technique providing a wide range of cell types for basic research and clinical applications. However, making the specific cells via iPSCs processing must aware of the tumor formation and the inability of iPSCs to convert into lineage specific progenitor cells. Here we report the generation of induced neural cells (iNCs) from human fibroblasts by direct reprogramming with a single transcriptional factor which is essential for neurogenesis during development of the central nervous system called Pax6. Therefore, the experiments were done based on a hypothesis that PAX6 overexpression would be valuable for transdifferentiation human fibroblasts into neural lineage cells. In this study we aim to identify and characterize fibroblast-derived neural lineage cells, determine their transdifferentiation capacity, and measure their functional phenotypic characteristics.

iNCs were produced by infecting fibroblasts using lentiviral transduction and a drug inducible system as defined doxycycline inducible transgenes. iNCs express neural lineage cell markers and wild-type neurons in their morphology and gene expression. The use of a single factor for direct reprogramming is a significant step toward the application of iPSCs tool for basic knowledge and can apply as patient-specific neural cell model using for studies of molecular pathogenesis. *This study is supported by FP7-PEOPLE-2012-IAPP scholarship.*

W-1314

ESTABLISHMENT OF A 3D CULTURE SYSTEM BASED ON A PORCINE-DERIVED BIOLOGICAL SCAFFOLD TO DIFFERENTIATE HUMAN IPS CELLS INTO PANCREATIC BETA-CELLS

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Diabetes mellitus type 1 is a highly prevalent disease worldwide. Currently, insulin-secreting β-cells generated from human pluripotent stem cells are a highly interesting cell source for therapeutic transplantation strategies. However, the low efficiency in generating functional and also mature β-cells is a major limitation of current in vitro differentiation approaches. One possible explanation for this could be the use of two-dimensional (2D) differentiation protocols that fail to mimic a natural microenvironment. Recently, several reports demonstrated that substrates and purified proteins derived from an extracellular matrix (ECM) component could increase the survival and function of pancreatic islets or individual β-cells in vitro. The aim of this study is to establish a three-dimensional (3D) culture system to differentiate human induced pluripotent stem cells (hiPSCs) on a biological vascularized scaffold (BioVaSc®) into β-cells. The BioVaSc® was generated from a porcine small bowel segment that first underwent a decellularization process followed by recellularization with microvascular endothelial cells. As the BioVaSc® is composed of vascular microstructures and preserves properties of extracellular matrix components, we assume a positive impact of this scaffold onto the generation of functional and mature β-cells from hiPSCs. In addition to the BioVaSc®-based 3D culture system, porcine-derived pancreatic tissue was decellularized to set up a pancreas-based 3D culture system. β-cell differentiation will be performed according to already published 2D protocols that also serve as control condition. Initially, 2D protocols will be applied 1:1 to the 3D cultures. In addition, modification of these protocols should further improve the differentiation potential of hiPSCs in the 3D culture systems. Differentiation outcome will be assessed by pancreatic lineage-specific gene expression profiles and immunohistochemical stainings for β-cell differentiation markers. Functionally, the generated β-cells will be analyzed onto their ability for insulin-secretion. Taken together, by establishing a 3D biological scaffold-based differentiation system we assume an improved β-cell differentiation potential of hiPSCs in vitro.

W-1315

INTERLEUKIN-3 SUPPORTS LARGE-SCALE MYELOID DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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Hematopoietic in vitro differentiation of hPSCs holds great promise for gene and cell therapy, and represents a valuable model system of embryonic hematopoietic development. Thus, we have established an embryoid body (EB)-based differentiation protocol employing IL-3 in combination with G-CSF or M-SCF to continuously (up to 5 months) produce large numbers (2-10x106/week/6 well plate) of >95% pure granulocytes (iPSC-gra) or monocyte/macrophages (iPSC-MΦ) via an intermediate "myeloid cell forming complex (MCFC)". IPSC-gra and iPSC-MΦ revealed typical morphology, surface phenotype, and functionality. Thus iPSC-gra migrated towards an IL8 or fMLP gradient, formed neutrophil extracellular traps, and produced ROS. IPSC-MΦ phagocytosed latex beads and secreted cytokines upon LPS stimulation. In our model production of myeloid cells was driven by a MCFC-resident, CD34+, clonogenic progenitor population. Furthermore, early embryonic events such as endothelial versus hematopoietic specification were recapitulated. In this line, analysis of MCFCs revealed expression of MIXL1, SOX17, KDR1, GATA2, and RUNX1, as well as early CD34+/CD45- cells undergoing transition to a CD34+/CD45+ and thereafter CD34-/CD45+ phenotype. The hypothesis of a primitive hematopoietic cell arising from a population with dual (hematopoietic and vascular epithelial) potential was supported by co-staining with VE-cadherin (CD144) and colony formation primarily by CD34+/CD45+/CD144- cells. Interestingly, cultivation of MCFCs with IL-3 only resulted into primarily myeloid progenitor cells, which retained the capacity of subsequent M- or G-CSF-driven terminal differentiation. Even more important, cells shedded from "IL3 only MCFCs" contained up to 15% CD34+ stem/progenitor cells and generated white and red colonies, which was not observed for IL3/G-CSF or IL3/M-CSF treated cultures. Thus, our in vitro PSC differentiation model allows for large-scale production of myeloid cells for cell therapy approaches and faithfully recapitulates key events in embryonic hematopoiesis. Due to its minimal cytokine requirements it additionally appears highly suited to study early hematopoietic development. We further provide evidence that IL3 constitutes a key cytokine driving the early hematopoietic specification of human PSC.

IPS CELLS: DISEASE MODELING

W-1317

HUMAN IPS CELL DISEASE MODEL OF MERTK-ASSOCIATED RETINITIS PIGMENTOSA

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Retinitis pigmentosa (RP) represents a genetically heterogeneous group of retinal dystrophies affecting mainly the rod photoreceptors and in some instances also the retinal pigment epithelium (RPE) cells of the retina. Clinical symptoms and disease progression leading to moderate to severe loss of vision are well established and despite significant progress in the identification of causative genes, the disease pathology remains unclear. Lack of this understanding has so far hindered development of effective therapies. Here we report successful generation of human induced pluripotent stem cells (iPSC) from skin fibroblasts of a patient harboring a novel Ser331Cysfs*5 mutation in the MERTK gene. The patient was diagnosed with an early onset and severe form of autosomal recessive RP (arRP). Upon differentiation of these iPSC towards RPE, patient-specific RPE cells exhibited defective phagocytosis, a characteristic phenotype of MERTK deficiency observed in human patients and animal models. Thus we have created a faithful cellular model of arRP incorporating the human genetic background which will allow us to investigate in detail the disease mechanism, explore screening of a variety of therapeutic compounds/reagents and design either combined cell and gene-based therapies or independent approaches.

W-1318

FUNCTIONAL PHENOTYPIC SCREENING FOR NOVEL PARKINSON'S DRUGS USING HUMAN IPSC-DERIVED DOPAMINERGIC NEURONS GROWN ON MICRO-ELECTRODE ARRAYS

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Phenotypic screening has led to the majority (7/8) of successfully launched CNS drugs in the last decade. Human iPSC-derived neurons are promising tools to increase predictability of cell-based in vitro assays. Yet, these cells still have to show their potential as a robust and reproducible screening platform. Our aim was to use human iPSC-derived neuronal networks containing dopaminergic neurons growing on micro-electrode arrays (MEAs) for functional validation of phenotypic in vitro screening Parkinson's (PD) relevant compounds. We cultured human iPSC-derived post-mitotic commercially available dopaminergic neurons and optimized culture conditions to record extra-cellular action potentials of single neurons forming spontaneously active and developing network communication patterns. We used the dopaminergic neuron-specific or sensitive toxin MPP+ to induce a functional pathophysiology and tested known neuroprotective compounds such as GDNF to prevent the functional impairment. Using multi-parametric data analysis of electrical activity patterns, we calculated a read-out able to capture, both, impairment and rescue of the network activity patterns. We present that the used neuronal cultures produce robust spontaneous activity in vitro showing a functional maturation into a synchronized network within 3 weeks. We further show that MPP+ induces significant functional effects which can be prevented by GDNF. We compare the results with those of primary mouse midbrain neuron/glia co-cultures using the same experimental design. Phenotypic and functional in vitro screening with human iPSC-derived dopaminergic neurons growing on MEAs enable the robust and reproducible screening assay for novel leads or advanced drugs (repurposing) for future PD therapies.

W-1319

IN VITRO MODELING OF HYPERPIGMENTATION ASSOCIATED TO NEUROFIBROMATOSIS TYPE 1 USING MELANOCYTES DERIVED FROM HUMAN EMBRYONIC STEM CELLS

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"Café-au-lait" macules and overall skin hyperpigmentation are early hallmarks of neurofibromatosis type 1 (NF1), one of the most frequent monogenic diseases, subsequently characterized by numerous benign Schwann cells-derived tumours. It has been well established that neurofibromin, the NF1 gene product, is an anti-oncogene that regulates negatively the oncogene RAS. In contrast, the molecular mechanisms associated to the alteration of skin pigmentation have remained elusive. We have taken the opportunity to differentiate human pluripotent stem cells into melanocytes to reassess that issue. In the present study, we demonstrated that NF1 melanocytes reproduce in vitro the hyperpigmentation phenotype, and further characterize the link between the loss of heterozygosity and the typical "café au lait" macules that appear over the generalized hyperpigmentation. Molecular mechanisms associated to those pathological phenotypes correlate with an increased activity of cyclic AMP-mediated protein kinase A and ERK1/2 signaling pathways, leading to the over-expression of the transcription factor MITF and of the melanogenic enzymes tyrosinase and dopachrome tautomerase, all major players in melanogenesis. Finally, hyperpigmentation phenotype could be rescued by using specific inhibitors of these signaling pathways. These results open new avenues for deciphering physiopathological mechanisms involved in pigmentation diseases and provide a robust assay for developing new therapeutic strategies to treat these diseases.

W-1320

MODELLING ALZHEIMER'S DISEASE IN 3D ENGINEERED NEURAL TISSUE FROM PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

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Patient-derived induced pluripotent stem cells (iPSCs) provide a useful tool to model the pathomechanism of certain CNS diseases in a patient-specific way. In this study fibroblasts and mononuclear blood cells from genetically and clinically well-characterized patients with Alzheimer's disease (AD) and healthy controls were reprogrammed into iPSCs. These cells were induced to become neural precursor cells (NPCs) and subsequently differentiated into neural cells. Using an air-liquid interface based, scaffold-free system we produced 3D engineered neural tissue (3D ENTs). After 6 weeks of differentiation we performed an in-depth analysis to characterize the 3D cultures. We studied the cellular composition of the 3D ENTs by the qualitative and quantitative expression of marker proteins (IHC, qPCR and WB), the morphology of the generated neurons and their spine density (Golgi-Cox-staining) as well as their functionality in terms of calcium signalling (calcium uptake assay) and electrophysiological properties (multi-electrode array, MEA). Gene expression analyses confirmed the presence of various neuronal and glial markers such as beta-III Tubulin, MAP2, NF200kD, GFAP, OSP and various synaptic proteins. These data demonstrate the presence of NPCs, neurons, astrocytes, and oligodendrocytes which seemed to have a rather homogenous composition. The generated neurons had a simple uni- or bipolar morphology with spines on their dendrites. MEA recordings and calcium imaging confirmed spontaneous firing activity, another evidence for the presence of functional synapses. Most importantly, the comparison of 3D ENTs derived from AD patients with those of healthy controls allowed the verification of the disease phenotype. 3D ENTs derived from AD patients produced more extracellular Aβ42 than healthy controls as measured by ELISA. Furthermore, we detected more Aβ deposits in diseased cultures than in control cultures by immunostaining. In conclusion, these findings demonstrate the suitability of 3D ENTs to examine the pathophysiology of neurological and psychiatric disorders such as Alzheimer's disease that may be also employed for drug development.

W-1321

N-BUTYLIDENEPHTHALIDE ATTENUATES ALZHEIMER'S DISEASE-LIKE CYTOPATHY IN DOWN SYNDROME INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONS

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Down syndrome (DS) patients with early-onset dementia share similar neurodegenerative features with Alzheimer's disease (AD). To recapitulate the AD cell model, DS induced pluripotent stem cells (DS-iPSCs), reprogrammed from mesenchymal stem cells in amniotic fluid, were directed toward a neuronal lineage. Neuroepithelial precursor cells with high purity and forebrain characteristics were robustly generated on day 10 (D10) of differentiation. Accumulated amyloid deposits, Tau protein hyperphosphorylation and Tau intracellular redistribution emerged rapidly in DS neurons within 45 days but not in normal embryonic stem cell-derived neurons. N-butylidenephthalide (Bdph), a major phthalide ingredient of Angelica sinensis, was emulsified by pluronic F127 to reduce its cellular toxicity and promote canonical Wnt signaling. Interestingly, we found that F127-Bdph showed significant therapeutic effects in reducing secreted Aβ40 deposits, the total Tau level and the hyperphosphorylated status of Tau in DS neurons. Taken together, DS-iPSC derived neural cells can serve as an ideal cellular model of DS and AD and have potential for high-throughput screening of candidate drugs. We also suggest that Bdph may benefit DS or AD treatment by scavenging Aβ aggregates and neurofibrillary tangles.

W-1322

TELOMERASE REACTIVATION IN WERNER SYNDROME IPSC AND THE PREVENTION OF PREMATURE AGING AT GROUND STATE

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Werner Syndrome (WS) is an adult progeria characterized by accelerated aging after puberty. WS patients exhibit short stature, bird-like face, gray hair, bilateral cataract, diabetes, osteoporosis, atherosclerosis, skin ulcer, hypoganadism, and high incidence of non-epithelial neoplasms. These clinical features demonstrate a severe deterioration in cells of the mesenchymal origin, whereas the central nervous system is less affected. Genetic studies indicate that WRN mutation contributes to the pathogenesis of premature aging. Loss of WRN protein impairs DNA replication and repair, and results in increased telomere loss in actively dividing cells such as fibroblasts. We suggest that WRN-dependent accelerated aging is lineage specific. To model premature aging, we reprogrammed WS fibroblasts to pluripotent stem cells (iPSCs). Reprogrammed WS iPSCs were corrected for their telomere defect, as revealed by successful telomerase reactivation and telomere elongation. Additionally, WS iPSCs were highly similar to wild-type iPSCs in gene expression and showed no slowdown of DNA synthesis. An explanation for this corrected cellular aging phenomenon is the prevention of telomere defect by telomerase at ground state. To understand the role of telomerase in protecting against accelerated aging, we differentiated WS iPSCs to mesenchymal stem cells (MSCs). Upon differentiation, telomerase activity decreased significantly. Newly derived WS MSCs divided actively in culture. However, WS MSCs entered senescence earlier than wild-type MSC and demonstrated accelerated telomere shortening and loss of sister telomeres at the lagging strand. Forced expression of hTERT (the catalytic unit of telomerase) or depletion of p53 rescued the accelerated senescence. Our data suggest a central role of telomerase in protecting specific lineage of stem cells from premature aging.

W-1323

A ROBUST PLATFORM FOR GENERATING GENE EDITED HUMAN INDUCED PLURIPOTENT STEM CELL LINES - FROM PATIENT SAMPLING TO CELL BANK.

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The potential of using induced pluripotent stem cells (iPSCs) as disease model systems has evolved extensively in recent years. The potential to observe disease phenotypes in the differentiated cells, potentially provides the pharmaceutical industry with optimized human models for drug discovery. Recently, gene editing (GE) has attracted extensive attention for precise genetic modulations using TALEN or CRISPR based technologies. The combination of iPSC technology with GE allows investigating the effects of pathogenic mutations in a specific human in vitro model. An essential parameter paving the way for GE-iPSCs in early discovery is a reliable production platform that integrates multiple quality control (QC) steps for iPSC production and characterization, GE tools and the actual GE process of iPSCs. We have established an effective process flow from patient sampling to master cell banking of a GE-iPSC line panel. The platform is based on integration-free iPSC generation using episomal technology and CRISPR technology for producing GE-iPSC lines in a quality to be used for early pharmaceutical discovery. The process platform involves a number of essential QC steps including plasmid integration analyses, short tandem repeat (SRT) analysis, sequence validation, karyotyping and comprehensive test panel for pluripotency. In parallel, a CRISPR QC flow has been implemented, including testing of CRISPR cutting efficiencies in HEK293T cells using flow cytometry, GE analysis using PCR, restriction digest and sequencing and evaluation of off-target effects. The result is a specification sheet covering complete GE-iPSC QC. The process platform is now being implemented into the EBiSC (European Bank for Induced Stem Cells) consortium - an IMI funded consortium, in which major pharmaceutical companies is involved.

W-1324

MODELING AUTISM USING CRISPR/CAS9 AND HUMAN INDUCED PLURIPOTENT STEM CELLS

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Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that is poorly understood at the gene level. As live neurons from human brain are not easily accessible, we generated a series of new iPS cell lines derived from fibroblasts obtained from members of seven different ASD families affected by loss-of-function mutations in ASD-associated genes. The seven candidate genes affected by these mutations are AGBL4, CAPRIN1, CNTN5, DLGAP2, EHMT2, KAL1 and NRXN1. We used the double-nicking type II CRISPR/Cas9 system to correct mutations in patient-derived iPS cells, hence providing isogenic controls to highlight the role of these genes in autism. Guide RNA (gRNA) sequences were devised in order to minimize the likelihood for off-target binding. In the presence of a single-stranded oligonucleotide (ssODN) template, cellular HDR machinery replaced the disease mutations for the wild-type sequences. Edited alleles were detected using specific DNA probes along with absolute quantification by droplet digital PCR (ddPCR). The frequency of modified alleles was amplified to 100% using sibselection steps in a 96 well format (Miyaoka Y, Nat. Methods, 2014;11[3]). We also adapted this editing system to specifically knock-out 8 additional ASD-associated genes, for which skin fibroblasts were not available to derive iPS cells from ASD patients. These include AFF2, ASTN2, ATRX, CACNA1C, CHD8, KCNQ2, SCN2A and TENM1. We hypothesized that artificial disruption of a candidate gene in iPS-derived neurons would promote a similar phenotype to that observed in iPS-derived neurons from probands. We preferentially targeted the earliest exon that is common to the different transcripts for each candidate gene. We inserted a 60bp DNA fragment that includes all-frame termination codons, a rare restriction site (Mre1) and a V5 epitope tag to possibly reveal truncated forms of proteins. This stop tag is synthesized as ssODN flanked by homology arms that are specific to each candidate gene. All iPS cell lines generated above are currently cultured in vitro under conditions favouring neuronal differentiation. RNAseq, immunostaining and electrophysiology will shed light on the effects of mutated ASD-associated genes on neuronal expression patterns and identity, dendritic complexity and synaptic function.

W-1325

DISSECTING THE ROLE OF THE RAS/MAPK PATHWAY IN NEURONS FROM 16P11.2 DELETION AND DUPLICATION CARRIER-DERIVED INDUCED PLURIPOTENT STEM CELLS

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One of the most common genetic causes of autism spectrum disorders is a 600kb deletion or duplication on chromosome 16. Many of the 28 genes at this locus are expressed in the brain and hence may contribute to the neurodevelopmental phenotype associated with 16p11.2 disorders. The duplication and deletion have opposite phenotypes such as micro- and macrocephaly, respectively. One of the genes at the 16p11.2 locus, MAPK3, encodes the kinase ERK1. ERK1 and ERK2 are effectors of the Ras/MAPK pathway that regulates important cellular functions such as proliferation and differentiation. In neurons, it regulates survival, differentiation and maturation. Rationally, overactive ERK signaling would correspond to increased proliferation causing macrocephaly (observed in several Mendelian disorders with hyperactive Ras/MAPK signaling). However, it may also result in rapid differentiation leading to microcephaly (observed in 16p11.2 duplications). To shed light on this issue, we decided to investigate the role of ERK1 and ERK2 in neurons from 16p11.2 duplication and deletion carriers. We generated patient-derived induced pluripotent stem cells (iPSC) and differentiated them into cortical neurons. Levels of activated ERK1 in neural progenitors appear slightly increased in duplications and reduced in deletions ($p<0.04$), as expected . Our data also show that - 1) the proportion of neurons is higher in duplication lines ($p<0.004$) and slightly lower in deletions compared to controls at early time points, 2) the duplication lines generate more early-born deep layer cortical neurons than controls or deletions ($p<0.0003$) and, 3) the number of cells exiting the cell cycle is higher in duplications ($p<0.002$) and marginally lower in deletions. This indicates that progenitors from duplications may undergo fewer self-renewing divisions and prematurely differentiate into cortical neurons while deletion

lines may cycle more and differentiate slightly slower compared to controls, consistent with observed micro- and macrocephalic phenotype. Overall, our preliminary results indicate dysregulated Ras/MAPK signaling and corresponding neuronal phenotypes of the 16p11.2 disorder and open up interesting avenues for further investigation of distinct roles for ERK1 and ERK2 in Ras/MAPK mediated processes in neurodevelopment.

W-1326

HUMAN INDUCED PLURIPOTENT STEM CELLS AND DIRECT EFFICIENT DERIVATION OF SKELETAL MUSCLE CELLS: A POWERFUL TOOL FOR FACIO-SCAPULO-HUMERAL DYSTROPHY MODELING

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Human induced pluripotent (hiPSCs) stem cells provide a powerful tool to investigate disease mechanism in vitro. In this context we created a repertoire of hiPSCs from healthy and patients affected with Facio-Scapulo-Humeral Dystrophy. In 95% of patients with FSHD, this pathology is linked to shortening of an array of macrosatellite elements, D4Z4, at the distal 4q35 locus (FSHD1). In the remaining 5% (FSHD2), the pathology is associated with mutation in the SMCHD1 gene for 2-3% of patients, while in the remaining 2-3%, the cause of the pathology remains undetermined. At the molecular level, the pathology is associated with hypomethylation of the D4Z4 repetitive sequence and activation of the DUX4 retrogene in a fraction of samples. SMCHD1 is known to play a key role in the X chromosome inactivation by maintaining CpG methylation. We have shown that most patients with the lowest level of methylation at the proximal region of D4Z4 present a SMCHD1 mutation and a loss of the H3K9me3 heterochromatin mark. We have produced and characterized a collection of hiPSCs clones corresponding to the different types of patients with FSHD and investigated the D4Z4 methylation dynamics after reprogramming and differentiation. Interestingly, we observe that hiPSCs cells are more methylated that somatic cells from which they are derived suggesting that D4Z4 methylation is dynamically regulated in pluripotency and differentiation. Results in contractile multinucleate skeletal muscle cells obtained from these hiPSCs will be discussed. In conclusion this repertoire of hiPSCs from FSHD1 and 2 patients provides us with new tools for investigating D4Z4 pathogenesis, and the epigenetic regulation of repetitive DNA.

W-1327

USING PATIENT SPECIFIC STEM CELLS TO UNDERSTAND AND DEFINE THE ROLE OF SOD1 PROTEIN MISFOLDING IN AMYOTROPHIC LATERAL SCLEROSIS

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Mutations in *superoxide dismutase 1 (SOD1)* were the first identified genetic link to amyotrophic lateral sclerosis (ALS), a neurodegenerative condition in which selective loss of motor neurons (MNs leads to progressive paralysis. SOD1 mutations contribute to 12-23 % of familial ALS, and in up to 6 % of all ALS cases. Mutations in *SOD1 l*ead to conformational changes within the protein, which is normally very stable. Accumulation of misfolded SOD1 leads to cytotoxicity and the further formation of misfolded SOD1 aggregates, which can spread from cell to cell via a prion-like mechanism. The presence of aggregates of misfolded SOD1, in both familial and sporadic ALS, suggests that misfolded SOD1 might represent part of a final common pathway leading to motor neuron death in multiple forms of ALS. Numerous studies have alluded to different, potential mechanisms of misfolded SOD1 toxicity. However, the precise nature of the toxic SOD1 species is not yet clear. The pathogenesis of ALS is proposed to be due to both cell autonomous mechanisms acting in motor neurons, and non-cell autonomous ones, where other cells types such as astrocytes are involved. We have used patient-derived induced pluripotent stem (iPS) cells to investigate SOD1 toxicity in vitro. iPS-derived motor neurons and astrocytes have been generated from different ALS patients carrying SOD1 mutations, as well from non-diseased controls. We have analysed the amounts of misfolded SOD1 in patient derived fibroblasts, iPS cells, iPS-motor neurons and astrocytes carrying mutations in *SOD1* as well as other ALS related genes including *Fused in Sarcoma (FUS), TAR DNA-binding protein 43 (TDP-43)* and sporadic ALS. The results of these analyses will be presented.

W-1328

DISTINCT NEURODEGENERATIVE CHANGES IN AN IPS CELL MODEL OF FRONTOTEMPORAL DEMENTIA CAUSED BY A MUTATION IN THE MICROTUBULE-ASSOCIATED PROTEIN TAU

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Frontotemporal dementia (FTD) is the second most common early-onset dementia after Alzheimer´s disease and can be caused by mutations in MAPT encoding the microtubule-associated protein TAU. These MAPT mutations result in excessive accumulation of phosphorylated TAU protein within neurons and glial cells of various brain areas including the frontal and temporal cortex and the substantia nigra leading to progressive degeneration and eventually cell death at these sites. However, despite thorough characterization of these histopathological changes, the underlying mechanisms

of neurodegeneration are poorly understood. Here, we derived induced pluripotent stem (iPS) cells from individuals with FTD-associated MAPT mutations and differentiated them into mature neurons. Patient iPS cell-derived neurons demonstrated pronounced TAU pathology, disturbed neurite outgrowth and an increased oxidative stress response to inhibition of mitochondrial respiration. Furthermore, FTD-neurons demonstrated distinct, disease-associated gene expression profiles in whole genome transcriptome analyses. We show that our patient-specific iPS cell model provides a suitable platform to shed light on underlying disease mechanisms of FTD, which could also serve as an invaluable drug screening tool to identify therapeutic compounds in FTD.

W-1329

ESTABLISHING A HUMAN INDUCED PLURIPOTENT STEM CELL MODEL OF PCDH19-FEMALE EPILEPSY

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A large number of neurodevelopmental disorders including intellectual disability, autism spectrum disorder and epilepsy, implicate altered cortical development as an underlying mechanism. We aimed to establish a model of human cortical development, which could be used as a robust tool to investigate the cellular role of genetic variation in patients with these disorders. To do this we built on previously published protocols to make them more reproducible across multiple pluripotent stem cell (PSC) lines. We used a cortical differentiation protocol based dual-SMAD inhibition to induce the neurulation of PSC. Our modified protocol differs from the previously published protocols with changes in seeding density, timing of passaging, concentration of inhibitors and the addition of a step to select for a pure population of cortical stem cells by manual cutting of neural rosettes. We have found this optimised protocol to be both robust and reproducible across both ES and iPSC lines. Having established the protocol, we tested it on iPSCs generated from patients with protocadherin 19 (PCDH19) mutations. Mutations in the X-chromosome linked gene PCDH19 cause the neurodevelopmental disorder, PCDH19-Female Epilepsy (PCDH19-FE). This disorder is predicted to result from mosaicism in the patient brain which stems from the gene being subjected to random X-inactivation. To generate iPSCs we extracted skin fibroblasts from 3 (two affected females and a carrier male) patients with 3 different PCDH19 mutations and proceeded to reprogram these into the pluripotent state. Interestingly, we were only ever able to isolate wild-type PCDH19 expressing iPSCs from affected females, suggesting selection in favour of cells with wild-type PCDH19 or the presence of a dominant X-chromosome. Using the male iPSCs, we have been able to differentiate them to cortical neurons using our protocol and subsequently use this protocol to model the expected mosaicism of the patient brain. We are now looking at the effect of this mosaic population of cells on the expression of key markers of cortical differentiation. Additionally, given the molecular interaction of multiple protocadherins with the WAVE complex, we are using this system to address the role of PCDH19 in the regulation of neural progenitor cell polarity through WAVE complex interaction.

W-1330

ASSESSING FUNCTIONAL EFFECTS OF A KCNT1 MUTATION ASSOCIATED WITH EPILEPSY USING HUMAN IPSC-DERIVED NEURONS

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The sodium-activated potassium channel Slack encoded by the gene *KCNT1* is expressed in neurons throughout the brain, including the frontal cortex, and mediates a sodium-sensitive potassium current (I_{KNa}). This outward current regulates neuronal excitability and determines how neurons respond to repeated high frequency stimulations, both of which are aspects of memory and learning. Mutations in *KCNT1* and alterations to the I_{KNa} current have patho-physiological consequences. Recent studies have described the emerging role of KCNT1 channels in cognitive deficits, and role of KCNT1 mutations in clinically distinct forms of severe early onset "childhood" epilepsies. The development of better therapies for neurological disorders has been hindered by limited access to clinically-meaningful cell for research and drug development. The advent of induced pluripotent stem (iPS) cell technology provides a platform to facilitate increased understanding of disease mechanisms in a physiologically-relevant human cell type. We have leveraged this technology to generate human neurons cells carrying the *KCNT1* P924L mutation and assessed the function effect of the mutation on physiology. To introduce the P924L allele, we genetically engineered a "control" iPS cell line from an apparently healthy female donor with no family history of neurological disorders and generated highly pure (>95% TUJ1-positive), and terminally differentiated cortical neurons from the KCNT1 P924L and isogenic control iPS cell lines. Here, we present data from the functional comparison of these human neurons (wild-type vs. KCNT1 P924L mutant), with a specific focus on the electrophysiological analysis using multi-electrode array (MEA). The ability to engineer isogenic wild type and disease associated alleles by genome editing human iPS cells gives researchers unprecedented access to models for neurological disorders. Our ability to produce pure populations of sub-type specific human neurons is revolutionizing our approach to studying diseases in vitro, and is opening new avenues to develop treatment for central nervous system diseases.

W-1331

INDUCED PLURIPOTENT STEM CELLS TO MODEL TREACHER COLLINS SYNDROME IN A DISH

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Treacher Collins Syndrome (TCS) is an autosomal dominant disorder characterized by craniofacial defects, including mandibular hypoplasia and conductive hearing loss. There is a wide spectrum of clinical variability and the most severe cases die soon after birth due to respiratory problems. Most cases are caused by heterozygous loss-of-function mutations in TCOF1, leading to truncation of a

nucleolar protein, TREACLE, that regulates ribosomal biogenesis. TCS phenotype seems to be the result of lack of neural crest progenitors during craniofacial development due to enhanced apoptosis. It is however still not well understood if cell properties are compromised in mesenchymal stem cells (MSCs) harboring TCOF1 mutations. Thus, our aim was to establish an in vitro cellular model to tackle this question. We compared proliferation, differentiation and TCOF1 expression between MSCs, dermal fibroblasts, and MSC cells differentiated from iPSCs (MSC-like cells) generated from fibroblasts obtained from TCS subjects and matched controls. MSCs and fibroblasts from TCS subjects showed reduction in TCOF1 expression (average of 31%, p=0.0213) in consistency with treacle haploinsufficiency, but similar proliferation rates and osteopotential. MSC-like cells from control and TCS subjects showed downregulation of pluripotency markers in vitro tri-lineage differentiation and mesenchymal immunophenotype. MSC-like cells from TCS subjects presented higher ALP activity (1.62 fold-change; p=0.0001) and matrix calcium deposition (1.31 fold-change; p=0.029) compared with matched controls during in vitro osteoinduction. During early in vitro chondrogenesis, MSC-like cells from TCS subjects presented lower expression of the early marker ACAN and higher expression of late markers SOX9 and COL2A1 when compared with controls. Therefore, MSC-like cells from TCS subjects presented a different pattern of osteo-chondrogenic differentiation due to either a pre-commitment of TCS or to a difference in their proliferation rates, not seen when adult MSCs are directly accessed. Our findings suggest the usefulness of iPSCs to model TCS in a dish and our preliminary data suggest that MSC cells of TCS present an altered biological function. (FAPESP, CNPq).

W-1332

INDUCED PLURIPOTENT STEM CELLS-DERIVED DOPAMINERGIC NEURONS AS A CELLULAR PLATFORM TO MODEL LESCH-NYHAN DISEASE

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Lesch Nyhan disease (LND) is a X-linked rare neurodevelopmental disorder affecting 1/380000 boys. LND is due to the mutation and loss of function of the HPRT gene coding for the Hypoxanthine-guanine phosphoribosyltransferase, an enzyme primarily implicated in the purine salvage pathway. At the neurological level, LND is associated with a dysfunction of the dopaminergic pathway. Consequently children develop symptoms involving abnormal movement disorders and self-injurious behaviors. So far, the relationship between HPRT loss of function and dopaminergic neurons dysfunction is not known which has considerably compromise the chance to develop relevant therapeutic approaches. To tackle this question, we reprogramed fibroblasts of healthy and LND individuals using Yamanaka factors: Oct4, Sox2, c-Myc and KLF4. We first confirmed that LND-derived iPSC have no detectable HPRT activity. We then develop a protocol of differentiation of IPSC into DA neurons that: i- respect key developmental milestone that maybe affected by HPRT deficiency, ii- was amenable to automated and image-based High Content Analysis technics. This should allow us to determine whether HPRT deficiency affect early commitment of mesencephalic progenitors, differentiation of dopaminergic neurons, network formation or terminal dopamine synthesis and secretion. Taken together, our work should lead to a better understanding of the etiology of LND and consequently to a more rational therapeutic strategies.

W-1333

IPSC BASED DISEASE MODELING AND CRISPR/CAS9 MEDIATED GENOME EDITING IN FAMILIAL MEDITERRANEAN FEVER

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Familial Mediterranean Fever (FMF) is a hereditary autosomal recessive autoinflammatory disease caused by mutations in the MEFV gene. MEFV encodes Pyrin, which primarily functions in neutrophils and macrophages, and regulates Caspase-1-dependent interlekuin-1 beta activation during inflammation. The exact role of mutant Pyrin during this process and its contribution to disease symptoms is currently under exploration. We reasoned that mechanistic and therapeutic studies on FMF would be greatly facilitated by availability of large quantities of disease-specific cells and genetically-matched controls. Therefore, we have established an induced pluripotent stem cell (iPSC)-based disease model of FMF and focused on the correction of the single point mutations in MEFV gene by using Crispr-Cas9 technology to produce genome-edited, genetically matched control iPSCs. FMF patient-specific fibroblasts were isolated and reprogrammed to iPSCs using episomal vectors expressing Oct4, Sox2, Klf4, c-Myc, Lin28 and shp53. Pluripotency of the generated iPSCs was demonstrated by the expression of pluripotency genes including Oct4, Sox2, Nanog as assessed by RT-PCR and immunofluorescence. FMF-specific iPSCs were also capable of extensive differentiation in teratomas upon implantation into SCID mice. Presence of the single point mutation in MEFV gene in FMF-specific iPSCs was confirmed by sequencing. To correct the p.Met694Val mutation by Crispr-Cas9 technology, guide RNAs targeting the mutated gene were designed. GFP reporter assay in 293T cells stably transfected with a MEFV-disrupted EGFP gene construct confirmed the functionality of MEFV-targeting guide RNAs, and homologous recombination was observed upon delivery of a truncated GFP template. Surveyor nuclease assays showed that MEFV-targeting gRNAs were able to generate double-stranded breaks at the endogenous mutant MEFV locus as well. These data indicate that gRNAs designed could be used for the correction of p.Met694Val mutation in patient fibroblast-derived iPSCs. Taken together, we demonstrate that fibroblasts from FMF patients can be reprogrammed to iPSCs, which will contribute to further studies to understand the mechanisms of disease and provide a platform for developing and testing new therapeutic approaches to autoinflammatory diseases.

W-1334

RNA-GUIDED CORRECTION OF LARGE CHROMOSOMAL INVERSIONS IN HEMOPHILIA A PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS TO RESTORE THE INTEGRITY OF THE BLOOD COAGULATION FACTOR VIII GENE

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Hemophilia A is caused by various mutations in the *F8* gene, which encodes the blood coagulation factor VIII. In particular, almost half of all severe hemophilia A cases result from two different gross (140-kbp or 600-kbp) chromosomal inversions, rather than point mutations, that involve the *F8* intron 1 or 22 homolog, respectively. We derived induced pluripotent stem cells (iPSCs) from patients with these inversion genotypes and used CRISPR/Cas9 nucleases to revert these chromosomal segments in hemophilia A patient-derived iPSCs, correcting these two recurrent genomic rearrangements in Chromosome X. We isolated several inversion-corrected iPSC clones with frequencies of up to 6.7% and showed that the *F8* gene was expressed in cells differentiated from inversion-corrected iPSCs but not in cells from original patient-derived iPSCs. Whole genome sequencing and targeted deep sequencing analyses showed that no off-target mutations were induced in the inversion-corrected iPSCs. Our results provide a proof-of-principle for correcting genetic defects caused by large chromosomal rearrangements including hemophilia A in patient-derived iPSCs.

W-1335

5'UTR EXPANSION MUTATIONS MAY INFLUENCE EXPRESSION OF ALTERNATIVE EIF4A3 TRANSCRIPTS IN IPSC AND DERMAL FIBROBLASTS

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Richieri-Costa-Pereira syndrome (RCPS) is an autosomal-recessive disease caused by expansion mutations in the 5'UTR of EIF4A3. This gene codifies an exon-junction complex protein involved in RNA splicing and nonsense-mediated decay; however, the relationship between the mutations and the proposed EIF4A3 loss of function remains unclear, and no pathogenic mechanism has been described to date. RCPS is characterised by cleft mandible, Robin sequence, and laryngeal and limb abnormalities; considering the craniofacial phenotype suggestive of impaired cranial neural crest cell function, disturbances in EIF4A3 expression in neural crest cells may lead to abnormalities in processing of transcripts important for craniofacial morphogenesis. We performed preliminary RT-qPCR assays with primers spanning three transcript sites (exons boundaries 1/2, 7/8 and 11/12) to assess EIF4A3 expression in control and patient-derived iPSCs and adult dermal fibroblasts. Results suggest a cell type- and disease-specific EIF4A3 exon usage profile, in which patient's iPSCs show down-regulation of transcripts retaining exons 7/8 and 11/12, and patient's fibroblasts show down-regulation of exons 1/2 and 7/8. To further explore these findings, we propose disease modelling with the generation of iPSC-derived neural crest stem cells (NCSCs; n=3) through Activin A/TGF-β blockade and WNT pathway activation. During differentiation, we observed mRNA down-regulation of OCT3/4, up-regulation of several NCSC markers (e.g. P75NTR, PAX3, TFAP2A, and SOX9/10), and stable expression of EIF4A3. Flow cytometry analysis showed populations positive for NCSC markers HNK-1 and P75, generated with variable efficiency (54.7%-87.5%). NCSCs were able to differentiate into cells with typical mesenchymal stem cell immunophenotype (negative for CD31, CD34, and CD45; positive for CD90, CD29, CD73 and CD105). Together, these observations suggest that, depending on cell type, RCPS 5'UTR mutations may influence expression of EIF4A3 alternative transcripts not previously described. Additional studies focusing on the transcriptional profile of patient-derived and EIF4A3 knockdown NCSCs will contribute to dissecting the pathogenesis of RCPS.

W-1336

GENERATION AND CHARACTERIZATION OF IPSC-DERIVED NEURONS FROM MULTIPLE SCLEROSIS PATIENTS

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Multiple sclerosis (MS) is an autoimmune demyelinating disorder of the central nervous system (CNS), of unknown etiology, in which focal lymphocytic infiltration causes damage to myelin and axons in genetically predisposed individuals. The possibility of generating neurons from induced pluripotent stem cells (iPSCs) opened a new avenue of research to test pathogenetic mechanisms in vitro. Our project is aimed at obtaining and characterizing iPSC lines derived from MS patients with different disease courses (RR, SP, PP) and healthy controls (HC), differentiating iPSC into functional neurons and evaluating their phenotype and functionality at baseline and after challenge with stressors to mimic the pathogenic process occurring within the CNS of MS patients. Of note, we will be able to compare also individuals with parental relationship (discordant twins). We have generated iPSC lines from 8 patients with different MS forms and 4 sex and age matched HC using non-integrating Sendai viral vectors, assessed their pluripotency for the expression of related markers and for the capacity of differentiating into cells belonging to the three germ layers. Further, we have induced neuronal differentiation in 3 MS- and 2 HC- iPSC lines using a dual SMAD inhibition protocol and characterized the neuronal populations obtained. We have assessed by immunostaining and RNA expression analyses that iPSCs from HC and MS patients can be efficiently differentiated into neural progenitors and neurons, although, with variable differentiation efficiency. The firing activity of neurons measured from day 60 to day 90 of differentiation by Micro Electrode Array (MEA) devices reached a peak at day 84 in terms of number of burst and spikes. Moreover, the spontaneous firing of these neurons was completely abolished upon tetrodotoxin (TTX) treatment. We are currently working on an optimized and standardized differentiation protocol to increase the percentage of mature neurons showing functional synapses. Once the protocol will be established, iPSC-derived neurons from both HC and MS patients will be challenged with

stressors (e.g. inflammatory molecules, ROS, NOS, Glutamate) and the mitochondrial properties and neuronal susceptibility to apoptosis will be evaluated.

W-1337

MODELING FAMILIAL CANCER WITH IPSC APPROACHES

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Li-Fraumeni syndrome (LFS) is a genetically inherited autosomal dominant cancer syndrome characterized by multiple tumors within an individual, early tumor onset and multiple affected family members. Germline mutations in the p53 tumor suppressor gene are responsible for LFS. Although there has been extensive research on cancer cell lines and even mouse models of LFS to study the role(s) of p53, these model systems do not fully recapitulate the range of human tumors or their properties. In vitro modeling of human disease has recently become feasible with the adoption of induced pluripotent stem cell (iPSC) technology. Here, we established patient-derived iPSCs from a LFS family and investigated the role of mutant p53 in the development of osteosarcoma. The osteoblasts, differentiated from LFS iPSC-derived mesenchymal stem cells, recapitulate osteosarcoma features including defective osteoblastic differentiation and tumorigenic ability, suggesting that our established LFS disease model is a "disease in a dish" platform for elucidating p53 mutant-mediated disease pathogenesis. The gene expression patterns of LFS osteoblasts are similar to those of tumor samples obtained from osteosarcoma patients and these tumorigenic features strongly correlate with shorter tumor recurrence times and poorer patient survival rates. Furthermore, the global transcriptome by mRNA-seq to reveal that LFS OBs exhibit impaired expression of the imprinted gene H19 during osteogenesis. Our functional studies implicate the essential H19 gene in normal osteogenesis and inhibition of tumorigenesis. In order to decipher the underlying mechanisms by which H19 mediates osteogenesis and tumor suppression, we characterized and analyzed the human imprinted gene network (IGN) and revealed the unidentified role of p53 in regulating the IGN culminating in osteogenic differentiation defects and tumorigenesis. In summary, these findings demonstrate the feasibility of studying inherited human cancer syndromes with iPSCs and also provide molecular insights into the role of the IGN in p53 mutation-mediated tumorigenesis.

W-1338

UNDERSTANDING THE ROLE OF LRRK2 IN PARKINSON'S DISEASE USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Mutations in leucine-rich repeat kinase 2 (LRRK2) is the most common identified cause of familial monogenic Parkinson's Disease (PD). However, the relevance of LRRK2 in other PD-linked genetic and pathogenic pathways is still not clear. Accumulations of alpha-synuclein and Lewy body formation in neuronal cells are common events in PD patients carrying a LRRK2 mutation, patients with mutated PARK1 gene (encoding triplication of the alpha-synuclein locus), and sporadic PD patients. Recent findings suggest that, more than LRRK2 kinase activity, levels of LRRK2 and alpha-synuclein are determinant for neuronal toxicity. We are using human induced pluripotent stem cell (hiPSC) lines derived from PD patients carrying a G2019S LRRK2 mutation and patients with the PARK1 mutation to establish functional links between LRRK2 and pathogenic pathways associated with alpha-synuclein. Using Western Blot analyses we compared levels of LRRK2 protein expression between controls, mutated LRRK2, and the mutated PARK1 hiPSCs. We observed a slightly increase of LRRK2 expression in the G2019S in both undifferentiated hiPSCs and the induced neuronal cells. Our studies focusing on human pluripotent stem cells derived from PD patients may help to give an insight into potential pathways shared by LRRK2 and alpha-synuclein.

W-1339

PATIENT IPSC-BASED MODELLING OF GENETIC RENAL DISEASE

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The reprogramming of somatic cells into induced pluripotent stem cells (iPSC) provides the potential to model human diseases in the laboratory, as has been applied extensively in the cardiac and neuronal fields. Nephrology has not yet benefited from these advancements primarily due to the lack of a robust kidney differentiation protocol. We have recently demonstrated a capacity to differentiate human iPSC to cells which self-organize into kidney organoids containing appropriate mesenchymal and epithelial components, including nephrons. Here we report the generation and characterisation of iPSCs from patients with clinical diagnoses of either Nephronophthisis (NPHP) or Medullary Cystic Kidney Disease (MCKD), both cystic kidney disorders with diverse co-morbidities. While many genes have now been associated with cystic kidney disease, the genetic aetiology remains unclear for many patients, including all cases reported here. iPSC have been derived from 5 families (5 probands and matched unaffected relative) in which the proband lacks mutations in any known disease-associated gene. In order to establish transgene-free iPSC lines, fibroblasts were isolated via skin biopsy and reprogrammed using non-integrating Sendai virus in a 21-day protocol. Established iPSC lines have a typical hESC-like morphology and express pluripotency markers after 4 (TRA1-60) and 15 passages (NANOG). Moreover, iPCS lines have cleared the Sendai virus vectors, as confirmed by RT PCR after only 7 passages. G-band analysis of 2 lines from each isolation confirmed that each of the derived lines had maintained the normal karyotype after reprogramming. We now aim to redifferentiate patient and control iPSCs lines towards kidney by employing our previously established protocol. Kidney organoids will be analysed using IF and FACS and subjected to transcriptional profiling. We hope

that these studies will uncover the biological consequences of novel genetic variants identified via NextGeneration sequencing, thereby beginning to explain the patient pathogenic phenotype.

W-1340

MIGRATION AND NEURODIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELL (IPS) OF PATIENT WITH FOCAL CORTICAL DYSPLASIA

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Focal cortical dysplasia (FCD) is one of the most frequent forms of malformations of cortical development, due to disturbance of cellular migration and/or neurodifferentiation and usually it is related with medically refractory partial epilepsies. The reprogramming of adult somatic cells is an attractive and promising approach due to the possibility of in vitro studies of complex genetic diseases. Direct cell reprogramming by the addition of four pluripotent genes in somatic cells, generate induced pluripotent stem cells (iPS), being very similar to embryonic stem cells with the characteristics of self-renewal and differentiation potential. The establishment of iPS using in vitro models could be an interesting approach to better understand the cellular and molecular mechanisms of these malformations. Fibroblasts were obtained from skin biopsies of patients enrolled in the Program of the Hospital São Lucas da PUC Plastic Surgery and Epilepsy Surgery (one patient from each program) after signing an informed consent. After observing the growth of fibroblasts, the cultures were tested for Mycoplasma contamination and the fibroblasts were grown up to the fifth passage. Cell migration test was carried out by cultivating the fibroblasts membrane inserts and induction migrating with fetal bovine serum at 30% for 24, 48 and 72 hours. Neurodifferentiation fibroblasts was performed by induction as described by Song et al 2008 and further characterization of neuronal structures using markers NeuN, NFH, β-tubulina-3 e MAP2. The expression of undifferentiated genes was detected by qRT-PCR. The iPS were generated through transfections by exposure to viral vectors containing the genes conference pluripotency (KLF4 / Oct4/ SOX2 / cMyc) using SENDAI kit feeder free protocol. The observed in vitro cell migration after 24 and 48 hours was higher in fibroblasts of patient with FCD ($p<0,001$) than the control however it was not sustained after 72 hours. In fibroblast of patient with FCD there is an increase of STX1A and SOX2 expression gene ($p<0,001$.) but we didn't succeed to induced neuronal differentiation in them. iPS clones could be observed from the 10th day after transfection and were expanded through manually pick.

W-1341

INDUCED PLURIPOTENT STEM CELLS (iPSCS) DERIVED FROM PATIENTS WITH SPINOCEREBELLAR ATAXIA TYPE 2 FOR PURKINJE CELL DIFFERENTIATION

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Spinocerebellar ataxia type 2 (SCA2) is a neurodegenerative disease primarily affecting neurons of the cerebellum such as Purkinje and granule cells. SCA2 is an autosomal dominant disease where 33 or more CAG repeats in the ATXN2 gene will result in a disease phenotype. As observed in all polyglutamine diseases, increased number of CAGs causes accumulation of the mutant protein in the cells, leading to neurodegeneration in specific brain regions. In case of SCA2, degeneration is seen mostly in the cerebellum, brain stem, and spinal cord, resulting in the loss of fine motor coordination. Life expectancy for SCA2 patients is around 10-15 years after onset of the disease. To date, no treatment is available to delay or halt the progression of SCA2. In this study, we generated induced pluripotent stem cells (iPSCs) of patient fibroblasts, which were subsequently gene corrected using CRISPR (clustered regularly interspaced short palindromic repeats) technology. These cells will be further differentiated into Purkinje neurons, the cell type most severely affected in SCA2 patients. Results obtained from experiments with skin fibroblasts from various SCA2 patients show that patient fibroblasts exhibit more oxidative stress, increased basal level of apoptosis, and impairment of the proteasomal function compared to control fibroblasts, indicating that these cellular pathways are involved in the disease mechanism of SCA2. These findings will be validated in our SCA2 iPSC model that will serve as a human neuronal model for further mechanistic studies on SCA2.

W-1342

DUAL STRATEGY FOR VARIANT PRIORITIZATION USING WHOLE GENOME SEQUENCING AND DISEASE-SPECIFIC IPSC TRANSCRIPTOMES IDENTIFIES NOVEL GENES ASSOCIATED WITH HYPOPLASTIC LEFT HEART SYNDROME

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The identification of genetic variants implicated in a complex multifactorial disease such as Hypoplastic Left Heart Syndrome (HLHS) often renders a long list of genes with unknown relevance in terms of pathogenesis. Complementing standard filtering strategies with additional datasets based upon biological evidence would create an innovative platform to prioritize relevant candidate genes and mutations. In this study, cardiac structure and function were evaluated by echocardiography in 5 members of a nuclear family which included a child with HLHS. DNA from all family members was processed and subjected to whole genome sequencing (WGS). Genetic variants were filtered according to rarity, functional impact and mode of inheritance leading to the identification of 34 genes with recessive or de novo variants potentially involved in the pathogenesis of HLHS. Simultaneously, induced pluripotent stem cells (iPSC) were derived from proband and parents and RNA-sequencing was performed in undifferentiated and spontaneously differentiated samples. Resulting from comparative transcriptional analysis, gene sets differentially expressed in proband compared to progenitors at each of those stages were used as additional filters. Out of 34 mutated genes, 10 displayed transcriptional differences in undifferentiated iPSC from the HLHS-affected individual while 16

out of 34 mutated genes showed significantly different expression levels in proband differentiated cells. Expression level dynamics for genes fulfilling both criteria (9 total) were further characterized in iPSC from proband and controls in a time-course of guided cardiac differentiation. Two genes, ELF4 and HSPG2, displayed significantly different profiles between differentiating HLHS-iPSC and control counterparts (p=0.04 and 0.008 in general linear model comparison), with PCDH11X also approaching significance (p=0.09). Temporal expression differences were detected for PRTG (p=0.02). Of note, none of these genes had been previously linked to HLHS. In summary, this innovative unbiased filtering strategy integrating WGS and a new layer of transcriptional information that leverages iPSC plasticity allows us to prioritize genes associated with HLHS in an in vitro model of disease.

W-1343

STEM CELL MODELING OF AUTOPHAGY DYSREGULATION IN X-LINKED SPINAL AND BULBAR MUSCULAR ATROPHY

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Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is a X-linked inherited neuromuscular disorder characterized by lower motor neuron degeneration leading to weakness and atrophy of bulbar, facial and limb muscles. A CAG repeat expansion in the first exon of the androgen receptor (AR) is the mutation that causes SBMA. This mutation encodes a polyglutamine (polyQ) stretch in the translated protein, and therefore places SBMA as one member of a family of nine polyQ repeat disorders. A common feature to all neurodegenerative disorders is the production of misfolded proteins that form aggregates, highlighting the key role of protein "quality control" systems, such as autophagy, in protecting neurons from misfolded protein stress. Autophagy dysfunction has been implicated in numerous neurodegenerative disorders, but its mechanistic basis in polyQ repeat diseases remains unclear. To determine the mechanistic basis of SBMA autophagy pathway dysfunction, we tested if polyQ-AR might be interfering with the function of a master autophagy pathway transcription regulatory protein known as TFEB, and we found strong evidence for an interaction between AR and TFEB. We also observed AR polyQ length-dependent repression of a TFEB response element (4X CLEAR) promoter-reporter in cell co-transfection assays. Analysis of SBMA-derived neural progenitor cells (NPCs) revealed autophagy flux impairment, accompanied by significant down-regulation of TFEB target-genes. Dysregulation of autophagic flux was confirmed in SBMA patient NPCs using a mCherry-EGFP-LC3 vector to quantify autophagosome (yellow puncta) and autolysosome (red puncta) formation. RT-PCR analysis of SBMA NPC RNA samples yielded significant reductions in TFEB target gene expression, further demonstrating polyQ-AR-mediated TFEB inhibition. When we tested if TFEB over-expression could rescue the autophagy pathway flux defect in SBMA NPCs, we found that TFEB could relieve the autophagic flux blockage present in SBMA NPCs. Our results thus implicate AR-mediated TFEB transcriptionopathy in autophagy pathway dysregulation in SBMA, and pinpoint altered CLEAR signaling pathway function as a pathological turning point in SBMA and potentially in other related neurodegenerative proteinopathies.

W-1344

HEMATOPOIETIC DIFFERENTIATION OF MURINE AND HUMAN IPSC REVEALS GM-CSF DEFECT IN PULMONARY ALVEOLAR PROTEINOSIS

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Following their discovery, induced pluripotent stem cells have proven applicability to various areas including disease modeling. Hereditary pulmonary alveolar proteinosis (herPAP) is a rare lung disease resulting from the inability of alveolar macrophages to clear the alveolar spaces from surfactant material. This defect is due to mutations within the genes encoding the α- or β-chain of the GM-CSF receptor, CSF2RA or CSF2RB, respectively. Given the limited treatment options in herPAP, we evaluated the suitability of iPSC-derived monocytes/macrophages (M/M) for disease modeling and, following gene correction, as a source for cell and gene therapy. For disease modeling we first generated iPSCs from Csf2rb deficient mice (miPAP) that displayed all major pluripotency criteria and could be differentiated into hematopoietic progenitor cells (HPC). In contrast to control iPSC-HPCs, miPAP-HPCs failed to form colonies in a clonogenic assay supplemented with GM-CSF only. HPCs could be further differentiated into M/M displaying the typical morphology and surface phenotype of bone marrow-derived macrophages. When miPAP-M/M were subjected to GM-CSF-dependent functional assays such as phosphorylation of STAT5 and GM-CSF-clearance, miPAP-M/M recapitulated the deficiency in GM-CSF signaling typical of herPAP. Based on these data, we next transferred this modeling approach into the clinically more relevant human system. Upon differentiation into M/M, patient specific PAP-hiPSCs carrying a CSF2RA mutation showed impaired GM-CSF dependent functions including CD11b activation, GM-CSF uptake, phagocytosis, and CSF2R-downstream signalling such as STAT5 phosphorylation. These functional defects could be effectively corrected upon transduction of PAP-hiPSC with a SIN-lentiviral vector expressing a codon-optimized CSF2RA-cDNA. Thus, in summary, we here established PAP-specific murine and human iPSC lines that upon differentiation comprise a functionally relevant disease modeling tool. Furthermore, after genetic correction PAP-hiPSC showed recovered CSF2R-dependent macrophage functions. Preliminary results in the murine model of herPAP also suggest that iPSC-M/M may constitute an interesting source for transplantation, and allow for innovative therapeutic applications.

W-1345

IPSC BASED CLINICAL TRANSLATION OF A MONOGENIC CHRONIC PAIN CONDITION; IPSC RECAPITULATE BOTH PHENOTYPE AND PHARMACOLOGICAL REVERSAL IN INHERITED ERYTHROMELALGIA

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Many patients suffering from neuropathic pain do not respond efficiently to current analgesics, such as patients with inherited Erythromelalgia (EM), a disease with burning pain sensations in the extremities that are often episodic and triggered by external stimuli like changes in heat and temperature. Inherited EM is caused by monogenic mutations in the voltage gated sodium channel SCN9A (Nav1.7). These mutations, of which 18 are currently known, result in hyperpolarizing shifts in voltage-dependent channel activation or inactivation. Development of a selective Nav1.7 blocker has so far been compromised due to lack of human patient material and surrogate human physiological models. Using iPSC technology, we have generated iPSC from four EM patients with different SCN9A mutations and subsequently differentiated several clonal lines per patient to nociceptive neurons. Despite comparable Nav1.7 activity, electrophysiology studies showed an increased excitability and spontaneous firing activity phenotype with aberrant responses to thermal stimuli relative to non-EM iPSC derived neurons. Both, the neuronal excitability level and spontaneous firing activity differed with individual EM mutations. These patient specific effects well recapitulated their clinical pain phenotype in terms of pain severity measures, demonstrating sufficient sensitivity of the iPSC based 'pain in a dish' approach. Increased excitability and spontaneous firing in iPSC derived EM neurons were reversed when a novel selective Nav1.7 blocker was applied. Blocking efficacy indicated convergence with its in vivo response efficacy in these patients, which has been assessed within the framework of a clinical trial. Taken together, this study for the first time proves utility of iPSC based disease modelling to translate chronic pain phenotypes and treatment responses from the clinic to an in vitro model. Ultimately, the range of clinical phenotypes and response efficacies among EM patients and their in vitro counterpart will open avenues to better understand individual genotype-phenotype correlations in the future for development of next generation analgesics.

W-1346

PSC-DERIVED HEMATOPOIETIC CELLS AS A NEW TOOL FOR EXPLORING LEUKEMIA PATHOGENESIS

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Onset of acute myeloid leukemia (AML) has been accounted for by cooperation between multiple genetic alterations. For example, AML1-ETO fusion (AE) generated by translocation (8;21) (q22;q22) is one of the common mutations observed in 20-40% of patients. AE affects transcriptional regulation associated with hematopoietic differentiation, while 60% of AE-positive AML cases are shown to have together other types of mutation of genes involved in cell proliferation, such as receptor tyrosine kinase (RTK) c-kit and FLT3. Those data are compatible with so-called "multi-step leukemogenesis" model. To elucidate unknown pathogenesis in reproducible manner, we established the novel disease modelling system using pluripotent stem cell (PSC)-derived hematopoietic culture in combination with reverse genetic approach. We induced hematopoietic cells from PSCs harbouring inducible AE fusion gene cassettes with or without other pathogenic genes such as RTK mutations, and performed in vitro and in vivo assay. First, serial replanting assays in methylcellulose-containing semisolid media as well as liquid culture revealed the strong tendency toward increased colony forming efficacy and suppressed differentiation. Especially, AE-positive myeloid lineage-committed progenitors as well as immature multipotent hematopoietic stem and progenitor cells (HSPCs) showed higher replating colony forming efficacy, which may indicate the representation of higher incidence of myeloblastic leukemia in AE-positive AML. In addition to in vitro assays, we next evaluated the in vivo phenotype by transplanting hematopoietic cells into immunodeficient NOG mice. Also in these experiments, we successfully observed the cooperation between AE and RTK mutations for increased engraftment with leukemia-like phenotypes when transplanting immature HSPCs. Those results indicated the successful recapitulation of pathogenic cooperation between AE and RTK mutations in our PSC-derived hematopoietic cells. In conclusion, we successfully established a novel system in vivo and in vitro to explore pathogenesis of leukemia using PSC-derived hematopoietic cells. We believe that our model must allow us to better match treatment to prognosis across the disease spectrum via comprehensive understanding of pathogenesis.

W-1347

CORRECTED MUTATION BY GENE TARGETING IN PATIENT-DERIVED IPS CELLS RESCUES DISEASE PHENOTYPES AND IDENTIFIES A CANDIDATE PHARMACOLOGICAL INTERVENTION

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Pelizaeus Merzbacher Disease (PMD) is a pediatric-onset progressive central nervous system disorder caused by mutations in PLP1 gene, affecting oligodendrocytes, the myelinating glial cells. Limited knowledge on disease mechanisms and the lack of treatment leads to lethality in patients affected by mutations associated with severe phenotype. We have collected fibroblasts from early-onset, severe PMD patients who participated in a clinical trial to receive non-autologous cell transplantation therapy (clinical trial identifier: NCT01005004) and modeled the disease from one patient by making iPS cells. The patient-derived iPS cells were genetically engineered to correct the disease causing point mutation resulting in one amino-acid change. When these cell lines were directed to oligodendrocyte differentiation under the strictly same protocol, the patient-derived mutant line showed reduced number of oligodendrocytes associated with morphological abnormality, maturational arrest, oxidative stress and apoptotic cell death, all of which were reversed in the corrected line. In human slice culture assay, the corrected cells were capable of differentiate to mature oligodendrocytes with similar morphology to endogenous human oligodendrocytes. We further tested a candidate pharmacological intervention inferred from rodent models of PMD. The intervention during oligodendrocyte differentiation rescued the disease phenotypes in oxidative stress and apoptotic cell death, allowing patient-derived mutant cells to differentiate

into mature oligodendrocytes. The intervention had no effect on the corrected line, suggesting specific role on the mutation. These findings demonstrate that corrected mutation in iPS cells can reverse disease phenotypes in oligodendrocytes, which implicates an alternative, autologous source for cell-based therapy. Moreover, a pharmacological intervention could be possible in an early-onset, severe PMD.

W-1348

PHENOTYPIC ABNORMALITIES IN FRIEDREICH ATAXIA-INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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Friedreich ataxia (FRDA) is an autosomal recessive disorder characterised by neurodegeneration and cardiomyopathy. It is caused by a GAA repeat expansion in the first intron of the FRATAXIN (FXN) gene that results in reduced FXN expression. Human induced pluripotent stem cells (iPSCs) are valuable cell models for FRDA, as these cells are of patient origin and can be differentiated into cell types of interest. To date, there are limited reports of a FRDA-related phenotype being identified in iPSC-derived cell types representative of disease pathology, such as cardiomyocytes (CMs). We used our previously reported FRDA-patient lines FA3 and FA4 as well as new iPSC lines. Here we undertook nucleofection to deliver episomal vectors containing OCT4, SOX2, KLF4, L-MYC, LIN28, shRNA against p53 and eGFP into FRDA-patient fibroblasts and derived 3 FRDA-iPSC lines, from individuals with different GAA repeat numbers: FA6 (female, 1077/1077); FA8 (male, 476/545); FA9 (male, 733/943). All clones expressed the pluripotency markers OCT4 and TRA-1-60 and could differentiate into cells of the three germ layers, as assessed by embryoid body formation. After reprogramming, we observed similar GAA repeat numbers as in original fibroblasts as well as retractions and expansions for all lines, with slight variations between clones of the same line. Importantly, the patient-fibroblasts and -derived iPSC lines maintained the reduced FXN expression that is characteristic of FRDA. Using a small-molecule based approach, we differentiated all iPSC lines (FA3, 4, 6, 8 and 9) and a control hESC line H9 into CMs. The FRDA-iPSC-derived CMs retained low levels of FXN and were mainly of a ventricular phenotype as assessed by the expression of MLC2v and MLC2a. These cells were then used for assessment of mitochondrial activity and electrophysiology. High-resolution respirometry showed abnormal oxidative phosphorylation (OXPHOS) with an impairment in Complex I activity and Multi Electrode Array revealed increased beat rate variability of FRDA-CMs, when compared to control hESC-derived CMs. Our data thus describe specific disease phenotypes in FRDA-CMs and demonstrate the value of FRDA-iPSC-derived CMs for successful disease modelling.

W-1349

NOVEL NEUROTHERAPEUTIC SYNERGISM AND BIOCHEMICAL INSIGHTS INTO THE GSK3B PATHWAY VIA HUMAN IPSC DERIVED NEURONS

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Mechanistic understanding and development of novel drug treatments for neuropsychiatric disorders has been restricted due to difficulties in acquiring live diseased human neural cells for experimentation. With advancements in stem cell technologies and neuronal differentiation, it is now possible to perform drug screens on human neurons derived from embryonic and induced pluripotent stem cells, including those from disease affected patients. The glycogen synthase kinase-3 beta (GSK3b) signaling pathway has long been associated with a myriad of neurodevelopmental and neuropsychiatric disorders, and modulation of GSK3b and its substrates' represents intriguing targets for novel neurotherapeutics. We have utilized human induced pluripotent stem cell (hiPSC) derived neurons to screen for compounds that modulate GSK3b. Unexpectedly, we found synergistic drug combinations that significantly impacted GSK3b and known direct GSK3b substrates. Fascinatingly, the efficacious drug synergisms we found are known to operate through divergent mechanisms of action, which appear to be converging on our proteins of interest. The effect of the compounds we found were compared between hiPSC derived neurons from individuals affected with a neuropsychiatric disorder and hiPSC derived neurons from unaffected individuals. Surprisingly, the affected hiPSC derived neurons had a different biochemical response to the screened compounds than the unaffected neurons, possibly highlighting key GSK3b pathway abnormalities in individuals with neuropsychiatric disorders. Our results identified a possible novel synergistic therapeutic approach for treating neuropsychiatric disorders caused by GSK3b pathway dysfunction, and identified compounds that may have the potential to be developed as neurotherapeutics.

W-1350

SITE-SPECIFIC GENE EDITING OF COL7A1 RESTORES TYPE VII COLLAGEN IN RDEB IPSCS

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Recessive dystrophic epidermolysis bullosa (RDEB) is an inherited blistering disorder caused by mutations in the COL7A1 gene encoding type VII collagen (C7), the major component of anchoring fibrils (AFs) at the dermal-epidermal junction. Loss of the AFs leads

to severe blistering, tissue cleavage and a high incidence of mortality through progression to metastatic squamous cell carcinoma. All previous therapeutic advances have had notable limitations and alternative approaches based on gene editing of disease causing mutations have recently become feasible. Furthermore, iPSCs provide an attractive alternative as a constant source of material for cell therapy for the treatment of degenerative diseases, such as RDEB, where the extensive need of tissue regeneration is required. Here we report the use of zinc finger nucleases (ZFN) for the targeted editing of COL7A1 and subsequent restoration of C7 expression in iPSCs derived from RDEB patient cells. ZFNs were designed to target a mutation hot-spot clustered around exon 4 of COL7A1. To improve the inefficiency of delivery of these DNA-binding nucleases we incorporated the ZFN pairs into a non-integrating lentiviral vector (NILV) platform, allowing for efficient yet transient expression. A dsDNA donor repair template encompassing a portion of endogenous sequence was designed and delivered via a third NILV. Introduction of silent mutations within the ZFN binding site of the template prevented ZFN-mediated cleavage, while incorporation of a restriction site within the intronic region allowed for PCR-based detection of integration. RDEB patient fibroblasts carrying a missense mutation in a nearby exon 3 (p.K142R) were reprogrammed into iPSCs which were subsequently transduced with a combination of NILVs delivering our ZFN pair and donor template. Direct sequencing revealed targeted template insertion by homologous recombination in 3/13 clones. The restoration of endogenous C7 expression was further demonstrated on the protein level. We now plan to employ our previously reported differentiation protocol yielding a pure population of keratinocytes from iPSCs. In theory, these iPSC-derived keratinocytes would provide an unlimited source for tissue regeneration for this debilitating skin disorder.

W-1351

MODELLING KOSTMANN DISEASE IN VITRO USING IPSC TECHNOLOGY

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Kostmann disease is a heritable disorder characterized by monogenetic lesion in HCLS1 associated protein X-1 (HAX1) gene. Patients with *HAX1*-deficiency show a promyelocytic arrest leading to the development of severe congenital neutropenia (SCN) and are predisposed to acute myeloid leukemia (AML). Here we employed the induced pluripotent stem cell (iPSC) technology to recapitulate the clinical phenotype of Kostmann disease in vitro. Skin fibroblasts carrying the HAX1^{W44X} mutation were reprogrammed to pluripotent state by ectopic expression of transcription factors OCT4, SOX2, KLF4 and the microRNA cluster 302/367. Generated iPSCs carried the original HAX1^{W44X} mutation and showed activation of pluripotency network. Neutrophilic differentiation capacity was impaired in patient-derived HAX1^{W44X}-iPSCs. Percentage of CD66b^{+} mature neutrophils was decreased whereas the percentage of CD11b^{+} monocytes was increased compared to control iPSC from a healthy donor or embryonic stem cell controls. Cytospins showed cells with neutrophilic and promyelocytic morphology in control sample, while the HAX1^{W44X}-iPSCs predominantly differentiated into monocyte-like cells. Genetic correction by lentiviral insertion of intact HAX1-cDNA could partially rescue the in vitro disease phenotype. Thus, the Kostmann disease can be recapitulated by patient-specific iPSCs in vitro. The disease model will serve as basis for further studies to shed light on the complex regulatory network of human hematopoiesis and its deregulation during leukemic transformation.

W-1352

SOMATIC CELL REPROGRAMMING: THE PROGRESS TOWARD ESTABLISHMENT OF INDUCED PLURIPOTENT STEM CELL AS A SCLERODERMA DISEASE MODEL

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Scleroderma -one of the important autoimmune diseases- leads to death of endothelial cells as one of the early events of this disease. The lack of repair after the loss of endothelial cells is observed in these patients and although it is a worldwide disease, its cause has remained unknown. Because of physiological differences between human and other species, a proper scleroderma animal model which represents all aspects of the disease has not been generated yet. So, making a patient-based system to mimic a developmental defect and evaluating its probable repair mechanism is considered as a necessity. In this study it was tried to generate patients' specific induced pluripotent stem (iPS) cells and characterize them. Patient's fibroblast cells -as an available and unlimited source of disease study in vitro- were isolated, cultured and transduction with retroviral vector containing Oct 3/4, Sox2, Klf4 and c-Myc. For characterization of generated iPS cells, their expression of endogenous pluripotent and silencing of retroviral genes were evaluated by RT-PCR technique and the existence of pluripotency proteins were assessed by immunostaining. Spontaneous differentiation into endothelial cells was done by embroid body formation and the expression of specific endothelial cell markers were examined by flowcytometery technique. RT-PCR analysis and immunostaining study of generated cells compared to human embryonic stem cells (royan H5) results showed that reprogrammed cells express specific markers similar to embryonic stem cells. EB formation results demonstrated that scleroderma pluripotent cells can differentiate into endothelial cells similar to human embryonic stem cell. Our study showed that scleroderma specific iPS cells can be indicated as an unlimited and available source for assessing molecular defects in vitro and finding regenerative methods to repair endothelial cells in scleroderma patients.

W-1353

MODELING AMYOTROPHIC LATERAL SCLEROSIS IN iPSC-DERIVED MOTOR NEURONS CARRYING MUTATIONS IN THE FUS GENE

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Induced Pluripotent Stem Cells (iPSCs) provide an opportunity to study human diseases in those cases where appropriate models are not available. Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease caused by loss of motoneurons (MNs), leading to progressive muscle atrophy. The recent discovery of several ALS-linked genes was promising for the identification of cellular and molecular defects underlying the pathology. However, in the case of the RNA-binding factor FUS, most in vitro studies rely on non-neural or neuroblastoma cell lines, in which the mutated protein is usually overexpressed. Such systems do not recapitulate the complexity of the MN and its microenvironment and imply non-physiological levels of protein. Here, we have derived iPSCs carrying ALS mutations in the FUS gene. Our iPSCs collection includes lines derived by reprogramming from patients (FUS-R514S and FUS-R521C) or raised by TALEN-directed mutagenesis (FUS-P525L), all of which express physiological levels of FUS. iPSC-derived MNs provided an in vitro model to study the behavior of the mutant proteins in the appropriate cellular and genetic background. We show that aberrant cytoplasmic localization of mutated FUS, which has been proposed as the initial step in ALS, was recapitulated in iPSC-derived MNs. Increased oxidative stress is thought to play a role in ALS pathogenesis and FUS cytoplasmic inclusions co-localize with stress granules (SGs) markers in ALS patients. Upon different kinds of stress, we detected aberrant recruitment of mutated FUS into SGs. Levels of FUS within SGs nicely mirrored the cytoplasmic delocalization phenotype of the different mutants, which is in turn inversely correlated with the age of ALS onset in patients carrying the same mutations. Therefore, the iPSC system presented here represents a suitable model for investigating the correlation between FUS mutations and ALS ethiopathogenesis.

W-1354

MODELING NEURODEGENERATIVE DISEASES BASED ON ISOGENIC GENE-CORRECTED IPS CELLS

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Induced pluripotent stem (iPS) cell technology allows studying diseases in a relevant cell type and therefore has the potential to capture pathomechanistic effects that may not be present in conventional in vitro model systems. iPS cells derived from patients with a pathogenic familial mutation can be used to investigate dysfunction of such mutations in a patient specific context without artificial overexpression. Gene-editing using TALEN or CRISPR technology is a powerful tool that enables the generation of isogenic controls differing for example in only a single DNA base. As such, the use of isogenic lines allows investigating the dysfunction of a pathogenic familial mutation. Here, we report the generation of iPS cells from patients with a familial form of Alzheimer's disease (AD), Fronto Temporal Dementia (FTD) and Spinocerebellar Ataxia 2 type 2 (SCA2). All patients harbored a genetic mutation (AD and FTD) or an elongated CAG repeat (SCA2) causing the respective disease. iPS cells were generated using a plasmid-based, non-integrative system. Characterization analyses revealed absence of reprogramming plasmids, normal karyotypes and expression of pluripotency markers. Through TALEN and CRISPR mediated gene-editing, we obtained both gene-corrected isogenic controls as well as healthy iPS cell lines with inserted hetero- and homozygous mutations. Upon differentiation to particular neuronal subtypes, we are investigating diverse disease-related phenotypes that can be rescued upon gene-correction. Interestingly, phenotypes are not detectable when patient iPS cell derived neurons are compared to neurons from age and gender matched healthy controls, potentially due to too high genetic variability. Our work therefore highlights the power of iPS cell derived neurons in combination with gene-editing techniques to uncover specific disease-related phenotypes in the culture dish.

W-1355

THE EARLY PATHOGENESIS OF DUCHENNE MUSCULAR DYSTROPHY EXHIBITED WITH PATIENT-DERIVED HUMAN INDUCED PLURIPOTENT STEM CELLS

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Duchenne muscular dystrophy (DMD) is a progressive and fatal muscle wasting disease caused by dystrophin deficiency. Effective suppression of primary pathology observed in DMD is the key to conquer this devastating disease. Patient-derived human induced pluripotent stem cells (hiPSCs) are becoming promising tool for developing novel drugs for intractable diseases. Here, we report a development of an in vitro evaluation system for a DMD therapy using hiPSCs for future drug screening. Skeletal myotubes generated from hiPSCs provides intact condition, allowing us to generate initial pathology of DMD in vitro. Induced wild type (WT) and DMD myotubes were substantially functional and morphologically comparable. However, electric stimulation to those myotubes as in vitro 'exercise' caused pronounced calcium ion (Ca2+) influx in DMD myocytes. Moreover, restoration of dystrophin by using exon-skipping technique suppressed Ca2+ overflow and reduced secretion of creatine kinase (CK) of DMD muscles. Thus, early pathogenesis of DMD can be exhibited with skeletal myotubes induced from patient-derived iPSCs effectively, which leads to a novel drug development for DMD.

W-1356

SYNAPTIC DYSREGULATION IN HUMAN INDUCED PLURI-POTENT STEM CELL-DERIVED NEURONS CARRYING THE A53T ALPHA-SYNUCLEIN MUTATION

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Parkinson's disease (PD) represents the second most common neurodegenerative disease of aging population. Even though a large number of transgenic animals and cell models have provided insight into the disease, it has been difficult to demonstrate that implicated mechanisms are also operating in neurons from affected individuals. Human induced pluripotent stem cell (iPS) technology has facilitated the investigation of phenotypes of patient-specific cells in vitro and offers a unique opportunity to study disease pathogenesis. In this study we haveused iPS cell lines derived from skin fibroblasts of Parkinsonian patients with a familial form of the disease, that carry the dominantly inherited G209A mutation in the α-synuclein gene (SNCA) encoding the A53T mutant α-synuclein protein (A53TαSYN), for directed neuronal differentiation, generating a cellular model of PD. Next generation sequencing (RNA-Seq) of mutant neurons has revealed a significant downregulation of genes involved in synaptogenesis, including transcripts encoding for i) presynaptic proteins such as SNAP-25, Synapsin 3, ALK and NRP2 and the CDC20-APC complex that is essential for the formation of the synaptic vesicle clustering, ii) trans synaptic adhesion molecules of the cadherin family such as CDH13, CDH15, CDH9 and CHRNG and iii) postsynaptic proteins such as DLGAP2 and SLITRKs. Another affected set of genes included members of the WNT family such as WNT1, DKK2, WNT7A and WISP1, that have been shown to induce synaptic degeneration and sensitivity to cell death, when impaired in animal models. The human mutant neurons exhibited profound sensitivity when exposed to various stress agents compared to control neurons suggesting defects in mitochondrial, protein degradation, oxidative stress and apoptosis. Finally, 26 out of 53 deregulated mRNA's involved in synaptic processes have already been linked to autism, schizophrenia and depression, the so called "diseases of the synapse". Overall our study reveals that the A53TaSYN dysregulation leads to synaptic dysfunction and increased susceptibility to environmental stress conditions, providing novel information regarding the mechanisms of the A53TaSYN pathology and possibly new targets for the development of therapeutics for synapse restoration and preservation for PD.

W-1357

DEVELOPMENT OF AN INDUCED PLURIPOTENT STEM CELL BASED MODEL OF ATAXIN-3 AGGREGATION

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SCA3 is a neurodegenerative disorder caused by expansion of polyglutamine (polyQ)-encoding CAG repeats in the ATXN3 gene. Like in all polyQ diseases, the CAG repeat length is inversely associated with the age at onset (AO) of the disease. Likewise, CAG-repeat length is proportionally related to the aggregation propensity of the ataxin3 protein, strongly suggesting that aggregation formation is a main disease-inducing factor. However, in patients with the same CAG repeat length, AO can vary up to more than 40 years, suggesting that other genetic or environmental factors must be involved. Studying such factors requires patient representative models. The recently discovery of the ability to reprogram fibroblast to pluripotent stem cells (iPSC) and the subsequent strategies to develop neuronal-like cells from them allows the studying the aggregation behaviour of the polyQ expanded ataxin3 protein within the patient's own genomic context. As a proof-of-concept, we here generated iPSC-derived neuronal lines from three SCA3 patients with similar CAG repeat length but with different AO. We show that iPSC generation and neuronal differentiation is possible and largely unaffected irrespective of the expression of the expanded polyQ-containing ataxin 3. We will report in how far aggregation-formation (spontaneous and induced by glutamate) and expression levels of aggregation-modulating chaperones differ between the different SCA3 iPSC-derived neurons.

W-1358

IDENTIFICATION OF CRITICAL FUNCTIONS OF DISC1 IN MAJOR MENTAL ILLNESS

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Schizophrenia is a debilitating psychiatric disorder that affects ~1% of the world's population. An improved understanding of the etiology of major mental illnesses will greatly facilitate development of disease-modifying treatments. Genetic and clinical association studies have identified the "disrupted in schizophrenia 1" (DISC1) as a strong candidate risk gene for schizophrenia and other major mental illnesses. DISC1 was initially associated with mental illness upon the discovery that its coding sequence is interrupted in a group of human subjects by a balanced t(1;11)(q42.1;q14.3) translocation that co-segregates with schizophrenia, bipolar disorder and major depression. DISC1 modulates many neuronal processes, including proliferation, Wnt signaling, synaptic maturation, neurite outgrowth, and neuronal migration. Adding to the complexity of DISC1 biology, over 50 DISC1 splice variants have been identified in the human brain. The relevance of a DISC1 loss-of-function versus gain-of-function model to the human disease state remains unclear. As the expression pattern of DISC1 differs between humans and rodents, exploration of disease-relevant DISC1 expression and function is best accomplished in human cells. We have explored the consequences of DISC1 interruption using TALE nucleases (TALENs) and CRISPR-Cas to disrupt the genome of human induced pluripotent stem cells at the DISC1 locus. Each line has been differentiated to neural progenitor cells (NPCs) and cortical neuronal fates and examined for altered gene expression, Wnt signaling, proliferation, and neuronal migration and morphology. Disease-relevant DISC1 interruption decreases expression of long DISC1 isoforms by nonsense-mediated decay, supporting a loss-of-function model of DISC1 disruption. Furthermore, DISC1 disruption increases baseline Wnt activity in NPCs, while reducing Wnt responsiveness. Our data suggest that alterations in WNT

activity result from subtle effects of DISC1 disruption on NPC fate. These data and future studies will help identify those functions of DISC1 which are likely to be perturbed in patients with the chr(1;11) translocation and which, when disrupted, contribute to the development of major mental illness.

W-1359

PROTECTION OF NOC18 AGAINST HIGH GLUCOSE CONDITION IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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Human induced pluripotent stem cells (hiPSCs) have the ability to differentiate into cardiomyocytes. However, little is known on their behavior in high glucose condition. Here we study the protective effect of the NO-donor NOC-18 and its downstream cellular signal pathway. Human induced pluripotent stem cell-derived cardiomyocytes were preincubated with or without NOC-18, a NO-donor, for 24 hrs, followed by 22mM high glucose condition or corresponding normal glucose condition. The following treatments were applied during high or normal glucose condition: the NO-donor NOC-18, the protein kinase G (PKG) inhibitor, glibenclamide (KATP channel blocker), LY294002 (PI3K inhibitor) alone or in combination. Cells Viability was examined by propidium iodide staining. NOC-18 attenuated high glucose-induced cell death in dose-dependent way, and the protection was attenuated by the inhibitor, such as PKG inhibitor, KATP channel blocker, or PI3K inhibitor. Our results reveal a novel molecular mechanism that NOC-18 protects hiPSC-derived cardiomyocytes again high glucose injury and KATP channel play a critical role in NOC-18/PKG/PI3K-signalling pathway. The novel hiPSC-derived cardiomyocytes-basing screening platform is a useful tool for discovering molecular regulation mechanisms of KATP channels again high glucose injury.

W-1360

MSH2 KNOCK DOWN TRIGGERS TRINUCLEOTIDE INSTABILITY IN MYOTONIC DYSTROPHY TYPE 1 HUMAN EMBRYONIC STEM CELLS

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Trinucleotide repeat (TNR) instability is the disease mechanism in a large number of neurodegenerative disorders such as myotonic dystrophy type 1 (DM1). The occurrence of instability has been associated with processes involving single stranded DNA such as DNA replication, repair, transcription and recombination. The role of mismatch repair (MMR) proteins MSH2, MSH3 and MSH6 is widely studied in different model systems. However conflicting results arose concerning the instability caused by different levels of mismatch repair expression. For this study, we focussed on *MSH2,* since this gene showed the most clear-cut difference of gene expression between hESC (high MSH2) and the osteo-progenitor-like cells (OPL, low MSH2) derived from them. We therefore used a knock-down of *MSH2* using lentiviral transduction of short hairpin RNA in DM1-hESC, obtaining a reduction in *MSH2* gene expression as low as 30% of the original expression in hESC as measured by real time PCR. TNR instability was measured by small pool PCR and denaturing Southern Blot, showing a higher instability in the *MSH2*-knock down samples compared to the wild-type hESC line and with scrambled short hairpin RNA. After growing single cell colonies, TNR instability was seen directly at the first passages suggesting the highly unstable state of the TNR in *MSH2*-knocked-down hESC. Although these results contradict the results of Du et al. (2013) on iPSC, these results support the hypothesis of Mason et al. (2014) and Goula et al. (2012), stating that a baseline level of MMR is required before TNR instability can occur. This means that OPL would not reach the baseline level of MMR expression to show TNR instability. When levels of MMR proteins rise to much higher levels, like in hESC, TNR instability would be present. However, the highest instability would be seen in cells with an MSH2 level in between OPL and hESC, the so-called 'suboptimal expression levels', which would be reached in our *MSH2* knock-down hESC. Our results would support the hypothesis of Goula et al., however, these results have to be confirmed on knock-out cell lines as well as on other DM1 hESC and iPSC lines.

W-1361

UTILIZING INDUCED PLURIPOTENT STEM CELLS (IPSCS) TO DELINEATE THE NEURODEVELOMENTAL GENOMIC BASE OF SCHIZOPHRENIA

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Schizophrenia is a neurodevelopmental disorder featuring complex aberrations in the structure, wiring, and chemistry of multiple neuronal systems. The abnormal developmental trajectory of the brain appears to be established during gestation, long before clinical symptoms of the disease appear in early adult life. In order to understand the effect of schizophrenia on neuronal differentiation, we utilized induced Pluripotent stem cells (iPSCs) derived from both schizophrenia disease(SCZD) patients (n=3) and normal patients (n=4). IPSCs were differentiated into neuronal progenitor cells. In order to elucidate the effects of schizophrenia on NPCs we preformed global RNA-sequencing to quantify differences in RNA expression. RNA-seq analysis shows 1375 dis-regulated genes in all SCZD iPSCs compared to control. Many of these genes are involved in important developmental pathways such as WNT/B-Catherin signaling, or in neuronal pathways, such as axonal guidance and glutamate receptor signaling. The majority of the deregulated genes are targeted by nuclear form of FGFR1, supporting the or of INFS in schizophrenia. The results of our study point to an early (preneuronal) developmental-genomic etiology of schizophrenia. The mechanisms underlying these transcriptional deregulations are under investigation. Supported by NYSTEM (C026415, C026714) and Patrick P. Lee Foundation and Esther Trachtman Foundation.

IPS CELLS: EPIGENETICS

W-1363

MYC AND SAGA REWIRE AN ALTERNATIVE SPLICING NETWORK DURING EARLY SOMATIC CELL REPROGRAMMING

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Embryonic stem cells are maintained in a self-renewing and pluripotent state by multiple regulatory pathways. As somatic cells reprogram to achieve pluripotency, a sequence of distinct transcriptional networks is activated. How epigenetic regulators modulate these networks and contribute to somatic cell reprogramming is not clear. Here we perform a functional RNAi screen to identify the earliest epigenetic regulators required for reprogramming. We find that components of the SAGA histone acetyltransferase complex, including Gcn5, are necessary for inducing pluripotency. Furthermore, we uncover that Myc initiates a positive feed forward loop within the first days of reprogramming to activate Gcn5 expression and trigger a novel Myc/SAGA-driven network centered on alternative splicing. Thus, these studies reveal a sequence of early events required for cellular reprogramming and expose a dynamic interplay between Myc and the SAGA complex that is critical to promote pluripotency.

W-1364

DNA METHYLATION AS A REPROGRAMMING MODULATOR: AN EPIGENOMIC ROADMAP TO INDUCED PLURIPOTENCY

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During cellular reprogramming to induced pluripotent stem cells (iPSCs), somatic cells rebuild their epigenetic architecture to acquire a steady self-renewing state. The biological significance and mechanisms of this somatic epigenetic remodeling have remained unclear. Here we characterize the epigenomic roadmap to pluripotency at base resolution by performing MethylC-seq, ChIP-seq (H3K4/K27/K36me3), and RNA-Seq on samples taken at several time points during murine secondary reprogramming. We investigated the changes in differentially methylated regions (DMRs) and integrated this with analysis of histone modifications. We observed that methylation gain in DMRs occurred gradually during reprogramming. In contrast, methylation loss in DMRs was achieved only at the transition to the ESC-like state. Supporting a prominent role for DNA methylation in reprogramming, DMRs were enriched for transcription factor binding sites (TFBSs) and histone mark H3K4me3. Cells exhibited focal DNA demethylation at the binding sites of activated reprogramming factors during high transgene expression. ESC-like pluripotent cells were distinguished by extension of demethylation to the wider neighborhood of these sites. Our data indicated contrasting modes of control for genes with CpG rich promoters, which demonstrated stable low DNA methylation and strong engagement of histone marks H3K4me3 and H3K27me3, and genes with CpG poor promoters whose repression was driven by DNA methylation. Such DNA methylation driven control is key to the expression of several ESC-pluripotency predictor genes, including Dppa2, Dppa5a and Esrrb. These results reveal the crucial role that DNA methylation plays in the epigenetic switch that drives somatic cells to pluripotency.

W-1365

IDENTIFICATION OF EPIGENETIC BARRIERS TO CELL REPROGRAMMING

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Induced pluripotent stem (iPS) cells are potentially an important source of cells for regenerative medicine. However, this technology is limited by the inefficiency of iPS cell generation. Previous studies have shown that reprogramming efficiency can be improved using epigenetic inhibitors, suggesting that epigenetic barriers to reprogramming exist. By using a targeted high content shRNA screen in immortalised Oct4-GFP reporter mouse embryonic fibroblasts containing DOX inducible Oct4, Sox2, Klf4 and c-Myc, we have identified epigenetic modifiers, which form epigenetic barriers to iPS cell generation. We are currently elucidating what role these epigenetic modifiers have in preventing differentiated cells re-establishing their pluripotent potential.

W-1366

HISTONE H3 LYSINE 36 METHYLATION IS A REGULATOR OF SOMATIC CELL REPROGRAMMING

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The ectopic expression of Oct4, Sox2, Klf4, and c-Myc (OSKM) are sufficient to reprogram fully differentiated cells into induced pluripotent stem cells (iPSCs). However, the efficiency of iPSC formation is low, suggesting the presence of multiple rate-limiting steps in the reprogramming process. To identify these steps, we had previously performed an shRNA screen to assess the effects of a selected set of chromatin modifying enzymes (CMEs) on reprogramming, and had shown that these enzymes can act as barriers or facilitators of this process. In particular, we had identified inhibition of the Histone H3 lysine 79 (H3K79) methyl-transferase Dot1L as a means to increase reprogramming efficiency. We have now extended this analysis to cover additional CMEs and identified two H3K36 methyl-transferases, Ash1L and SetD2, as potent suppressors of reprogramming. H3K36 methylation is found on gene bodies and associated with active gene expression and transcriptional elongation. ShRNA-mediated knock-down of Ash1L

and SetD2 increased the number of iPSC colonies by 2.5 to 3 fold as assessed by Tra-1-60 positivity. This observation suggests that loss of H3K36 methylation facilitates the acquisition of pluripotency. Consistent with this notion, inhibition of the de-methylases for this modification, Kdm2A and Kdm2B, resulted in significantly reduced numbers of iPSCs upon OSKM transduction. Conversely, overexpression of Kdm2b mimicked the effects observed upon Setd2 and Ash1L knock-down. Interestingly, suppression of H3K36 methylation had an additive effect with loss of H3K79 methylation (achieved by Dot1L inhibition) on reprogramming. Taken together, these findings suggest that removal of active marks on somatic cell-specific genes may be an important rate-limiting step during transition to pluripotency.

CHROMATIN IN STEM CELLS

W-1367

EXPRESSION AND EPIGENETIC STATES OF HUMAN EMBRYONIC STEM CELLS UNDER ENDOGENOUS OXYGEN TENSION

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Cells of the blastocyst inner cell mass are exposed to a hypoxic environment and phenotypic markers suggest that human embryonic stem cells (hESC) cultured in reduced oxygen environments are characterized by increased pluripotency. We sought to explore the impact of oxygen tension on the hESC regulatory landscape by culturing hESCs under normoxic (20%) and reduced oxygen tension (8%, hypoxic) and annotating the expression and epigenetic states of these cell types using massively parallel sequencing-based assays. Hypoxia specific enhancer regions, which showed an enrichment for H3K4me1, were defined in the cells cultured under reduced oxygen tension with MACS2 algorithm using the normoxic sample as control. We found that the genomes of hESCs cultured under 8% oxygen tension showed an increase in H3K4me1 genome occupancy and more pluripotency potential compared to the normoxic condition. Nearest gene analysis of the reduced oxygen tension showed a statistically significant enrichment in transcription factor targets of Nanog, Sox2, and Oct4 (FDR q-value <4.9e-64). The hypoxic specific enhancer regions also showed enrichments for transcription and organ development terms including nervous system development associated genes (FDR q-value <2.2e-227). We found a striking asymmetry in differentially expressed (DE) genes with a majority (384 out of 420 DE genes) over-expressed in hESCs cultured under reduced oxygen tension. HIF-1 and -2-alpha transcription factor networks were also enriched in under reduced oxygen tension (FDR q-value <1.7e-11). Integrin-linked kinase pathway, a hypoxia-induced anti-apoptotic factor, and TGF-β/ ALK1/ SMAD2/3 signaling pathway were among the enriched pathways in the cells cultured under reduced oxygen tension (FDR q-value <3.7e-52). Homeobox and homodomain were the enriched Integrative Protein Signature terms in the hypoxic specific enhancers (FDR q-value <7.7e-103) confirming an increase in pluripotency potential of the cells. Intersection of methyl-cytosine levels derived from genome bisulfite sequencing datasets showed that the enhancer elements linked to pluripotency associated genes were hypo-methylated (CpG methylation < 30%). In summary, hypoxia induces genomic hypo-methylation in pluripotent regulatory regions and an increase in gene expression.

W-1368

GEMININ REGULATES NEURAL PROGENITOR AND NEURONAL SUBTYPE GENERATION

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The embryonic development of the cerebral cortex involves the sequential generation of different neural progenitor cells reside in two main neurogenic regions, the ventricular and subventricular zone. In the developing mouse cortex, neuroepithelial cells (NECs) and Radial Glial cells (RGs) are the main neural progenitor cells. RGs undergo self-renewal divisions and subsequently they switch to neurogenic divisions in order to produce the cortical neurons that migrate radially towards their final position within the cortex. Cortical neurons are organized in six layers (I-VI) which are formed in a strict temporal order; deep layers first (V-VI) upper layers last (II/III-IV). A new model has been recently proposed according to which, neural progenitors are committed to specific neuronal subtypes even before the onset of neurogenesis. Moreover, epigenetic modifications mediated by the Polycomb Group (PcG) have been suggested to play an important role in neuronal subtypes generation. We have previously shown that conditional inactivation of Geminin in the developing cortex, leads to a transient expansion of neural progenitor cells which, subsequently affects the output of inner and upper layer neurons. Furthermore, we have shown that Geminin is an important mediator of PcG repression that regulates the transition of Hematopoietic Stem Cells to committed progenitors. Here, we demonstrate that upon deletion of Geminin in the developing mouse cortex the transition of NECs to RGs is delayed. Chromatin immunoprecipitation experiments have been used to examine the role of Geminin in altering epigenetic modifications mediated by PcG affecting fate decisions of neural progenitors and neuronal subtype generation in the developing cortex.

W-1369

ISOFORM-SPECIFIC ROLES FOR DNMT3A IN EPIGENETIC REGULATION OF EMBRYONIC STEM CELLS

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DNA methylation is one of the major epigenetic mechanisms classically associated with gene silencing at promoters. DNA methylation is catalyzed by a family of DNA methyltransferases (DNMTs), however, the division of roles between family members is not well understood. To investigate the role of individual DNMTs, we generated DNMT triple-knockout (TKO) mouse embryonic stem cells (ESCs) and stably reconstituted DNMT individually, including different isoforms. By mapping DNA methylation, we find that the de novo enzymes, DNMT3a and DNMT3b, share a high degree of genomic targets, suggesting functional redundancy. By contrast, profiling histone modifications genome-wide reveals different roles of histone regulation between the two de novo enzymes. Strikingly, reconstitution of DNMT3a2, the dominant isoform found in ESCs, was sufficient to phenocopy the wild-type chromatin state while other enzymes had minimal effect, indicating DNMT3a2 isoform as the foremost enzyme in shaping the ESC epigenome. For example, TKO ESCs exhibit a profound redistribution of H3K27me3, especially at bivalent promoters and intragenic regions, which is restored only by DNMT3a2. In addition to regulating H3K27me3 occupancy, we also observe widespread changes in H3K27ac distribution that is causally linked to DNMT3a2. This result may suggest DNMT3a2 regulation of enhancer activity. Finally, using a catalytically null DNMT3a2, we demonstrate these effects were independent of DNA methyltransferase activity. Overall, these results indicate that Dnmt3a2 isoform is a predominant enzyme in shaping the ESC epigenome.

W-1370

PROLONGED FEMALE HUMAN ESC REPROGRAMMING BY NAïVE CONDITIONS PARTLY REACTIVATES X CHROMOSOME

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Female human Pluripotent Stem Cells (PSCs) have variable status of X-chromosome inactivation (XCI). One of the X-chromosomes in PSCs may either be inactive (Xi) or display active state markers during cultivation. When using PSC to study X-linked diseases it is necessary to determine the status of X. It has been reported that maintaining human PSCs in the so-called naïve or ground state results in reactivation of Xi. However, previously published data on Xi activation in naïve conditions are ambiguous. The aim of our study was to verify the possibility of X reactivation in previously established human embryonic stem cells (hESCs) by prolonged cultivation of cell lines in naïve culture conditions. We cultivated five female hESC lines with various states of XCI in 5% O2 in 4i-containing medium supplemented with bFGF and LIF. Reprogramming to naïve state by culture conditions during 10 passages resulted in significant morphological changes. Colonies acquired tight edges, were effectively replated by trypsinization during passaging, and showed changes in gene expression verifying the transition towards naïve state. At the end of reprogramming we analyzed changes in X chromosomes status by H3K27me3, H3K9me3, and 5-hydroxymethylcytosine (5-hmC) staining, XIST expression, replication timing, and biallelic gene expression. We found that all cell lines lost the H3K27me3 foci and XIST-cloud (the marks of the Xi), except for one cell line which kept the inactive state of X. We analyzed the replication timing and found that naïve conditions facilitate synchronous X replication except two regions of Xi, namely pericentromeric region and the distal region of q-arm. These regions replicate asynchronously and keep their inactive status, i.e. retain H3K9me3 and are not enriched with 5-hmC in contrast to the active X chromosome. Interestingly, the same regions have been previously shown to remain inactive in several induced PSC cell lines with partial reactivation of Xi. The reasons of such resistance of these two Xi chromosome loci to reactivation need to be investigated.

W-1371

IS SKEWED X CHROMOSOME INACTIVATION IN HUMAN EMBRYONIC STEM CELLS DRIVEN BY A CULTURE ADVANTAGE?

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Female human embryonic stem cell (hESC) cultures display variable X chromosome inactivation (XCI) patterns already at the undifferentiated state. Whereas in normal human development, cells randomly inactivate either the maternal X chromosome or the paternal one, most of the reported hESC lines display a strongly skewed pattern of XCI. It is however unknown whether hESC have a preferential pattern of XCI upon derivation or whether the XCI skewed pattern is acquired de novo during long-term culture and if the acquired XCI pattern is maintained upon differentiation. To determine how this inactivation bias is acquired we studied the XCI patterns for 22 female hESC lines at sequential passages during long-term culture and after differentiation. Methylation analysis by methylation-sensitive DNA restriction and PCR revealed XCI in all investigated lines. All lines but one displayed a completely skewed (non-random) inactivation pattern. For 10 hESC lines for which DNA of the donors was available, the parental origin of the inactivated X chromosome was identified. From those ten lines, the male donor allele was inactivated in six lines, while in the other four the female donor allele was inactivated. We have thus not found evidence for a predominant inactivation of either the X chromosome inherited from the female or the male donor, strongly suggesting that there is no imprinting event directing the choice for XCI. Skewing was detected in early passages (earliest passage studied: P3) and maintained during long-term culture (latest passage studied: P144) as well as upon differentiation into osteoprogenitor-like cells and the trophoblast lineage. In the one line in which XCI was not completely skewed at early passage, we describe a transition from a random towards a non-random XCI pattern. Massive parallel bisulphite sequencing revealed partially methylated profiles for ARX, ZDHHC15 and SLITRK4 genes, suggesting erosion of methylation. Repressing histone marks and XIST expression were present in some lines at early passages, but rapidly lost after a few passages. Remarkably, we observed that the loss of histone marks occurred in a colony-specific manner. Taking all the evidence together, we hypothesize that the XCI skewing pattern derives from a culture advantage conferred by a specific XCI pattern.

W-1372

FUNCTION OF KDM6B IN DPSC POTENCY AND ALCOHOL-INDUCED OSTEOPOROSIS

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Alcoholism is a detrimental disease that has been linked to osteoporosis. Ethyl alcohol (C2H5OH), also known as ethanol, is the addictive organic compound contained in alcoholic beverages that destroys osteoblasts. Ethanol has been shown to affect growth factor signaling and down regulation of mRNAs and proteins via hypermethylation of cell cycle genes. One epigenetic regulator involved in osteogenic commitment that is severely downregulated by ethanol is KDM6B, a histone lysine demethylase that plays critical roles in controlling cell fate of stem cells. Mesenchymal stem cells (MSCs) are adult stems cells that can proliferate and differentiate into a variety of cell types. Dental pulp stem Cells (DPSCs) are easily accessible and an abundant source of MSCs with the ability to produce odontogenic, chrondrogenic, adipogenic, and osteogenic lineages under different culture conditions in vitro. Initial analysis on the role of ethanol on KDM6B in DPSCs has shown reduced potency levels as well as dysregulation of osteogenic marker expression. We hypothesize that by overexpressing KDM6B, the effects of ethanol on DPSCs can be blocked.

GERMLINE CELLS

W-1374

DNA METHYLTRANSFERASE 1 IS REQUIRED FOR MAINTAINING METHYLATION DURING PGC REPROGRAMMING

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Whole genome reprogramming of DNA methylation is an essential process that occurs in pre-implantation embryos, primordial germ cells (PGCs) and during the reversion of embryonic stem cells (ESCs) to the naïve ground state. The reprogramming of DNA methylation in these contexts is required to establish the correct epigenetic landscape and to prevent the inheritance of epialleles that will impact differentiation potential, or in the case of the germline, future child health. However, during the removal of DNA methylation genome wide, some loci are protected from cytosine demethylation and the mechanism for this process remains unknown. In the current study we tested the hypothesis that Dnmt1 is responsible for maintaining methylation by being recruited at specific genomic sites during whole genome demethylation in the absence of its major cofactor Uhrf1. To address this, we created a conditional germline knockout of Dnmt1 using the Dnmt12lox/2lox strain crossed to Blimp1-Cre (BC) together with incorporation of the OCT4-Gfp reporter tool for sorting germ line cells. Analysis of OCT4 positive BC; Dnmt11lox/1lox PGCs revealed that Dnmt1 is the major methyltransferase that functions during whole genome demethylation to maintain DNA methylation at discreet genomic regions including intracisternal A particle (IAP) transposons, as well as maternal and paternal imprinting control centers. Furthermore, the absence of DNMT1 from the start of PGC development not only results in hypomethylated germ cells that lack the presence of 5-hydroxymethylcytosine, but this uncharacteristic epigenetic landscape results in abnormal differentiation that leads to germ cell loss in both male and female embryos. Taken together, we propose a model in which maintenance of cytosine methylation by Dnmt1 in the absence of Uhrf1 is essential to maintain cytosine methylation at discreet regions of the genome during whole genome DNA methylation reprogramming.

W-1375

IN VITRO DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO OVARIAN FOLLICLE-LIKE CELLS

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Oocyte is a rare population of gametes in adult ovary that is destined to fuse with sperm and become a totipotent zygote. Understanding the unique mechanisms of human oogenesis and eventually benefiting women experiencing infertility such as premature ovarian failure all awaiting an in vitro differentiation system from pluripotent stem cells into oocytes. The most recent report of obtaining oocyte-like cells in mice required an aggregation of primordial germ cells (PGCs) and somatic cells from fetal gonads, followed by transplantation into ovarian bursa. The requirement of fetal ovary tissues for transplantation would be difficult for human studies and would encounter ethical issues. Therefore, a more complete in vitro differentiation approach without the procurement of human tissue is much desired. We have developed an in vitro differentiation system from human embryonic stem cells (hESCs) into ovarian follicle-like cells. First, we found that an RNA binding protein specifically expressed in germ cells, DAZL, regulates exit of pluripotency and entry into meiotic during differentiation of hESCs. Overexpression of DAZL down-regulate multiple pluripotency genes including OCT4, NANOG, PRDM14, but late germ cell marker VASA and meiotic marker were up-regulated. These results were obtained by mRNA expression analysis, protein expression analysis, 3'UTR luciferase assay and RNA-IP experiments. Combination of DAZL and BOULE overexpression further induced these hESCs to enter meiosis and express PRDM9, γH2AX, SCP3, and MLH1 on meiotic spreads. Upon induction by ovarian growth factors GDF9 and BMP15, these meiotic germ cells started to form ovarian follicle-like cells (FLCs) in vitro, including the presence of oocyte-like cells and granulosa-like cells. These FLCs expressed oocyte and granulosa cell proteins including ZP2, NOBOX, and AMH. To test if FLCs can further mature into late follicles, we transplanted FLCs into kidney capsule of immune-deficient mice. FLCs in the transplant resembled primary follicles of human fetal ovary with expressions of NOBOX and AMH. In summary, we have developed a robust in vitro system that allows us to differentiate pluripotent hESCs to ovarian FLCs, and studying the unique mechanisms therein.

W-1376

IDENTIFICATION AND CHARACTERIZATION OF EPITHELIAL CELLS DERIVED FROM HUMAN OVARIAN FOLLICULAR FLUID

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Follicular fluid is important for follicular development and oocyte maturation. Evidences suggest that follicular fluid is not only rich in proteins but cells. Besides oocytes, the follicular fluid contains granulosa, thecal, and ovarian surface epithelial cells, and both granulosa and thecal cells were well-characterized. However, scant attention has been shown to epithelial cells in follicular fluid. This study is first to describe that epithelial cell could be isolated from human ovarian follicular fluid retrieved in the assisted fertilization program. Herein, follicular fluid samples were collected from 20 women undergoing in vitro fertilization (IVF). Among them, epithelial cell cultures were established from 18 samples. A small population of epithelial cells expresses germ-line stem cell markers, such as OCT4, NANOG, and DDX4 by immunofluorescence staining and FACS assay. In the epithelial cell culture system, cell colonies were formed that resembled early ESC colonies and could maintain and proliferate in an undifferentiated way on the feeder layer which expressed pluripotency markers, including OCT4, NANOG, SSEA4, Tra-1-60 and Tra-1-82. These colonies differentiated in vitro into various somatic cell types in all three germ layers, but did not form teratoma when injected into immunodeficient mice. Furthermore, using the hepatocyte differentiation system, theses epithelial cells could be differentiated directly to functional hepatocyte-like cells, which not existed in ovarian tissues. Intriguingly, the epithelial cells derived from follicular fluid could form oocyte-like cell structure spontaneously in vitro with a diameter of 100-200 µm, comparable to naturally occurring oocytes, and prominent nucleus and peri-nuclear organelle accumulation were also observed. These oocyte-like cells expressed DAZL, STELLA, ZPC, SCP, and GDF9 transcription markers and were further confirmed by immunofluorescence staining. Some of the oocyte-like cells developed a zona pellucida-like structure and blasto-like structure. In summary, the epithelial cells derived from follicular fluid are an integral part of the ovarian surface epithelium and a potential stem cell source with a pluripotent/ multipotent character for safe application in oogenesis and regenerative medicine.

TOTIPOTENT/EARLY EMBRYO CELLS

W-1378

DEPLETION OF H3K9ME3 CAUSES MATERNAL XIST DEREPRESSION DURING EARLY PREIMPLANTATION EMBRYOS IN MICE

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During mouse preimplantation development, maternally derived X chromosome (Xm) maintains active state by repressing Xist, which is a large non-coding RNA and essential for establishment X chromosome inactivation (XCI). The preferential inactivation on paternal X chromosome (Xp) is called as imprinted XCI. In mice, Xp-Xist starts to express at the 4-cell stage onward and Xm-Xist is silenced at the same period by the imprinted XCI regulation. Interestingly, Xm-Xist escapes the activation even in the presence of an Xist activator RNF12/RLIM in oocytes during the preimplantation stage. In the present study, we aim to identify and characterize the imprinting XCI factors, and explore developmental function in mouse model. To achieve our goal, we have developed two novel experimental systems which are a micromanipulation with parthenogenetic uniparental embryos and a chromatin immunoprecipetation combined with quantitative polymerase chain reaction method (embryo ChIP-qPCR: eChIP-qPCR) enabling to reduce amount of cell numbers required for analysis. We focus on histone modifications specifically imposed on maternal genomes and screen them correlated with Xm-Xist derepression of parthenogenetic embryos. Depletion of histone H3 lysine 9 tri-methylation (H3K9me3) by ectopic expression of lysine demethylase Kdm4b induces derepression of Xm-Xist at the 4-cell stage. The eChIP-qPCR analysis reveal that H3K9me3 levels at promoter regions of Xm-Xist at 4-cell stage are high compared with those of morula stage, at which Xm-Xist of control parthenogenetic embryos is derepressed. We demonstrate that ectopic Kdm4b expression causes promoter demethylation of H3K9me3 on Xm-Xist at the 4-cell stage. Thus, promoter H3K9me3 modifications are involved in Xm-Xist silencing. In addition, the acquisition of Xm-XCI of parthenogenetic embryos by loss of maternal H3K9me3 can escape high incidence of embryonic lethality immediately after implantation. Taken together, our findings highlight the role of maternal H3K9me3 for imprinted Xist regulation and lack of Xm-XCI is roadblock for post-implantation development of parthenogenenetic embryos. Our results also shed light on epigenetic reprogramming machinery operating of somatic cell nuclear transplantation.

W-1379

THE CIRCADIAN OSCILLATOR PERIOD 2 IS EXPRESSED IN EARLY CLEAVING MOUSE EMBRYOS AND EMBRYONIC STEM CELLS

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The role of core circadian oscillators in early development and stem cell differentiation is unknown. The influence of 24-hour light/dark cycles on physiological processes is mediated through the suprachiasmatic nucleus (SCN) which entrains the interaction of a set of transcription factors, one of which is Period 2. Approximately 12% of gene expression is under circadian control. Peripheral tissues maintain individual circadian oscillations in gene expression, unique to the tissues. The discovery that 8-cell human embryos, but not cultured human embryonic stem cells nor human fibroblasts before and after induced pluripotency, express core circadian oscillators suggests that circadian signals play a role in early embryo cleavage. One possible role is stabilizing cell division since key cell cycle checkpoint, Rb and Wee1, are silent in early human embryos. To understand the possible role of the core circadian oscillators in

stem cell differentiation, we derived two embryonic stem cell lines from the mouse transgenic for Period 2 linked to the reporter gene Luciferase, "Per2Luc". Prior luminometry studies revealed that undifferentiated cells emit Period 2-luciferase activated light, and that the light becomes circadianly expressed with differentiation. The luminometer did not detect light from fewer than 50,000 cells, nor from individual embryos. With differentiation, luminometer tracings of Per2-light production showed a 24-hour rhythmicity with peaks approximately two-fold higher production than valleys. Once entrained, the cells maintained their circadian light emissions following trypsinization and re-plating into new dishes. With the aid of the Olympus LV200, more recent studies have detect Per2-luciferase light emitted both from individual Per2Luc stem cells and Per2Luc fertilized eggs. Pilot studies have revealed: (1) Period 2 is detectable from the late 2-cell stage to the morula stage, but is lacking in blastocysts; (2) post hatching and attachment, Period 2 is detected in inner cell masses and very brightly in early embryonic stem cells; (3) approximately 10% of established Per2Luc embryonic stem cells emit light at any one time, 1% appear to emit light continuously, and 20% emit detectable light during a circadian cycle. Period 2 may play multiple roles during early embryonic development.

W-1380

ESTABLISHMENT IN CULTURE OF MOUSE EXPANDED POTENTIAL STEM CELLS

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The zygote and blastomeres of a cleavage-stage mouse embryo have the capacity to differentiate into all lineages of both the embryo proper and the extraembryonic tissues, and are thus considered totipotent. Mouse embryonic stem cells (ESCs) can differentiate into embryonic lineages but have a restricted potential to become trophoblasts. We find that cultures of a new type of expanded-potential stem cells (EPSCs) can be established from in vitro-cultured mouse preimplantation embryos and individual blastomeres, and from directly converting mouse ESCs or genetically reprogramming somatic cells. This is achieved by chemical modulation of the genetic pathways involved in the segregation of the first embryonic cell lineages, ICM and trophectoderm. EPSCs differentiate into trophoblasts in vitro, and a single EPSC contributes to both the extraembryonic lineage, including the placenta trophoblasts, and the embryo proper in chimeras. Single cell transcriptomic analysis reveals that EPSCs express core pluripotency factors as in ESCs, but have enriched preimplantation blastomere signatures. We have thus established for the first time stable and homogenous cultures of expanded-potential stem cells. Our data suggest that it may be possible to derive similar cell lines from other mammalian species.

EMBRYONIC STEM CELL DIFFERENTIATION

W-1381

CHARACTERIZATION OF NEURAL PROGENITOR CELLS GENERATED FROM HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSC) and their differentiated derivatives provide new opportunities to overcome several limitations of currently used cellular model systems, such as tumorous character, species differences, and limited availability of ex vivo tissue samples. However, the hPSC-derived models are also burdened by numerous challenges, since most differentiation protocols are time consuming, consist of several steps of treatments, and result in a mixed population of desired cell types. These limitations especially hold true for neural lineages differentiated from human pluripotent stem cells; therefore, we started to establish methods for generation of various types of human neural model cells from hPSCs. Functional assays such as calcium-imaging are suitable for addressing this type of variability in hPSC derived neural cells. For optimization and standardization purposes, i.e. to overcome the cellular variability, the differentiation protocols are divided into well-defined stages, adding up to a several week long procedure. First the neural progenitor cell (NPC) populations are generated. We adapted and optimized previously published differentiation protocols and established NPC cultures for the generation of mixed neural cell types, as well as PROX1-positive hippocampal neurons from different hPSCs. Next the NPC cultures were characterized by morphology, immunostaining, and mRNA expression profiling for specific markers including SOX2, PAX6, and Nestin. NPCs expressing a Ca-indicator protein (GCaMP3) have also been generated by using a transposon-based gene delivery system. Specific ligand-induced and spontaneous calcium transients were compared in GCaMP3-expressing and in Fluo4-loaded NPCs. Based on these technologies and using patient-derived iPSCs we plan to generate efficient cellular model systems for studying the cellular basis of various neurological and psychiatric diseases. *This work was supported by the Hungarian Scientific Research Fund [NK83533]; Hungarian Brain Research Program [KTIA VKSZ_12, NAP-A-1.10] and by the National Research, Development and Innovation Office KTIA_AIK_12-1-2012-0025, KMR_12-1-2012-0112].*

W-1382

MODELLING NEUROMUSCULAR CIRCUITS IN VITRO WITH STEM CELL-DERIVED TISSUE

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All motor behaviour are controlled by the neuromuscular system. Motor neurons are the specialized cells in the central nervous system which control skeletal muscle contraction. The neuromuscular junction is the synaptic connection between motor neurons and muscle fibres. In this work we developed a neuromuscular circuitry model based in embryonic stem cell derived tissue to study nerve-muscle connectivity in health and in neuromuscular disorders. We have genetically modified murine and human stem cells to allow the sorting of motor neurons and glia from mixed poorly defined differentiation cultures. The cellular components of these circuits (motor neurons, astrocytes and muscle) were assembled in vitro where motor neurons connect to their synaptic targets. This approach will be applied to study Amyotrophic Lateral Sclerosis, a fatal neurodegenerative disease without effective treatment. Understanding neuromuscular junction development and stabilization looking at both pre- and post-synaptic compartments may shed light in mechanisms involved synapse deterioration.

W-1383

COMPREHENSIVE CHARACTERIZATION OF THE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSC) are self-renewing multipotent cells which hold great potential in reconstructive medicine and have been shown to be beneficial for the treatment of a variety of diseases. MSC can be derived from multiple adult tissues but have limited expansion capacity in cell culture. Highly proliferative ESC-derived MSC may be an alternative source for MSC but to date no standardized protocol exists which meets clinical standards. We extended and improved a published protocol for the differentiation of human embryonic stem cells (ESC) into highly-proliferative MSC. ESC-derived MSC were tested for characteristic surface markers and the differentiation capabilities typical for MSC (bone, fat, cartilage) were verified. To characterize the differentiation process in-depth we performed comprehensive large-scale proteomic and phosphoproteomic profiling experiments using quantitative high resolution mass spectrometric analysis based on reductive dimethylation. Phosphoproteomic profiling was performed according to the TiSH protocol including phosphopeptide enrichment by titanium dioxide combined with sequential immobilized metal affinity chromatography. Proteomics data was complemented by transcriptome analysis derived from next generation RNA sequencing. Differentiation was followed over a time course of 30 days including sampling on days 0, 1, 2, 5, 15, and 30. ESC-derived MSC were compared to adult tissue-derived MSC (bone marrow MSC) as well as to their origin (ESC). We identified 9,470 proteins with 8,600 proteins quantified. In total, 4,325 proteins were quantified in all samples at all stages. The phosphopeptide enrichment strategy yielded 10,575 phosphosites, with 1681 commonly quantified in all samples. For validation purposes and to optimize quantification of phosphorylation we included transcriptomic data derived from next generation RNA sequencing. The established differentiation protocol is highly reproducible and may be adapted for clinical use. The comprehensive analysis of the differentiation process will improve understanding of MSC biology and therefore directly benefit MSC-based therapies.

W-1384

TOWARDS A MODEL OF PROSTATE ORGANOGENESIS: IN VITRO DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS INTO CELLS OF THE PROSTATE

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The prostate is an exocrine gland of the male mammalian reproductive tract. It functions to secrete constituents of seminal plasma. The endoderm differentiates into the urogenital sinus, which in turn differentiates into the prostate. However, the mechanisms regulating prostate organogenesis are poorly defined. This is in part due to the absence of an *in vitro* model. Mouse embryonic stem (mES) cells are extremely useful tools for modelling embryogenesis due to their capability to differentiate into all cell types of the three germ layers: the ectoderm, the mesoderm and the endoderm. We aimed to direct the differentiation of mES cells into mature functional cells of the prostate. Endodermal progenitors were obtained in serum-free conditions with the addition of Activin-A and Wnt-3a. Lineage specification of these endodermal progenitors into urothelial lineages was then induced by the addition of retinoic acid. Generation of urothelial progenitor was confirmed by expression of *uroplakin 1b* and 2. To induce terminal prostate differentiation, FGF-10, TGF-β and dihydrotestosterone were added into urothelial progenitor cultures. Further differentiation was identified by detection of *Ar* (androgen receptor) gene expression, a morphoregulatory factor critical in prostate development. Unexpectedly, at the time of urothelial progenitor differentiation, these endoderm-specific culture conditions spawned a large number of adipocytes. Adipocytes are a cell lineage thought to be generated from the mesoderm. We propose that these mesoderm-derived adipocytes may act to support the differentiation of urothelial and prostatic cells. We aim to translate this system of co-differentiation for 3D cultures to assess the formation of mature prostatic acini.

W-1385

AN ONTOGENY-RECAPITULATING PROTOCOL FOR THE GENERATION OF MEDIUM SPINY NEURONS FROM HES AND HIPS CELL LINES

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An ontogeny-recapitulating protocol for the directed differentiation of hPS cells toward the striatal fate has been developed using knowledge of human developmental biology. By applying the developmental principles that orchestrate ventral telencephalic determination, we have successfully recapitulated the process of striatal neurogenesis and instructed hPS cells toward region- and transmitter-specific striatal MSNs. Our more recent study of human fetal brain development provided further resources and opportunities to gain global understanding of how transcriptional and functional processes converge to specify human striatal during development. The observations obtained from this systematically analysis has identified new markers of striatal specification which have been included in our in vitro protocol to further document the differentiation of human pluripotent stem cells toward MSNs. Immunocitochemistry and counts for additional specific markers have been performed and validated by multiple investigators on one hES cell line and two human iPS lines. This detailed analysis of each step of our striatal differentiation protocol has allowed both to characterize in an accurate manner the neuronal population obtained and to further implement the protocol in order to increase the percentage of striatal neurons. In the context of an international collaboration, this new panel of neuronal markers will be applied to characterize the specific neuronal population obtained from HD patient-specific iPS cells, with the aim to investigate the effect of the presence of mutant huntingtin on cell survival and differentiation.

W-1386

HYPERGLYCEMIA IMPEDES HESC DIFFERENTIATION INTO DEFINITIVE ENDODERM THROUGH MODULATION OF HISTONE METHYLATION PATTERNS

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Type 2 Diabetes Mellitus (T2D) is a metabolic syndrome characterized by hyperglycemia and insulin resistance. Fetal exposure to maternal diabetes is a possible cause for T2D, as offsprings of diabetic mothers had higher prevalence of impaired glucose tolerance and T2D at adulthood. Epigenetic changes could be the underlying mechanism, since the epigenome is vulnerable and susceptible to environment-induced dysregulation during early fetal development. In light of these, we hypothesized that intrauterine hyperglycemic environment led to persistent epigenetic modifications associated with impaired fetal pancreatic differentiation. The human embryonic stem cell line (hESCs), Val3, was selected as in-vitro model in this study, because of its efficiency of differentiation into definitive endoderm (DE), an important intermediate stage along the pancreatic lineage. From our results, we found that the expressions of DE markers (SOX17, FOXA2, CXCR4 and EOMES) were significantly suppressed when induction was done in the presence of high glucose concentration (50mM D-glucose). Upon DE differentiation, the repressive histone methylation mark H3K27me3 initially bound to the promoters of the DE markers in hESCs were lost. Interestingly, these marks were partially retained under hyperglycemic condition (25 mM and 50 mM). Further investigation into the temporal methylation patterns during DE differentiation revealed the upregulation of H3K27me3 binding on DE markers appeared as early from day 2 onwards upon hyperglycemic treatment. Treatment with adenosine-2',3'-dialdehyde (Adox), a methyltransferase inhibitor, from day 2 onwards significantly rescued the expression of DE markers and restored normal histone methylation patterns upon hyperglycemic treatment. Our findings was further proved by an in-vivo diabetic mouse model induced by low dose of streptozotocin that embryos at 7.5 dpc born to diabetic mothers had lower percentage of Sox17+ Foxa2+ Cxcr4+ cells when compared to control. To conclude, our findings suggested hyperglycemia impeded differentiation of hESCs into DE by affecting the histone methylation patterns. The impairment of DE development by hyperglycemia was observed in a diabetic mouse model.

W-1387

HIGH-THROUGHPUT SCREENING IDENTIFIES A CORE GENE REGULATORY NETWORK THAT CONTROLS THE FORMATION OF MULTIPOTENT MESODERM PROGENITORS FROM PLURIPOTENT STEM CELLS

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Multipotent mesoderm progenitors (MMPs) represent an attractive cell population for cell therapy as their differentiation potential includes skeletal and cardiac muscle and vascular endothelial cells. A major challenge resides in identifying the regulatory network of genes that can be manipulated to efficiently and robustly produce MMPs from pluripotent stem cells. We have previously published that MMPs can be efficiently produced by inhibiting Activin Receptor 1B signaling (siAcvr1b). We reasoned that effectors of mesoderm differentiation should be upregulated within 24 hours post siAcvr1b transfection. Using a mRNA profiling approach we identified that 14 genes were significantly upregulated in response to siAcvr1b. Next we asked which of these genes are required for mesoderm differentiation using a siRNA-mediated knock-down strategy. Our data show that a minimal set of 3 genes are required for mesoderm differentiation. Next, we determined which, if any, of these 3 genes is sufficient to induce mesoderm differentiation. We created ESC lines that stably overexpressed all possible combinations ("whole combinome") of the 3 candidates using lentiviruses. The results show that the overexpression of a single gene induces mesoderm differentiation at levels that are similar to siAcvr1b, showing that alone it fully phenocopies siAcvr1b (~80% Kdr+ cells). Next we evaluated the differentiation potential of induced-MMPs (iMMPs) in vitro. Our results show that they spontaneously differentiate into at least 3 lineages including skeletal, cardiac and endothelial cells. Moreover, we show that their differentiation potential can be directed using ectopic cues as overexpressing Myod1, leads to the differentiation of myotubes at high efficiency (>95%). Next, in order to test their regenerative potential, we transplanted iMMPs into injured TA muscle of adult mice and assessed cells for survival, integration and differentiation. 15 days post transplantation, we observe that iMMPs are able to survive, integrate into host muscle and differentiate into at least 3 population of cells including Pax7 expressing muscle progenitor cells, newly formed myofibres and vascular endothelial cells suggesting that iMMPs retained their differentiation capacity in vivo and represent a promising source of cells for cell therapy

W-1388

PRODUCTION OF CD45+ MATURE HEMATOPOIETIC STEM CELLS WITH GREAT CLONOGENIC POTENTIAL FROM HUMAN EMBRYONIC STEM CELLS

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Transplantation of hematopoietic stem cells (HSCs) is the most successful type of cell therapy. Despite the importance of isolated HSCs of adults in clinical practice, obtaining and using these cells have some limitations. The discovery of alternative sources of HSCs to supply the demand for transplants is necessary. An alternative to overcome this problem would be the cultivation and differentiation of human embryonic stem cells (hESCs). Current protocols for hESC differentiation and HSCs production are not efficient. CD34+ HSCs obtained by almost all the research groups represent a very primitive population of HSCs with poor in vivo functional capacity. Recent studies have demonstrated that mature and functional HSCs present CD45+CD43+CD34low/- phenotype with low expression of CD34 and high expression of CD45 and CD43. We developed a differentiation system based on co-culture of hESC with murine stromal cells in differentiation medium supplemented with fetal bovine serum and cytokines/hematopoietic growth factors in low concentrations. As a result, we were able to produce a mixed population of cells enriched in hematopoietic stem/progenitor cells positive for CD45 marker, which proved to be co-expressed with other hematopoietic markers (CD43, CD31, CD71 and CD38), and mature hematopoietic cells positive for erythroid (CD235a) and myeloid (CD14, CD15, CD16)-specific markers, all of them with morphological characteristics typical. We demonstrated that it is possible to obtain a population cell CD45+ CD43+ CD31+ CD34low by this protocol. It was shown also that these cells expressed genes related to primitive and definitive hematopoiesis (CD45, CD31, runx1, TAL1, LMO2, prom1, CD34 and NOTCH1), and had clonogenic potential in vitro comparable to umbilical cord blood mononuclear cells. They were able to generate BFU-E, CFU-GEMM and CFU-GM hematopoietic colonies. Serial in vitro clonogenic analysis and in vivo transplantation are being conducted to better evaluate hematopoietic reconstiturion capacity of these cells. Scanning electron microscopy analysis revealed the interaction of hematopoietic cells with stromal cells within the microenvironment created in vitro. These results demonstrate that it is possible to obtain mature HSCs from hESCs and represent a promise for future clinical applications.

W-1389

THE ROLE OF MICRORNA AND HMGB PROTEINS IN CELL CYCLE REGULATION AND DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELL-DERIVED NEURAL STEM CELLS

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Over the past decade, miRNAs were identified as crucial post-transcriptional regulators of gene expression. Recently, miRNAs were also directly linked with human embryonic stem cell (hESC) physiology and are likely to regulate key stem cell properties: self-renewal and pluripotency. Importantly, we and others have previously connected self-renewal and differentiation with regulation of cell cycle. However, the role of miRNAs in these pathways is only beginning to emerge. Therefore we aim to study the role of miRNAs in differentiation of hESCs into self-renewing neural stem cells (NSCs) and terminally differentiated neurons. We hypothesize that differentiation-associated miRNAs contribute to cell cycle regulation in NSCs and help to maintain their unlimited proliferative potential. We initiated our experiments by high throughput analysis (miRNAseq/RNAseq, Illumina) of gene and miRNA expression in undifferentiated hESCs and their differentiated counterparts. Our results show that expression of several cell cycle regulatory molecules (such as p15/ CDKN2B and p16/ CDKN2A) remain low or undetectable in hESCs as well as self-renewing NSCs while they are markedly upregulated upon induction of terminal differentiation of NSCs into neurons. Functional experiments with DNA-binding proteins (i.e. HMGB proteins) also suggest their previously unreported role in NSC differentiation. Furthermore, results from miRNA microarray (Exiqon) analysis show 26 significantly upregulated miRNAs upon induction of differentiation of hESCs into NSCs. The most dramatically (>15 fold) upregulated miRNAs with differentiation were miR-21, miR-221, miR-125a, and miR-145 and this was also confirmed by qPCR. Some of these miRNAs are strongly associated with differentiation of hESCs and were shown to play a role in cell cycle regulation, i.e. miR-145 and let-7c. Other miRNAs have been strongly associated with various types of cancers, such as miR-21, miR-221/221 or miR-143. However, their role in differentiation of hESCs was, to our knowledge, never studied. Our investigations are therefore ongoing to reveal their target mRNAs and functions in cell cycle regulation and NSC phenotype. *This study was supported by GJ15-18316Y and MUNI/A/1558/2014, SoMoPro II-no. 4SGA8684; IGA MZ CR NT11218-6/2010*

W-1390

MANIPULATION OF MIRNA EXPRESSION DURING ESC OSTEOGENESIS AS A MEANS TO DEVELOP CLINICALLY RELEVANT OSTEOPROGENITORS

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Low bone density is a rapidly growing problem; the U.S. Surgeon General predicts that by 2020, over half of all Americans will have weak bones. The advent of tissue engineering from embryonic stem cells (ESCs) has begun to provide viable solutions and therapies for formerly devastating diseases and injuries in a variety of organs. However, due to the molecular intricacy and limited understanding of bone development, scientists have yet to pinpoint a feasible way to apply these newfound therapies to patients with low bone mass. The discovery of the microRNA (miRNA, miR) and its implication as a key regulator of protein expression during embryonic development has opened up many possibilities as to the manipulation of stem cell differentiation. In order to identify miRNAs that regulate the specification of osteogenic precursors, our lab previously conducted

a miRNA screen using osteogenically differentiating ESCs. From 25 differentially regulated miRNAs, two stood out, miR-361 and miR-690, as their overexpression resulted in the significant increase in ESC calcification, a hallmark of maturing osteogenic cultures. In order to elucidate the role of these two miRNAs in modulating osteogenesis, we identified prickle1/2 as a direct target of miR-361, and beta-catenin (CatnB) as a direct target of miR-690. As an upstream negative regulator of Dishevelled (Dvl), prickle is implicated in convergent extension movements by affecting CatnB. Western blots and immunocytochemistry suggested that overexpression of miR-361 in ESCs achieved a downregulation of nuclear CatnB levels concomitant with an up-regulation of Dvl1 levels. This occurred at a time point of differentiation at which mesenchyme is specified from neural crest cells that have the capability to subsequently differentiate into osteoprogenitors. Similarly, data shows that cells overexpressing miR-690 promote neural crest specification in conjunction with enhanced bone formation. In conclusion, overexpression of miR-361 and miR-690 may be exploited in vitro to enhance the efficiency of directed differentiation of pluripotent stem cells into osteoprogenitors, which then may be employed clinically to treat patients with low bone mass.

W-1391

HARNESSING THE POTENTIAL OF HUMAN PLURIPOTENT STEM CELLS FOR ARTICULAR CARTILAGE REPAIR

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In the current study our aim was to determine the unique gene expression profiles that specify the chondrogenic, osteogenic, tendogenic, myogenic and ligamentogenic lineages in humans. In addition, we defined the transcriptional and epigenetic profiles of human chondrocytes isolated at different stages of human ontogeny in humans and also human pluripotent stem cell (hPSC) derived chondrocytes. Although each skeletal lineage showed a unique molecular signature, significant overlap exists between ligamental and cartilage cells and also between myoblasts and tendoblasts. Minimal overlap was found between osteoblasts and chondrocytes from 17-week old fetal limbs, suggesting different developmental origins. 14-day old hPSC-derived cells were highly similar to human primary embryonic cartilage cells from 5-6 weeks, while less similarity was found between hPSC-derived chondrocytes and adult cells, fetal myogenic and osteogenic cells. Two gene ontology categories of particular interest were statistically significantly upregulated in both hPSC-derived and embryonic chondrocytes with p values < 1 X 10-6: "embryonic morphogenesis" and "embryonic limb morphogenesis". Furthermore, hierarchical clustering of expression values for hPSC-derived chondrocytes, embryonic chondrocytes and fetal skeletogenic lineages over a set of genes known to be involved in early limb development in mice demonstrated the highest degree of similarity between hPSC-derived and embryonic chondrocytes; hPSC-derived chondrocytes cultured in vitro for 60 days showed higher level of molecular similarity with fetal chondorocytes. Embryonic chondrocytes and 14-day hPSC-derived chondrocytes implanted into nude rats showed no ability to form cartilage, while 60-day hPSC-derived chondrocytes and 17-week fetal chondrocytes showed robust cartilage formation in vivo. Altogether, these data define molecular signatures for human skeletal lineages and also suggest that hPSC-derived cells have the potential to mature into functional cartilage cells under appropriate conditions. These results add a critical functional dimension to the isolation of cartilage-committed progenitors from hPSCs that is especially relevant in light of phenotypic differences present during the hPSC differentiation process

W-1392

AN INDUCIBLE GAIN-OF-FUNCTION SYSTEM UNCOVERS NOVEL ROLES FOR GSX2 AND EBF1 IN HUMAN NEURAL PROGENITORS AND PROMOTES MSNS DIFFERENTIATION FROM HES CELLS

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The coordination of embryonic human brain development is a process involving multiple players, among which transcription factors (TFs) play a fundamental role. Medium spiny neurons (MSNs) are a key population in the basal ganglia network, and their degeneration causes a severe neurodegenerative condition, Huntington's disease. Understanding how the human ventral telencephalon drives neuroepithelial progenitors towards this specific neuronal lineage is critical for regenerative medicine to develop specific differentiation protocols using human pluripotent stem cells. Studies performed in murine models have identified a handful of transcriptional determinants, including Gsx2 and Ebf1. Here, we have generated human embryonic stem (hES) cell lines inducible for these TFs, with the aim (i) of studying their biological role in human neural progenitors, and (ii) of incorporating TFs conditional expression in a developmental-based protocol for generating MSNs from hES cells. Using this approach, we found that Gsx2 promotes cell cycle retention, while Ebf1 co-expression reverses this phenotype. Moreover, we show that the inducible Gsx2-Ebf1 combined expression in a specific temporal window drives MSNs differentiation from hES cells. *This study received funding from Cure Huntington's Disease Initiative (CHDI, U.S.A)*

W-1393

IMPROVED VENTRAL MIDBRAIN DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS UNDER XENO-FREE CONDITIONS

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Current pharmacotherapies and surgical intervention provide limited benefit in the treatment of neurodegenerative disorders such as Parkinson's disease (PD). Cellular replacement therapy represents a promising intervention that has proven successful in patients using human fetal tissue; however, ethical alternative source material is required for widespread clinical application. Stem cell biology provides a powerful alternative. Key to this approach is the derivation of clinical grade, disease relevant populations. Current protocols are dependent on feeder layers and xenogenic components and are consequently prone to high variation. Here we describe a xeno-free, feeder-free protocol for the improved differentiation of human pluripotent stem cells into ventral midbrain dopaminergic precursors and neurons. In addition to significantly reduced variability, immunocytochemical and gene expression

analyses confirm increases in yield and purity of LMX1A+, OTX2+ and FOXA2+ progenitors as well as mature NURR1+, PITX3+ and tyrosine hydroxylase (TH)+ neurons - genes essential in developing or mature midbrain dopamine neurons. Pilot transplantation data into Parkinsonian rodents confirms survival and indicates improved engraftment of cells derived from this refined protocol. Our bulk culture, xeno-free, feeder-free system efficiently generates dopamine neurons for PD that are one step closer to GMP compliance and clinical translation.

W-1394

WELL DEFINED DIFFERENTIATION OF HUMAN EMBRYONIC STME CELLS DERIVED HEMANGIOBLASTS BY EMBRYOID BODY FORMATION WITHOUT ENZYMATIC TREATMENT

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Human hemangioblasts exist only during the early embryonic developmental stage thereby limiting the adult cellular source from which to obtain such cells for study. To overcome this hemangioblast studies have focused on utilizing human embryonic stem cell (hESC) derivatives but current methods are cell line dependent. Single cell dissociation of a hESC colony promptly led to cell death in most hESC lines due to enzyme treatment, which in turn reduced induction potential and hemangioblast differentiation efficiency. Therefore, we sought to effectively improve the process of cell dissociation that is adaptable to various hESC lines and increase the initial induction potential of embryoid body (hEB). As a result, we determined an effective cell dissociation method through a comparison study involving various reagents which demonstrated successful dissociation regardless of cell line and enhanced hemangioblast differentiation efficiency. *This study was supported by grant (2011-0019487) from the Bio and Medical Technology Development Program of the National Research Foundation (NRF), grant (PJ00995602 and PJ00933303) from the Next-Generation BioGreen 21 Program of Rural Development Administration, and grant (PJ009103) from The Ministry for Food, Agriculture, Forestry and Fisheries, Cooperative Research Program for Agriculture Science and Technology Development, Rural Development Administration and, a grant of the Korea Healthcare technology R andD project through the Korea Health Industry Development Institure (KHIDI), funded by the Ministry for Health and Welfare (grant number : HI14C3365), all funded by the Korean government.*

W-1395

INVESTIGATING THE SIGNALING RATIONALE UNDERLYING CARDIAC INDUCTION OF HUMAN PLURIPOTENT STEM CELLS

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Understanding the mechanisms underlying differentiation of human pluripotent stem cells (hPSCs) into specific fates may enable improved protocols for the generation of desired cell types and also allow insights into early human development. However, gene regulatory events underlying directed differentiation protocols are poorly understood in many cases. We have developed a directed differentiation procedure for converting hPSCs into cardiomyocytes at high efficiency. Through systematic optimization, this allowed us to define the minimal signaling requirements driving this process. One key requirement for efficient cardiac induction of hPSCs is inhibition of the WNT pathway, following the initial induction of mesoderm. The WNT inhibition step is evolutionary conserved and shared with other reported protocols but the underlying rationale is not understood. Using global time-course gene expression analysis, we have elucidated the gene-regulatory consequences underlying WNT inhibition for promoting cardiac induction in our protocol. Unexpectedly, WNT inhibition does not seem to promote the activation of procardiac factors in the cells. Rather, we show that it serves to suppress anti-cardiac regulators that would otherwise, i.e. without extrinsic WNT inhibition, become strongly upregulated by default. Several such candidate regulators were identified using unbiased subtractive filtering of global gene expression data. Their overexpression in the presence of a WNT inhibitor compromised cardiac induction and promoted differentiation into alternative fates, demonstrating their anti-cardiac functions. The CRISPR/Cas9 system was used to simultaneously knock out these genes in hPSCs. Triple knock-out hPSCs displayed facilitated cardiogenesis at limiting concentrations of the WNT inhibitor, thus demonstrating that suppression of anti-cardiac regulators is necessary - and partially sufficient - for promoting cardiomyocyte formation. Our data hence reveal an unexpected repressive roadblock in the human cardiac induction lineage, which might have universal validity.

W-1396

SCLERAXIS KNOCK-DOWN PREVENTS TENDON DIFFERENTIATION BY EQUINE EMBRYONIC STEM CELLS

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Tendon injuries are one of the most common orthopaedic injuries in human and equine athletes. They require long recuperation periods which have a large financial impact and re-injury rates are high due to healing via the formation of biomechanically inferior scar tissue. The structure and function of tendons are very similar in horses and humans and they share many of the same risk factors for tendon injuries. Horses may therefore provide a relevant large animal model for studying the natural repair process and evaluating novel therapies. For the efficient application of stem cell therapies, the processes regulating tenocyte differentiation must be better understood. We previously demonstrated that equine embryonic stem cells (ESCs) undergo tenocyte differentiation in response to 3D culture and/or TGF-beta 3. We have now established that these signalling mechanisms are conserved in equine induced pluripotent cells (iPSCs) generated by expression of Oct4, c-myc, Klf4 and Sox2 via retroviral vectors or *piggyBac* transposons. Scleraxis (SCX) is a tendon specific transcription factor which is required for normal tendon formation during development but its role in regeneration and repair is not understood. A short hairpin RNA against SCX (shSCX) was expressed in equine ESCs, adult tenocytes and fetal

tenocytes and reduces SCX expression to undetectable levels but has no effect on the ability of adult tenocytes to generate artificial tendons in 3D culture. In contrast, SCX knock-down in ESCs and fetal tenocytes blocks their ability to remodel a 3D construct entirely and is associated with a significant reduction in their survival in 3D. In 2D cultures SCX knock-down has no effect on the proliferation and morphology of ESCs, fetal or adult tenocytes. However, gene expression analysis revealed a significant downregulation of collagen I and cartilage oligomeric matrix protein (COMP) in fetal tenocytes expressing shSCX. No change was observed in the expression of tendon genes in adult tenocytes expressing shSCX. These results reveal that SCX has a changing role during development which can be studied in vitro using the cell culture system described to understand developmental and reparative mechanisms. This work has important implications for stem cell therapies for tendon injuries in both humans and horses.

W-1397

NEW MARKERS FOR TRACKING ENDODERM INDUCTION AND PATTERNING FROM HUMAN PLURIPOTENT STEM CELLS

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Of the different cell types that can be generated from human pluripotent stem cells (hPSCs), substantial effort in recent years has been directed at the generation of endoderm derivatives such as hepatocytes, pancreatic and intestinal cells. One of the earliest and most important steps for the differentiation of a highly enriched culture is the induction of a proper definitive endoderm population as even modest inefficiencies at this stage result in the development of mixed end stage populations. Optimization of endoderm induction is therefore essential for all hPSC lines and is ideally monitored quantitatively by flow cytometric analyses of the expression of germ-layer specific surface makers. To date, the cell surface markers used to assess endoderm induction in hPSC differentiation cultures, including CXCR4, are not endoderm specific, and strategies to identify and isolate endoderm subpopulations predisposed to different endoderm lineages do not exist. In this study, we generated mouse monoclonal antibodies against hESC-derived definitive endoderm with the goal of identifying cell surface markers that can be used to track the development of this germ layer. Through this approach, we identified one endoderm-specific antibody, HDE1, which stains the emerging definitive endoderm with a unique pattern. HDE1 allows to isolate specific endoderm sub-populations with distinct cell fate potentials. Later, as the foregut gets specified, the endodermal cells lose HDE1 positivity while hindgut cells remain HDE1+, and CDX2 expression in late cultures can be tracked with HDE1. HDE1 represents therefore a powerful tool to track for the appropriate endoderm population and monitor proper patterning, and thus optimize the generation of the endoderm derivatives of interest from any hPSC line.

W-1398

PROLIFERATION DYNAMICS, BRAIN MATURATION AND TRANSCRIPTION FACTOR EXPRESSION IN THE DEVELOPING SALAMANDER BRAIN

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Among adult vertebrates, salamanders display the largest regenerative repertoire. They can even regenerate brain tissue, reactivating ependymoglial quiescent cells that proliferate and differentiate leading to functional regeneration. Many aspects of regeneration in Urodele amphibians may recapitulate developmental programs, but little is known about the proliferation and neurogenesis dynamics in their developing brain. By means of immunohistochemistry and EdU-chasing experiments, the proliferation and neurogenesis events in the growing brain of two salamander *(Notophthalmus viridescens* and *Pleurodeles waltl)* larvae have been analyzed within the frame of the prosomeric model, which is based on combinatorial gene expression patterns and allows comparisons among distant vertebrate species. Interestingly, in relation to the acquisition of behavioural events and glial maturation, several proliferative zones become quiescent as development proceeds, whereas others maintain extensive proliferation. Moreover, the cell cycle length varies in different regions in species-specific manner. In addition, after migrating from the ventricular zone, the new born cells start to express neuronal markers, showing regional and temporal dependent specification. In this context, the development of several dopaminergic cell populations was analyzed both during normal development and regeneration after chemical ablation. These results may help to unravel the potentially common mechanisms underlying development and regeneration of nervous tissue in Urodele amphibians, which can become of great significance in future therapeutical approaches for brain injury and disease.

W-1399

CHANGES IN THE EXPRESSION OF TRANSCRIPTION FACTORS NANOG, OCT4, SOX2 IN HUMAN EMBRYONIC STEM CELLS DURING EARLY DIFFERENTIATION INITIATED WITH SODIUM BUTYRATE

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Transcription factors NANOG, OCT4, and SOX2 regulate self-renewal and pluripotency in human embryonic stem (hES) cells. During differentiation, the levels of these transcription factors are modulated through mechanisms involving epigenetic modifications. In this study we used multiparameter flow cytometric assay to detect all three transcription factors simultaneously at single cell level and monitored the changes in NANOG, OCT4, and SOX2 expression during early differentiation towards endodermal lineage. Differentiation was initiated by using sodium butyrate, which inhibits histone deacetylases and induces hyperacetylation of histone. At the beginning of the differentiation the co-expression of NANOG, OCT4, and SOX2 at high levels (90% NANOG+ OCT4+ SOX2+ cells) was detected in hES cells. However, during differentiation the level of OCT4 and NANOG expression decreased (39% and

17% of NANOG+ OCT4+ cells by day 3 and 4, respectively), while SOX2 expression maintained at high level (from 98% at the beginning of differentiation to 86% by day 4). The number of SSEA-3 expressing cells decreased rapidly from 95% to 30% during 4 days, pointing at the changes on the surface of the cells. The co-expression of SOX17 and SOX2 increased from 61% to 81% as detected by day 3 and 4, respectively. NANOG expression in these SOX17+ SOX2+ cells decreased from 66% (day 3) to 13% (day 4). The differentiation markers (GATA4, GATA6, SOX17, SOX9) specific to early differentiation into endodermal lineage were first detectable in hES cell subpopulation co-expressing pluripotency markers NANOG, OCT4, and SOX2 and later in cells expressing SOX2, but without NANOG and OCT4 expression. The expression of both pluripotency markers and differentiation markers in a single cell at the same time showed the gradual mode of developmental transition. Different regulation of transcription factor SOX2 during early differentiation events was detected pointing that SOX2 might be important for self-renewal of hES cells during differentiation.

W-1400

MICRORNA 361 ATTENUATES THE MIGRATION OF NEURAL CREST CELLS BY THE NUCLEAR LEVELS OF -CATENIN AND REPRESSOR REST DURING OSTEOGENIC DIFFERENTIATION

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Malformations of craniofacial regions can be attributed to the complex events occurring during embryonic development. The bone and cartilage of the facial skeleton and the vast majority of facial connective tissues are derived exclusively from cranial neural crest (NC) cells. The understanding pathways that control normal bone development is imminent for deducing novel therapeutic targets, which could be aimed at during disease intervention in the clinic. Osteoblast differentiation is a key step in proper skeletal development and acquisition of bone mass. All of the major developmental signals including Wnt and Notch signaling, along with an increasing number of transcription factors such as Runx-2 and osterix, have been shown to regulate the differentiation and/or function of osteoblasts. However, the role of microRNAs (miRs) to turn stem cells or progenitors into osteoblasts has been reported, but remains inefficient. In order to elucidate the role of miRs in osteogenesis, we have turned to murine embryonic stem cells (mESCs), which are an ideal model to study the earliest events of pre-osteogenic specification. Using this model, we focused on osteogenic lineage committing miRs, which are known molecular regulators of other developmental processes. We performed a global microRNA profiling using CombiMatrix miRNA arrays to identify candidate miRs that have the potential to play a role as regulators of early NC-osteogenesis, Overexpressing and knocking down the miR candidates at the late NC stage identified miR-361 as the most potent pro-osteogenic miRNA. MiR-361 caused changes in calcium deposition, ALP activity and osteogenic marker gene expression. Moreover, miR-361 presence resulted in a reduction of Prickle1 and Prickle 2 expression levels which are important downstream components of the Wnt pathway and nuclear translocator receptors for RE-1 silencing transcription factor (REST). To demonstrate the necessity and the requirement of miR-361 for the specific stages of NC development we performed PCR analysis of NC markers. Enhanced levels of miR-361 resulted in significant increases of Snai1, Slug, Sox9, Sox10, CD73, CamK2, Twist1 and Pax3 expression. Unraveling the regulatory circuits of miRNAs is a great challenge, but may provide attractive targets for mechanism-based treatment of bone diseases.

W-1401

GENERATION OF A CILIARY MARGIN-LIKE STEM CELL NICHE FROM SELF-ORGANIZING HUMAN RETINAL TISSUE

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In the developing neural retina (NR), multipotent stem cells within the ciliary margin (CM) contribute to de novo retinal tissue growth. We recently reported the ability of human embryonic stem cells (hESCs) to self-organize stratified NR using a culture technique known as SFEBq. Here we report the emergence of CM-like stem cell niches within human retinal tissue. First, we developed a culture method for selective NR differentiation from hESCs by timed BMP4 treatment. We then found that inhibiting GSK3 and FGF-signaling induced a transition from NR tissue to retinal pigment epithelium (RPE), and that removing these inhibitions facilitated the reversion of this RPE-like tissue back to the NR fate. This step-wise 'induction-reversal' method generated tissue aggregates with RPE at the margin of central-peripherally polarized NR. We demonstrate that the NR-RPE tissue boundary further self-organizes a niche for CM-like stem cells that functions to expand the NR peripherally by de novo progenitor generation. Thus, the 'induction-reversal' method may prove useful in future studies examining optic tissue transplantation, pathogenesis and drug discovery.

W-1402

EFFECT OF SUBSTRATE AND ROCK INHIBITOR (Y27632) ON THE DIFFERENTIATION POTENTIAL OF HUMAN EMBRYONIC STEM CELLS GROWN AS NON-COLONY TYPE MONOLAYER

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We have shown previously that pluripotent stem cells may be routinely cultured as a monolayer on Matrigel when the ROCK inhibitor, Y27632, is included in the plating medium at passage. We have also shown that cells may be cultured on Laminin-521 (LN-521) in the same manner without this inhibitor albeit with lower plating efficiency. Since Y27632 has the capacity to affect several signaling pathways including cell cycle, SMAD and JAK/STAT, we speculated that it could influence differentiation potential. In addition, we have observed that the LN-521 substrate itself alters the growth characteristics of the cells which may bias the cells toward or away from certain lineages. To investigate these observations, H9 human embryonic stem cells (hESCs) were cultured in parallel on Matrigel and LN-521 (with or without Y27632). At the third passage under these conditions cells were subject to differentiation conditions

described in the literature which did not involve the formation of embryoid bodies. Cells were differentiated to definitive endoderm using a commercially available kit; to neurectoderm using the 'dual SMAD' protocol (Chambers et al); and to mesoderm using the protocol of Lian et al. for cardiomyocytes. RNA was extracted from each condition as well as from the undifferentiated cells, labeled and hybridized to Agilent One-color microarray slides (4x44K). Preliminary analysis of the data indicates that there is no apparent difference at the gross level when studying all genes with the undifferentiated cells being most closely related. Comparison to our StemCellDB gene expression database and, in particular, the 'EB_ecto-' and 'EB_mesend'-specific gene lists previously described, show minor differences in differentiation propensity which may not reach significance. These data will be examined in depth and, if verified, fully explored.

W-1403

THE SWITCH FROM SELF-RENEWAL TO ASYMMETRIC CELL DIVISION IN EMBRYONIC STEM CELLS

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Stem cell self-renewal is mediated by a genetic network, comprised of several autoregulatory feed-forward loops, that maintains embryonic stem (ES) cell identity. Stem cell differentiation is triggered by repression of the transcription factors needed to establish and maintain their pluripotency and self-renewal. While searching for genes highly expressed in ES cells and downregulated during stem cell differentiation, we identified Dido, a gene complex that encodes three proteins (Dido1, Dido2 and Dido3) generated by alternative splicing. The largest isoform, Dido3, is expressed ubiquitously, whereas the two smaller splice variants are transient. Dido3 comprises a plant homeodomain (PHD) finger, a transcription elongation factor S-II subunit M (TFSIIM) domain, a Spen paralogue and orthologue (SPOC) module, and a long C terminal region (CT). By targeting the murine Dido amino-terminal domain, we showed that mice develop a transplantable disease with features of myeloid dysplasia syndromes (MDS) and myeloid proliferation (MPD), very similar to human MDS/MPD. We also reported embryonic lethality of mice with a mutation in the Dido3 carboxy-terminal domain (Dido3ΔCT-RFP), which coincides with failure of epiblast cells to differentiate. Stem cells derived from Dido3ΔCT-RFP mutant mouse blastocytes neither downregulate stemness genes nor upregulate differentiation genes in vitro. Differentiation is restored by ectopic expression of wt Dido3 and constructs bearing distinct Dido domains. Our results show that the Dido PHD domain participates in the induction of stem cell differentiation, which is associated with Dido1 upregulation. We also observed that shRNA inhibition of Dido1 expression prevents wt ES cell differentiation. The Dido3 PHD domain binds euchromatin through H3K4Met3, and through the SPOC domain then binds the transcriptional repressor complex, which contains HDAC3, SMRT and NCoR. A Dido3 PHD mutant unable to bind H3K4Met3 does not displace Dido3ΔCT-RFP, which remains bound to the repressor complex. Displacement is due to competition for H3K4Met3 binding by Dido1 or the PHD, both of which lack the SPOC domain, and therefore the repressor complex.

W-1404

DYNAMICS OF TERMINAL CELL FATE PROGRAMMING

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Direct programming of terminal cell fate by the activity of transcription factor (TF) combinations promises to be an instrumental tool to control cell differentiation for clinical applications. However, how multiple TFs coordinate their activity and transform the transcriptional and epigenetic landscapes remains obscure. By taking advantage of an efficient system that programs motor neurons from embryonic stem cells via the forced expression of Ngn2, Isl1 and Lhx3, we have mapped the dynamics of gene expression, programming TF binding and chromatin modifications within the 48 hours that are required for stem cells to program into postmitotic motor neurons. We find that programming factor binding is dynamic and can be divided into three behaviors: stable across the time series, early binding associated with stem cell genes and late binding associated with terminal factors. As programming progresses, enhancers rapidly change their chromatin state in response to the programming TFs, while promoters show a much slower kinetics. By integrating all data sets, we aim to describe how the early expressed genes modify the cellular state to facilitate complete cell programming. These results suggest that direct programming of terminal fate is the consequence of a transcriptional and epigenetic cascade rather than a unique step. Understanding these events will allow for the rational improvement of direct programming strategies for the application of stem cell technologies to the clinic.

W-1405

GLYCOLYSIS-MEDIATED CHANGES IN ACETYL-COA AND HISTONE ACETYLATION CONTROL THE EARLY DIFFERENTIATION OF EMBRYONIC STEM CELLS

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Loss of pluripotency is a gradual event, whose initiating factors are largely unknown. Metabolic processes are leading candidates for regulating this switch, as chemical fluxes change within minutes and can affect both transcriptional and epigenetic mechanisms. Here, we sought to identify the earliest metabolic changes induced at the first hours of differentiation of embryonic stem cells. High-resolution NMR analyses identified 44 metabolites and a distinct, gradual transition in metabolism during early differentiation. Metabolic and transcriptional analyses showed that pluripotent cells produced acetyl-CoA through glycolysis and rapidly lost this function in the first hours of differentiation. Importantly, modulation of this metabolic pathway blocked histone de-acetylation and associated differentiation in human and mouse embryonic stem cells. Acetate, acetyl-CoA precursor, was found to delay cell differentiation and block early

histone de-acetylation in a dose-dependent manner. Inhibitors upstream of acetyl-CoA caused differentiation of pluripotent cells, while those downstream delayed differentiation. Thus, we demonstrate a metabolic switch during the very first steps of differentiation, causing a loss of histone acetylation and concomitant loss of pluripotency. It highlights the important role metabolism plays in pluripotency and suggests that a glycolytic switch controlling histone acetylation can release stem cells from pluripotency.

W-1406

HOXA GENE EXPRESSION DEFINES DEFINITIVE FETAL HAEMATOPOIETIC CELLS DIFFERENTIATED FROM HESCS

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The production of definitive haematopoietic lineages from human embryonic stem cells (hESCs) remains a significant challenge. To dissect the requirements for definitive blood, we tracked haematopoiesis from hESCs using Green Fluorescent Protein (GFP) reporter lines targeting the haematopoietic isoform of RUNX1. GFP+CD34+ progenitors homed to the bone marrow, but did not engraft, prompting us to compare their transcriptome with cord blood (CB) CD34+ cells. We discovered that hESC-derived progenitors lacked expression of HOXA cluster genes, suggesting an underlying mesoderm patterning defect. Using HOXA expression as a guide, we found that modulation of WNT and ACTIVIN signaling was sufficient to produce HOXA+ endothelial and haematopoietic precursors that robustly generated T cells and KLF1+BCL11A+ definitive fetal-like erythroid cells. Our finding that HOXA codes reflect cellular potential highlights the importance of appropriate embryonic patterning for the generation of definitive hematopoietic cells.

W-1407

ASSEMBLY OF hES CELL CLUSTERS AND DIFFERENTIATION THEREOF IN CELL CULTIVATION BAGS

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The differentiation of hES cells to generate ß-cells for treatment of type 1 diabetes patients will preferably be performed in suspension culture to facilitate scale-up, which will be a challenge. Based on islet transplantation studies it is assumed that ~10^9 cells will be needed to treat one patient. It has been shown that the survival of cells is significantly improved by formation of multi-cell clusters from a single cell suspension. Mixing conditions during cluster formation are crucial to the survival and later differentiation of the cell culture. In search for a gentle and efficient mixing system for this process shaked cultivation bags were tested. The cluster formation in bags was studied at different working volumes and mixing speed set points. It was possible to identify conditions where cluster formation and cell yield were comparable to the performance in reference cultures in shaked 6 well plates. Further it was shown that the clusters formed in bags could be directed to step-wise differentiation towards beta cells. Since the bag cultivation systems are considered scalable this study indicate a possible solution to the scale-up challenge of bringing the cell therapy into pre-clinical and clinical development.

Acknowledgements: The methods applied in this study are based on protocols developed in collaboration between Novo Nordisk A/S Department of Stem cell biology and Takara Bio Europe, Cellartis AB Gothenburg, Sweden.

W-1408

REGULATION OF WNT ANTAGONISTS BY SP5 AND ITS EFFECTS ON WNT SIGNALING IN HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESCs) must adhere to a strict developmental trajectory to generate a fully functional organism from a single fertilized egg. Many indispensible signal transduction pathways contribute to this process, including Wnt signaling. Wnt signaling is required throughout development and is vital for maintenance of tissue specific stem cell populations including intestine, skin, blood, and brain. Misregulation of this pathway can lead to cancer, developmental abnormalities, or diseases in which tissues fail to renew properly. To identify important Wnt target genes in hESCs, we used RNA-seq to examine changes in gene expression over time following treatment with purified Wnt3a. A transcriptional repressor, SP5, was observed to be one of the most highly upregulated genes in our dataset. We confirmed this observation by qPCR and Western Blot. To examine the role of SP5 in global gene regulation, we performed ChIP-seq analysis on hESCs. Interestingly, we found evidence that SP5 displaces SP1, a global transcription factor, upon Wnt stimulation, thereby significantly altering the transcriptional landscape of hESCs. Furthermore, this analysis revealed that a significant subset of SP1/5 target genes encode Wnt antagonists, including AXIN2, ZNRF3, CTNNBIP1 to name a few. Upon Wnt signaling, elevated levels of SP5 repressed expression of Wnt antagonists, thereby increasing Wnt signaling in hESCs. Conversely, mutagenesis of SP5 in hESCs using CRISPR/Cas9 led to elevated expression of Wnt antagonists. Taken together, we have uncovered a novel mechanism by which Wnt signaling, via transcriptional upregulation of SP5, augments its own signaling output. These findings have profound implications for Wnt's role during development, in stem cell self renewal and differentiation and in cancer.

W-1409

NEURAL TUBE MORPHOGENESIS IN SYNTHETIC 3D MICROENVIRONMENTS

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The earliest steps of development are characterized by cellular reorganization and differentiation within a three-dimensional (3D) microenvironment. This 3D context allows for a complex spatial interplay between biochemical and physical signals, and governs important cellular rearrangements leading to morphogenesis. In-vitro approaches have attempted to recapitulate key features of these processes, and it has now become possible to generate an increasing variety of self-organizing multicellular tissue constructs termed organoids. While important aspects of the 3D in-vivo organization have been recreated in these organoid systems, such studies have been exclusively performed in MatrigelTM, a poorly defined proteinaceous mixture whose properties cannot be readily modulated. As such, the uncharacterized interactions between cells and this extracellular matrix (ECM) have proven to be a major challenge to understanding the underlying regulatory mechanisms governing morphogenesis. In this work, we employ highly tunable synthetic ECM hydrogels in order to disentangle the contributions of biochemical and physical components of the microenvironment in the specification of pluripotent stem cell fate and morphogenesis. We develop a high-throughput approach to systematically generate combinatorial interactions between various components of the cellular microenvironment in 3D and deploy it to elucidate the mechanisms controlling early neuroepithelial development from single mouse embryonic stem cells. We show the synergistic roles of matrix elasticity, degradability and ECM protein composition in specifying neural fate and initiating apico-basal polarity. We explore how matrix characteristics relate to dynamic symmetry-breaking events in such multicellular constructs, and show how apico-basal polarity is required for initiating subsequent dorso-ventral patterning. We demonstrate that these morphogenetic processes are tightly coupled to the physical characteristics of the matrix, and demonstrate for the first time that a patterned neural tube-like organoid can be generated within an optimized, fully synthetic matrix. This systematic approach should be applicable to other organoid systems.

W-1410

INITIATION OF VASCULAR COMMITMENT OF HESCS THROUGH ACTIVATION OF BMP9/ALK1 SIGNALLING PATHWAY

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In order to study human vascular development, human embryonic stem cells (hESCs) provide an excellent tool to characterize molecular mechanisms. The Transforming growth factor beta (TGFbeta) superfamily plays fundamental roles in mouse mesodermal development including vascular commitment, but little is known about the molecular mechanisms that regulate cardiovascular development in humans. The Bone morphogenic protein (BMP) 9 binds to the Activin like kinase receptor 1 (ALK1) and Endoglin in endothelial cells (ECs), causing phosphorylation of Smad1/5/8 and amongst others upregulation of Id1. Both pro- and anti-angiogenic effects of BMP9 have been reported in mouse and human system. In hESCs limited data exist for the TGFbeta family in mediating vascular commitment. We investigated the role of BMP9/ALK1 pathway in hESC vascular differentiation. hESCs were differentiated towards the vascular lineage using very efficient monolayer cultures for expression analysis but inducing embryoid bodies (EBs) using the SpinEB method in serum free medium (BPEL) to assess EB sprouting. In addition, BMP4 and bFGF were used to induce mesodermal differentiation, since it is essential for vascular development. Afterwards, EBs were stimulated with different members/inhibitors of the TGFbeta superfamily to study hESC derived vascular differentiation. We assessed the expression of vascular markers and the activation of the VEGF pathway. Furthermore, endothelial properties were analysed by assessing proliferation, migration and EB sprouting angiogenesis. Here, we demonstrate that BMP9/ALK1 promotes vascular commitment of hESCs via Smad1/5/8 activation and Id1 upregulation. We used the neutralizing anti-hALK1 antibody [PF-03446962] to clarify the specific effect of ALK1 in BMP9 induced human vascular development. We verified that BMP9/ALK1 induced EB sprouting is inhibited when EBs are subjected to the neutralizing human ALK1 antibody.

W-1411

IMPROVEMENT OF CARDIAC FUNCTION BY EMBRYONIC STEM CELL DERIVED CARDIOMYOCYTES IN DOXORUBICIN INDUCED CARDIOMYOPATHY MODEL

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Doxorubicin (Dox) is a widely used chemotherapy drug with limited usage due cardiotoxicity that may progress to heart failure. This study aims to evaluate the role of cardiomyocytes derived from mouse embryonic stem cells (CM-mESC) in the treatment of Dox-induced cardiomyopathy (DIC). mESC line E14TG2A was cultured and characterized according to karyotype analysis and expression of pluripotency-associated genes by RT-PCR and Immunofluorescence. Then, cells were stably transduced with a viral construct expressing luciferase under control of the Murine Stem Cell Vector (MSCV) promoter. After that, cells were submitted to cardiac differentiation and the efficiency of protocol was evaluated. Dox (7.5mg/kg) was delivered into CD1 mice cardiac left ventricle, guided by echocardiogram (ECHO), once a week for 3 weeks. After 26 days, mice were submitted to intramyocardial injections of CM-mESC (8x10e5 cells) and tracked by bioluminescence assay. Cardiac function was evaluated by ECHO. Cultured mESC presented a normal Karyotype (40 chromosomes). Oct3/4, Sox2 and Nanog expression by RT-PCR and SSEA-1 and Oct3/4 by immunofluorescence assays confirmed mESC pluripotency stage. After 8 days of cardiac differentiation protocol, beating cells were observed. On 14 day, differentiated cells presented high expression of Troponin T (80.6 ± 12.9, n=5) and functional characteristics of cardiomyocytes with prevalence of ventricular cells. Mice that received Dox presented DIC after 21 days. Ejection fraction (EF) decreased in Dox-group, n=23 (38.87±1.2%) when compared to Placebo-group, n=9 (58.15 ± 1.9%). Dox-group treated with CM-mESC showed a significant increase in EF on 5 and 30 days after treatment [(EF5d: CM-mESC-Dox: 51.57 ± 1.9% versus Placebo-Dox: 39.42 ± 1.6%; $p<0.05$) (EF30d: CM-mESC-Dox: 54.66 ± 4.7% versus Placebo-Dox: 41.89 ± 2.9%; $p<0.05$)]. No difference was observed in EF between Control and CM-mESC-Dox-groups on 5 and 30 days

after treatment [(EF5d: Control: 53.86 ± 2.6% versus CM-mESC-Dox: 51.57 ± 1.9%, p>0.05) (EF30d: Control: 54.92 ± 1.7% versus 54.66 ± 4.7%, p>0.05)]. Transduced CM-mESC was detected up to 11 days after intramyocardial injection. These results show that CM-mESC transplantation contributed to cardiac function improvement in an experimental model of Dox-induced cardiomyopathy.

W-1412

STIMULATION OF VASCULOGENESIS AND LEUKOPOIESIS IN EMBRYONIC STEM CELLS UPON EXTRACELLULAR RIBO-NUCLEIC ACID TREATMENT

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Released nucleic acids from damaged cells are enriched at the site of tissue injury and support inflammation and stem cell activation. Here, the impact of extracellular ribonucleic acid, especially transfer RNA (tRNA), on vasculogenesis and leukopoiesis of mouse embryonic stem (ES) cells was investigated. Extracellular tRNA (ex-tRNA) and whole cell RNA as well as ribosomal RNA (ex-rRNA) but not DNA increased CD31-positive branching points in embryoid bodies. Ex-tRNA and ex-rRNA treatment increased cell numbers of VEGFR2+, CD31+ and VE-cadherin+ vascular cells as well as CD18+, CD45+ and CD68+ cells, indicating leukocyte/macrophage differentiation. This was paralleled by mRNA and protein expression of hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor-165 (VEGF165), neuropilin 1 (NRP1), and α-smooth muscle actin as well as phosphorylation of phosphatidyl inositol 3-kinase (PI3K) and VEGF receptor 2 (VEGFR2). Furthermore, ex-tRNA increased protein expression of the pro-angiogenic semaphorin B4 receptor plexin B1 as well as the ephrin-type B receptor 4 (EphB4) and ephrinB2 ligand. Ex-tRNA enhanced cell migration, which was inhibited by the VEGFR2 antagonist SU5614 and the PI3K inhibitor LY294002. This likewise abolished the effects of ex-tRNA on vasculogenesis and leukopoiesis of ES cells. Ex-tRNA increased Nox-1, Nox-2 and Nox-4 mRNA and boosted the generation of reactive oxygen species (ROS) which was inhibited in the presence of radical scavengers and the NADPH oxidase inhibitors apocynin, VAS2870, ML171, and plumbagin. The latter abolished the stimulation of vasculogenesis and leukopoiesis upon ex-tRNA treatment. Our findings indicate that ex-tRNA treatment induces vasculogenesis and leukopoiesis of ES cells via ROS generated by NADPH oxidase, activation of VEGFR2 and PI3K. Released RNAs at the site of tissue injury may act as mediators of healing and/or remodeling of damaged tissue via stimulation of vasculogenesis and leukopoiesis.

W-1413

GENERATION AND CHARACTERISATION OF NEURONS DERIVED FROM DLG2 KNOCKOUT HESCS

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There is accumulating evidence that mutations conferring increased risk of neurodevelopmental disorders as schizophrenia converge on specific biological pathways. In particular multiple studies implicate synaptic proteins involved in the regulation of plasticity, especially those modulating glutamate receptor function such as postsynaptic density. Implicated genes include members of the DLG (disks large) family of membrane associated guanylate kinases (MAGUKs) and related synaptic proteins. In addition, recent genome-wide association studies found individuals with DLG2 deletion have an increased risk of developing schizophrenia. The aim of the study is to find the cellular phenotypes on neurons derived from DLG2 knockout hESCs and to define underlying mechanisms. Currently the knockout cell lines were derived using the new genome editing technology called clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system. The first round of differentiation is on going. Neural induction, cellular proliferation, survival, differentiation, and maturation including synaptogenesis will be studied on differentiated neural precursors and neurons with immunocytochemistry and quantitative PCR. In addition, neuronal activity and connectivity will be measured by patch clamp and microelectrode array. There is no result about the phenotypic differences of mutant cells at this point but some results will be able to be shown at the time of the event.

W-1414

THE ROLE OF GEMININ IN THE DIFFERENTIATION OF RADIAL GLIAL CELLS DURING LATE EMBRYOGENESIS

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Ependymal cells are located on the lateral walls of the brain ventricles. Ependymal cells carry a large number of motile cilia on their surface which beat in a coordinated way. This rhythmic beating affects the direction of the flow of the cerebrospinal fluid and facilitates the distribution of neurotransmitters, contributing to the differentiation of neural stem cells. During embryogenesis, the generation of the multiciliated ependymal cells begins with the differentiation of a subpopulation of radial glial cells to the ependymal lineage. Geminin is a protein that controls cell cycle and differentiation. It binds to the licensing factor Cdt1 and inhibits DNA replication, while it interacts with transcription factors and chromatin remodeling complexes and regulates cellular differentiation. We have previously shown that Geminin regulates self-renewal and differentiation timing of cortical progenitor cells during mid-embryogenesis. In the present study we have used conditional knock out mice in order to investigate the role of Geminin in the differentiation of late radial glial cells. Immunofluorescence experiments and pulse labeling experiments have been employed in order to define differences in the differentiation of cortical progenitor cells at the end of embryogenesis that take place in the absence of Geminin.

W-1415

ENDOGENOUS WNT SIGNALS MEDIATE BMP-INDUCED AND SPONTANEOUS DIFFERENTIATION OF EPIBLAST STEM CELLS AND HUMAN EMBRYONIC STEM CELLS

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Therapeutic application of human embryonic stem cells (hESCs) requires precise control over their differentiation. However, spontaneous differentiation is prevalent and growth factors induce multiple cell types, e.g. the mesoderm inducer BMP4 generates both mesoderm and trophoblast. Here we identify endogenous WNT signals as BMP targets that are required and sufficient for mesoderm induction, while trophoblast induction is WNT independent, enabling the exclusive differentiation towards either lineage. Furthermore, endogenous WNT signals induce loss of pluripotency in hESCs and their murine counterparts, epiblast stem cells (EpiSCs). WNT inhibition obviates the need to manually remove differentiated cells to maintain cultures, and improves the efficiency of directed differentiation. In EpiSCs, WNT inhibition stabilizes a pregastrula epiblast state with novel characteristics, including the ability to contribute to blastocyst chimeras. Our findings show that endogenous WNT signals function as hidden mediators of growth factor-induced differentiation and play critical roles in the self-renewal of hESCs and EpiSCs.

W-1416

DIRECT ANTAGONISTIC INTERACTIONS BETWEEN PLURIPOTENCY FACTORS AND LINEAGE-SPECIFIC TRANSCRIPTION FACTORS DURING PARAXIAL MESODERMAL SPECIFICATION OF HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (PSCs) have the capacity of unlimited self-renewal and ability to differentiate into cell types of all three germ layers. Therefore, they are good models to study the molecular mechanisms involved in germ layer-specific lineage commitment. Using two different types of PSCs, human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), we investigated the molecular mechanisms involved in paraxial mesodermal differentiation. Previous studies have indicated that BMP and Wnt signaling pathways are involved in commitment to this germ layer and lineage. It is known that PSCs highly express the transcription factors Oct4, Sox2, and Nanog to maintain pluripotency. However, how signaling pathways interact with pluripotency transcription factors to begin the process of differentiation is incompletely studied. Thus, we sought to elucidate the interactions between the pluripotency factors and signaling pathways involved in paraxial mesodermal differentiation. We found that introduction of BMP and Wnt ligands lead hESCs and iPSCs to differentiate into paraxial mesoderm with clear changes in cell morphology and decreases in the transcriptional levels of pluripotency factors. This was simultaneously coupled with transcriptional increases in several paraxial mesodermal-related transcription factors, including Brachyury and Runx2, but not lateral mesodermal or other germ layer-specific transcription factors such as TAL1, Sox17, Sox1, and GATA1 , etc. Our current findings demonstrate that direct antagonistic interactions between pluripotency factors and lineage-specific transcription factors are involved during the differentiation process, highlighting the complex interplay between different levels of transcription factors during early development and lineage specification.

W-1417

ANALYSIS AND COMPARISON OF EMBRYOID BODY FORMATION FROM HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) have the potential to become an effective resource for regenerative medicine. In this study, we compared global changes of gene expression during the differentiation by DNA microarray using six human pluripotent stem cell lines, human ES cell: khES1 (Kyoto), khES2 (Kyoto), khES3 (Kyoto), H1 (Wisconsin) and human iPS cell: 253G1 (Kyoto), 201B7 (Kyoto) and two somatic cells: human adipose-derived stem cells (Lonza), human fibroblasts. To induce differentiation, we produced spherical multicellular aggregates known as embryoid bodies (EBs) and found that some genes were commonly up- or down-regulated during the EB formation. These genes are assumed to induce differentiation or to be sensitive to the EB formation. In addition, the six pluripotent stem cell lines showed the different tendency to differentiation. As the efficiency of differentiation depends on the quality of EB formation, we compared the production of EBs in rotary orbital suspension culture at various speeds with that in a simple static suspension culture. Although all of six pluripotent stem cells express some differentiation markers EBs formed in static suspension culture, none of the outgrowths of EBs developed beating. In contrast, outgrowths of EBs formed at 100 rpm had the high rate of beating, 70%, and increased the expression of Nkx2.5, a master gene of cardiomyocyte differentiation. Outgrowths of EBs developed at slower rotational speeds showed more endoderm gene expression and that of faster speeds showed the increased expression of ectoderm markers. A computational hydrodynamic simulation showed that at 100 rpm, the shear stress was uniformly distributed in the dish. Our results suggest that shear stress induces the differentiation of specific cell types from hPSCs depending on rotational speed.

W-1418

TRANSCRIPTION FACTOR BINDING DYNAMICS DURING HUMAN ESC DIFFERENTIATION

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Pluripotent stem cells provide a powerful system to dissect the

underlying molecular dynamics that regulate cell fate changes during mammalian development. Here we report the integrative analysis of genome wide binding data for 38 transcription factors with extensive epigenome and transcriptional data across the differentiation of human embryonic stem cells to the three germ layers. We describe key regulatory dynamics and show the orchestrated lineage specific behavior of selected factors. For instance, GATA4 appears to act as a master regulator in early mesoderm, while its targets in the endodermal population enrich for poised enhancers. GATA4's divergent binding in the two lineages could be the result of a mesoderm specific interaction with SMAD1, which acts downstream of BMP signaling during the mesoderm induction. We also find that the binding of several transcription factors is strongly associated with specific loss of DNA methylation in one germ layer and in many cases a reciprocal gain in the other layers. Taken together, our work shows context-dependent rewiring of transcription factor binding, downstream signaling effectors, and the epigenome during human embryonic stem cell differentiation.

W-1419

MONITORING CHANGES IN GENE EXPRESSION WITHIN LIVE CELLS DURING EMBRYONIC STEM CELL DIFFERENTIATION

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Understanding the level of pluripotency within a stem cell is critical to embryonic stem cell research. More importantly monitoring changes in pluripotency during differentiation to progenitor cells and even to a terminally differentiated state within live cells are imperative to performing meaningful downstream assays. Traditionally this has been accomplished through the use of antibody staining or qRT-PCR on a subset of the population of interest. Unfortunately, these techniques are traditionally endpoint assays and the results are assumed to be consistent across the entire population which does not account for any heterogeneity within the sample. The use of a live cell RNA detection method allows for greater flexibility in enriching for cells with high and low expression profiles by FACS analysis or microscopy. Specific RNA probes can be generated toward pluripotent, multi-potent, or even terminally differentiated target RNA's allowing researchers to understand the "state" of the individual cells. Since the probes are nontoxic, they can be used to enrich cells based on RNA expression level and those same cells can be subjected to follow-up assays. Here we present data showing H9 human ES cells following addition of OCT4 RNA detection probes compared to neural progenitor cell lines illustrating the idea of monitoring expected changes in RNA levels. To further validate this approach, a live cell RNA probe for GFAP was added to neural progenitor cells (NPCs) throughout a 14 day directed differentiation to an astrocyte lineage. The level of GFAP mRNA was observed to increase steadily during the first 72 hours followed by a sustained levels through day 7 with peak increases occurring by day 8 and throughout the 14 day time-lapse experiment. The ability to monitor gene expression during NPC differentiation to astrocytes in a live cell setting removes the need to set up duplicate wells for downstream studies and enhances the relevance of the data generated. Additionally this approach can be utilized during the transition from adult fibroblasts to an induced pluripotency state as in the generation of IPS cells. Coupled with the ability to enrich for cells with a given expression level, these live cell RNA probes provide researchers with valuable information and confidence about the cells used in subsequent assays.

W-1420

COMPARISON OF IMMUNE-RELATED GENE EXPRESSION PROFILES BETWEEN PLURIPOTENT STEM CELLS, THEIR DERIVATIVES AND SOMATIC CELLS

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Human pluripotent stem cells, including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are virtually unlimited cell sources for clinical applicability. Our previous reports indicated that human ES cells and iPS cells are characterized by immune privilege, and the differential immunogenicity may exist in terms of the MHC-related molecules and NK receptor ligand. In the present study we therefore aim to investigate the global immune-related gene expression profiles among human ES cells, iPS cells and somatic cells to identify the candidate immune-related genes that potentially lead to an altered immunogenicity. The cDNA microarray was undertaken to measure the expression levels of immune related genes (Immunome list) in undifferentiated stem cells, differentiated stem cells (14days in vitro spontaneous differentiation), and 3 types of somatic cells including dermal papilla cells, ovarian granulosa cells and foreskin fibroblast cells. The array data were analyzed by IPA and gene ontology biological function. To further investigate the role of mTORC1 signal pathway, differentiated stem cells were treated with rapamycin and the immune responses were measured by human mix lymphocyte reaction. Several high variance genes including CD14, CD59, GATA3, IDO1, IL6, HMOX1, THBS and OLR1 were overlapped with the selected immune-related gene lists. These genes are consistently up- or down-regulated when undifferentiated stem cells were compared with differentiated stem cells and all the 3 types of somatic cells, suggesting that these candidate genes are potentially critical ones for regulating the immune privilege of pluripotent stem cells/derivatives. Furthermore, these genes are associated with the gene ontology terms for ECM-receptor interaction and TGF-β signaling pathway. The immunogenicity of differentiated stem cells is significantly negative regulated by mTOR inhibitor rapamycin. Our findings herein suggest that the transcriptome signature of immune-related genes including immune privilege-associated genes support the development of human pluripotent stem cells differentiation in vitro, toward a pattern more or less to the features and significantly different from somatic cells.

EMBRYONIC STEM CELL PLURIPOTENCY

W-1423

HUMAN AMNIOTIC EPITHELIAL CELLS AS FEEDER LAYER FOR DERIVATION OF HUMAN EMBRYONIC STEM CELLS FROM POOR-QUALITY EMBRYOS

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Data from the literature suggest that human embryonic stem cells (hESC) lines used in research do not genetically represent all human populations because these lines are derived exclusively from European and Middle Eastern natives. Nonetheless, differences in the epidemiology of diseases and susceptibility between different ethnic groups have been reported. To this end more efforts are needed to derivate and study new human lines with emphasis in underrepresented populations, including the Latin-American. However, the derivation of human ESC through conventional methods, involves the destruction of viable human embryos. During in vitro fertilization (IVF) process, embryos are discarded based on poor morphology and because they produce low percentage of full-term pregnancies. Nevertheless, these embryos have been considered a viable source to derive hESC lines. On the other hand, the use of mouse embryonic fibroblasts as a feeder layer cells of hESC has several drawbacks, including the high risk of xeno-contamination during the in vitro derivation and propagation process. For this reason, the use of alternative feeder layers like the human amniotic epithelial cells (hAEC) has been proposed. These cells can maintain mouse and hESC in an undifferentiated state. Our laboratory proposes that hAEC could support the derivation and maintenance of human ESC from poor quality embryos. The IVF poor quality embryos donated and the hAEC were collected according to National Institute of Perinatology Ethics Committee as well as the Guidelines for the Conduct of human ESC Research. The embryos were cultured on a hAEC feeder layer. We observed the embryos outgrowth at the 4 day after plating. After two weeks, the outgrowth was passaged mechanically on a hAEC new layer until getting several colonies. The colonies were positive for transcription factors as well as cell surface markers of pluripotency detected by immunofluorescences and RT-PCR. Through the formation of embryoid bodies, we evaluated their ability to differentiate into the three embryonic layers, using specific markers for the mesoderm, endoderm and ectoderm. Moreover, the colonies presented a normal karyotype. These results suggested that hAEC can be a source of feeder layer to derivate and maintain new lines of hESC. *Funding by Conacyt (202717) and InPER (21071).*

W-1424

KAP1 PRESERVES EPIGENETIC MEMORY IN PLURIPOTENT STEM CELLS

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The corepressor KAP1 plays a central role in controlling expression of endogenous retroelements and maintaining parent-of-origin DNA methylation at imprinting control regions (ICRs) during the wave of genome-wide reprogramming that precedes implantation. In order to investigate the molecular mechanisms of these events, we first used a complementation assay with a library of Kap1 deletion mutants in ground state murine embryonic stem cells (ESC). Our data suggest that the RBCC domain is required for KAP1 recruitment to the DNA, while the HP1 box and the PHD-Bromodomain are dispensable for this process but necessary for heterochromatin formation and maintenance of DNA methylation. Given the well-described crosstalk between histone modifications and DNA methyl transferases (DNMTs), we hypothesised that KAP1-mediated recruitment of histone methyltransferases (HMTs) is central to preserve the epigenetic landscape at ICRs. We identified Setdb1 as the main HMT responsible for deposition of H3K9me3 at imprinted loci, although its knockdown did not lead to a decrease in histone and DNA methylation levels of a magnitude comparable with that observed upon removal of KAP1. Following KAP1 knockdown we could detect an increase in the levels of 5-hydroxymethylcytosine (5hmC) at several KAP1-bound loci indicating that active demethylation by TET proteins contributed to the erasure of epigenetic memory noted at these loci. We are currently investigating the mechanism by which KAP1 counteracts the activity of TETs. These experiments unveil a critical role for KAP1 in protecting specific loci from both active and passive demethylation during early development, shedding new light on how epigenetic information is transmitted from the gametes to the embryo.

W-1425

THE INVESTIGATION OF PLURIPOTENCY MAINTENANCE IN STIRRED SUSPENSION BIOREACTORS

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Pluripotent stem cells (PSCs) including embryonic stem cells and induced pluripotent stem cells are capable of self-renewal and differentiation into any cell type. The adherent culture system currently used to generate PSCs does not efficiently produce an adequate number of cells and lacks culture control leading to culture heterogeneity. To overcome this, we have developed stirred suspension bioreactors (SSBs) to culture PSCs as aggregates. Cells are stirred at a shear stress level of 6 dyne/cm2 to maintain an optimal size for mass transfer of oxygen and nutrients. Using murine PSCs, we have observed that pluripotency is maintained in the absence of leukemia inhibitory factor, an obligate pluripotency maintenance factor. We have been able to replicate this in 100 mL and 10 mL SSBs. Our results suggest shear stress may play a role in the maintenance of pluripotency. We hypothesize that shear stress alters cell signalling and gene expression via mechanotransduction, which alters adherens junctions and allows β-catenin to translocate

to the nucleus and regulate pluripotency. We have examined the nuclear translocation of β-catenin in murine ESCs using a TCF/LEF GFP reporter system and have observed a 40-fold increase in the amount of nuclear β-catenin in cells exposed to higher shear stress levels. Confocal microscopy reveals that β-catenin activation occurs throughout the aggregate, suggesting that the affect occurs uniformly. This observation is consistent with a recent report using other cells indicating that E-cadherin may have mechano-sensing properties, which lead to a restructuring of adherens junctions and concomitant nuclear translocation of β-catenin.

W-1426

EXAMINATION THE EXPRESSION PROFILE OF STEM CELL SPECIFIC MICRORNAS IN RABBIT EMBRYOS AND PLURIPOTENT STEM CELLS

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The rabbit pluripotent stem cells (rabPSCs) represents an alternative animal model to study human diseases since rabbit PSCs are more similar to primate PSCs than mouse PSCs. Several groups have reported the establishment of rabbit ESCs and iPSCs, but these rabPSCs were defined as primed state stem cells while they were rarely competent to contribute to chimeras in contrast to naive state mouse PSCs. MicroRNAs have been identified as important regulators of embryonic stem cell properties and they expression profile are different in primed and naïve state of stem cells. To discover the expression profile of pluripotency-associated miRNAs in rabbit embryos and rabPSCs, we had to identify the rabbit specific miRNA sequences. By applying SOLiD deep sequencing technique we could identify more than 1600 rabbit miRNAs based on comparison to known miRNA databases. We detected and characterized all miR-302 cluster members. We found that the ocu-miR-302 cluster is highly similar to its human homolog both in mature and pre-miRNA sequence level and in the identical linear order arrangement on its corresponding chromosome. The ocu-miR-302 cluster began to express at 3.5 dpc embryonic stage embryonic stage and increased through the blastocyst stages. The ocu-miR-290 cluster comprises only three mature miRNAs, ocu-miR-290-5p, ocu-miR-292-3p and ocu-miR294-3p. The expression of ocu-miR-290 cluster was initiated at 4-cell stage in rabbit embryos and steadily increased through the blastocyst stage. The highest expression of ocu-miR-290 cluster was observed at 3.5 dpc embryonic stage. Three homologs of human C19MC cluster (ocu-miR-498, ocu-miR-520e and ocumiR-512-5p) are differentially expressed in embryonic epiblast, hypoblast and trophoblast of 6dpc rabbit embryos. Comparison the miRNA profiles revealed that rabbit ESCs and iPSCs exhibit very different miRNA signatures. Differential expression of several miRNAs, including members of miR290-295, miR17-92, miR302-367 was observed. Altogether, these data suggest that miRNA signatures provide an important sensor for the primed compared to the naive state of rabbit PSCs.

W-1427

HIGH VIABILITY AND RECOVERY OF DISSOCIATED PLURIPOTENT STEM CELLS CRYOPRESERVED IN AN OPTIMIZED FREEZING MEDIUM

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Efficient recovery of cryopreserved cells is critical to achieve maximal benefit from frozen tissue banks. Human pluripotent stem cells (hPSC) are typically harvested from culture as cell aggregates during passaging, and these aggregates can be cryopreserved using standard protocols. However, poor post-thaw cell viability often results from the limited diffusion of critical medium components to the aggregate core. To improve on current cryopreservation protocols, we have developed a freezing medium, FreSR™-S, that is optimized for freezing hPSC as single cell suspensions and allows the rapid re-establishment of colonies in culture after thawing. The performance of FreSR™-S compared to a commercially available freezing medium (control) was assessed by examining post-thaw viability, recovery, and the ability of recovered hPSC to re-establish colonies in culture. To initiate the experiments, hPSC previously cultured as aggregates in mTeSR™1 or TeSR™-E8™ were dissociated into single cell suspensions using Gentle Cell Dissociation Reagent for 8 min at 37°C, re-suspended in FreSR™-S or control freezing medium at 1 × 10e6 cells/mL, and dispensed into cryotubes. Cryotubes were cooled to -80°C at -1°C/min using a Mr. Frosty™ device before being placed in liquid nitrogen for up to 12 months. Upon thawing, recovered cells were assessed for viability using trypan blue exclusion and seeded at approximately 7 × 10e4 viable cells/cm2 in TeSR™-E8™ medium containing 10 μM Y-27632. Cells were subsequently fed daily with TeSR™-E8™ and passaged as cell aggregates for up to five passages using standard culture techniques. Single hPSC suspensions were recovered from FreSR™-S at higher percentages (78 ± 6% of input cells) after thawing compared to the control medium (34 ± 4%; n = 18, p > 0.0001). The viability of the recovered cells was also higher in cells frozen in FreSR™-S (79 ± 2%) compared to control (56 ± 3%; n = 18, p > 0.0001). Recovered hPSC readily established colonies 2-3 days after seeding, demonstrated high expression of undifferentiated cell markers by flow cytometry (Oct4, Tra-1-60 > 90%) and retained a normal karyotype (n =4). In summary, FreSR™-S supports the efficient post-thaw recovery of cryopreserved single cell hPSC suspensions, and recovered cells were capable of rapidly re-establishing colonies upon re-plating.

W-1428

REVERSION TO EMBRYONIC CD44 SPLICE VARIANT 3 EXPRESSION ENHANCES LEUKEMIA STEM CELL SELF-RENEWAL

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Deregulation of CD44 plays a vital role in leukemia stem cell (LSC) maintenance in the malignant microenvironment. However, extensive alternative mRNA splicing in humans results in expression of multiple CD44 isoforms, some of which have been implicated in cancer invasion and metastasis. We investigated the role of CD44 splice variant expression on human embryonic stem cells (hESCs) and on human blast crisis LSC maintenance in the malignant niche. As a key arbiter of cell migration and survival, increased CD44 expression and splice isoform switching have been linked to poor prognosis in several types of cancer. By lentiviral enforced expression on hESCs we have identified that CD44 splice variant 3 (CD44v3) is involved in maintaining self-renewing hESCs in an undifferentiated state, while loss of CD44v3 affects the cells ability to propagate and self-renew. Moreover, CD44v3 gene expression was highly expressed in undifferentied hESCs while differentiated hESCs had low expression, suggesting CD44v3 to be important for pluripotency. Coincident with the deregulation of a hESC alternative splicing gene network, human chronic myeloid leukemia (CML) progenitors selectively express CD44v3. The CD44 splice isoform expression pattern in CML progenitors was determined by whole transcriptome RNA sequencing (Illumina HiSeq 2000) on FACS sorted chronic phase (CP; n=8) and blast crisis (BC; n=8) CML progenitors as well as their normal counterparts from cord blood (CB) (n=7) and adult peripheral blood (NPB; n=4). While whole gene expression analysis revealed upregulation of CD44 in blast crisis compared with chronic phase and normal progenitors, a plethora of CD44 transcript variants were also detected including variants 3, 4 (CD44s), 5, 6, 7, 8. Notably, RNA Seq isoform analysis detected a higher expression of CD44 transcript variant 3 in BC compared to CP and CB and NPB. Upregulation of an embryonic splice variant of CD44, variant 3, expands pluripotent stem cell populations and promotes malignant reprogramming of CML progenitors into self-renewing LSC. In addition, these observations strongly suggest that CD44 transcript variant 3 upregulation serves as a biomarker of progression from CP to BC as well as the generation of resistant LSCs, with the potential of being a more specific target for future combination therapies.

W-1429

OSCILLATING EXPRESSION OF ENVIRONMENTAL SENSOR RECEPTOR IN MOUSE EMBRYONIC STEM CELLS DEREGULATES MITOTIC PROGRESSION AND CAUSES PLURIPOTENCY LOSS

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The G1-shortened cell cycle of embryonic stem (ES) cell is closely related to their self-renewal; whereas the pluripotent state of ES cells is under tight control of pluripotency factors (PF) that regulate gene expression through protein interaction and cooperating with reversible epigenetic and transcriptional machineries. Fluctuating PF expression creates various responses to developmental stimuli within ES cell population. The aryl hydrocarbon receptor (AHR) is an environmental sensor but also play role in regulating cell cycle progression. Besides, emerging evidence suggested the importance of AHR during embryonic development. Our previous study showed that repressed Ahr in ES cells through direct PF binding is essential to maintain stemness; however due to the hyper-plasticity of ES epigenome, leakiness of AHR expression may exist. Using ES cells in which eGFP expression was driven by the Cyp1a1-promoter, we identified 6 - 10% AHR-expressing ES (ESCAE) cells oscillating within the population. Dominant marker selection of ESCAE cells showed that they express lower levels of OCT4 and NANOG. Transcriptomic analysis of ESCAE cells predicts that AHR connects to cell proliferation, cell differentiation, and epigenetic modifications. Consistently, ESCAE cells have reduced S-phase cell population, accumulate at G2/M-phase due to abnormal progression of mitosis, and proliferate slower than normal ES population. Cell synchronization and immunostaining of mitosis-related histone modification confirmed the accumulation of ESCAE cells at M-phase. Changes of protein levels for cell cycle components in ESCAE cells, including lower CDK1, lower pCDK2, lower inhibitory-pCDK1, lower cyclin A, lower pRB, and highly elevated CDC25B are in agreement with their altered cell cycle distribution. Modulating genetically AHR expression levels in ES cells confirms the slower proliferation rates and elevated CDC25B expression as a consequence of AHR expression. Furthermore, ESCAE cells formed smaller embryoid bodies and differentiate with a remarkable decline of cardiomyocyte contractility. In conclusion, our data suggest that oscillating AHR expression in ES cells disrupts the pluripotent state through interfering the ES-specific cell cycle and contributes to altered responses to differentiation stimuli.

W-1430

DIOXYGENASES MEDIATING OXIDATION OF DNA AND HISTONES BLOCK OXIDATIVE STRESS INDUCED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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Prior studies have supported a role for hypoxia and 2-oxoglutarate (2OG)-/Fe(II)-dependent dioxygenases (mediating oxidation of DNA and histone tail residues) in murine and human pluripotency. The purpose of our study was to explore the relationship between these two apparently conflicting activities in human embryonic stem cells (hESC) using two independent lines (H9 and RH1) in feeder-free culture. We observed that sRNAi targeting of either JMJD2C (a histone demethylase), and TET family members (mediating hydroxymethylation of methyl-cytosine [hMC]) induced hESC differentiation as shown by: i) loss of colony morphology, ii) down-regulation of OCT4, NANOG, and/or SOX2 by Q-PCR and immunocytochemistry, and iii) up/downregulation of trophoblast (CDX2, CGα, PL1), endoderm (AFP, HNF4α, GATA4, 6), mesoderm (BMP2, VEGF, GATA2) and ectoderm (Pax6, Nestin, Tubulin III, NF-200) lineage markers, with conserved and variable target-specific effects across lines ($p<0.05$). This correlated with a reduction in genomic hMC levels as measured by ELISA ($p<0.01$). As compared with culture for 7 days under normoxia (20% O2) vs hypoxia (0.5% O2), the latter yielded: i) more cell growth ($p<0.05$); ii) elevation of reactive oxygen species (ROS), confirmed by quantification of H2DCF-DA fluorescence ($p<0.01$), and iii) upregulation of Hypoxia-Inducible Factor (HIF)-1α expression ($p<0.001$). After establishing cytotoxic concentration ranges for oxidative stress inducing sodium arsenite (NaAsO2) for 7 days under normoxia, we observed that exposure to arsenite at sub-cytotoxic concentrations (IC10 and up to 3 log-fold lower) under normoxia induced differentiation, whereas under hypoxia did not. This correlated with elevations under hypoxia of i) JMJD2C, TET1, HIF1α and antioxidant gene (GPX1, 4, SOD1,2) expression by Q-PCR ($p<0.0001$), ii) hMC

content ($p<0.001$), and iii) ROS production ($p<0.0001$). Transient episomal expression of JMJD2C or TET1 protected both lines against differentiation induced by sub-cytotoxic concentrations of NaAsO2 under normoxia, and this correlated with elevated hMC ($p<0.05$) and blockage of NaAsO2 -induced elevation of ROS ($p<0.05$). Our study implicates histone and DNA dioxygenases as hypoxia-inducible responses to oxidative stress which prevent oxidative stress-induced differentiation.

W-1431

OVEREXPRESSION OF OCT4A ORTHOLOG ELEVATES ENDOGENOUS XIST EXPRESSION LEVELS IN PORCINE PARTHENOGENIC BLASTOCYSTS

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X-chromosome inactivation (XCI) is an epigenetic process for compensating dosages of X-borne genes between male and female eutherians. This process is observed in the early eutherian embryo development with species-specific manner. Until recently, various pluripotent factors, like non-coding have been suggested to regulate XCI process by repressing XIST expression, which is master inducer for XCI. However, insights of the process and its regulators have been restricted in mouse species despite evolutionary diversity on the process and molecular mechanism among the species. Therefore, in here, we examined the relation between one of the most represent pluripotent factors, OCT4A, which is gate-keeper for maintaining pluripotency and suggested to XIST repressor, and three X-linked genes, XIST, LOC102165544, and RLIM, of which orthologs in mouse were known to regulate XCI, in porcine preimplantation embryos. To know their relation, expression levels of OCT4 and the three genes in blastocysts were correlatively compared. Unexpectedly, expression levels of OCT4A were positively correlated with XIST and LOC102165544 in female blastocysts. And also, overexpression of exogenous human OCT4A in cleaved parthenotes generated blastocysts with increased XIST expression levels. However, the increased XIST expression was not observed in blastocysts which were obtained exogenous OCT4A at early blastocyst. These results suggest the possibility that OCT4A would be directly or indirectly involved in XIST expression at earlier stage embryos than blastocysts in pigs.

W-1432

DIFFERENT EXPRESSION PATTERN OF CYCLIN-D1 MRNA BETWEEN HUMAN AND MOUSE PLURIPOTENT CELLS

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Cyclin D1 is one of important molecule involved in the cell cycle regulation in normal and cancer cells. It plays roles as well as a key sensor and integrator of extracellular signals of cells in S to G1 phase that mediate its function through binding the CDKs for modulating local chromatin structure of the genes that are functions in regulation of the cell proliferation and differentiation. Although many studies reported that Cyclin D1 is frequently overexpressed in cancers, but its expression and biological functions in stem cells are not clear. Because the proliferation of the pluripotent cells is a characteristic cell cycle structure with a particular rapid rates and truncated gap phase comparing normal somatic cell cycles, we speculate that Cyclin D1 may play novel function in regulation of cell proliferation and differentiation in stem cells. In this study, we investicated Cyclin D1 gene expression in human embryonic stem cells (hESCs) and mouse embryonic stem cells (mESCs) with comparing mouse embryonic fibroblasts (MEF) and human newborn foreskin fibroblasts (NuFF) by using quantitative reveres transcription polymerase chain reaction (qRT-PCR). Compared MEF, expression of Cyclin D1 mRNA were significantly decreased (28%) in mESCs. However, there were no significant differences in Cyclin D1 mRNA expression between NuFF and hESCs. Our results indicated the different expression level of Cyclin D1 gene between human and mouse stem cells. We hypothesize that Cyclin D1 might be the different gene expression pattern with heterologous differences cause by different mechanisms of cell cycle states.

W-1433

XIST RNA-MEDIATED SILENCING OF X-LINKED AND AUTOSOMAL GENES: DOES GENOMIC POSITION MATTER?

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X chromosome inactivation (XCI) is the epigenetic mechanism by which mammals compensate for differences in X-linked genes expression between females (XX) and males (XY). XCI starts early during female mouse embryonic development when the X-linked gene *Xist* is up-regulated from one X chromosome. Xist RNA spreads on the X *in cis* and triggers chromosome-wide gene inactivation. Studies involving Xist transgenes have indicated that spreading is less robust when Xist is integrated on an autosome, suggesting a sequence specific model for *Xist* function. Recently, a different model has been proposed where the 3D spatial organization is instructive in Xist spreading along the X. To better understand Xist function, we developed a doxycycline responsive *Xist* expression system that allows control of *Xist* expression from different genomic contexts in XX ES cells. First, we integrated a dox-inducible Xist transgene either at the *Xist* endogenous locus or at different positions on the X. These clones allow us to investigate the linear and spatial properties of Xist mediated silencing. Second, we randomly integrated an Xist dox-inducible transgene on one copy of chromosome 12 of a trisomic cell line (41, XX dup12). In these lines, similar to inactivation of one X in female cells, silencing of one chromosome 12 will result in a viable cell, allowing us to study whether Xist spreading and silencing abilities rely on a sequence specific mechanism. Xist qPCR and RNA FISH analysis show up-regulation of Xist transgenes upon dox induction in all ES clones. Interestingly, X-linked gene silencing is more efficient when *Xist* is induced from the endogenous locus compared to other positions along the X. Moreover, Xist spreads *in cis* on chromosome 12 but gene silencing is variable between different clones. Although a chromosome 12 trisomy is welltolerated in ES cells, ES differentiation leads to cell death. This phenotype was partially or completely rescued upon *Xist* induction on chromosome 12, reflecting different silencing efficiencies for Xist transgenes integrated at different

positions. Currently, we are combining allele specific RNA-seq data with Targeted Chromatin Capture (T2C) to relate Xist silencing capabilities to chromosomal position and 3D environment.

W-1434

A MINIMAL CO-EXPRESSION NETWORK DESCRIBES TRANSITION THROUGH THE STEM CELL HIERARCHY

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As pluripotent cells proliferate during mammalian development, they transition through a hierarchy of phenotypic states marked by a stepwise restriction in developmental potential and self renewal capacity. This is characterized by changes in the activity of key elements in pluripotency and lineage-specific regulatory networks. A high degree of heterogeneity persists in pluripotent stem cell cultures, with recent debate focused on whether molecular variability between individual stem cells is a direct consequence of the microenvironment, cell-cycle asynchrony, or a necessary feature of the pluripotent phenotype. While extrinsic singling modulates the pluripotency network, the contribution of cell signalling and adhesion pathways to the maintenance of core transcriptional programs is often overlooked, perhaps due to their contribution to a diverse range of cellular functions. We have previously applied a co-expression strategy to expand current pluripotency networks to incorporate signalling, cytoplasmic, cell-cell adhesion and plasma membrane associated elements. Here, we identify the minimal subset of this network sufficient to describe a stem cells transition from self-renewal to lineage commitment. We further characterize single cell and population-based gene expression patterns within the stem cell hierarchy, and explore the association between self-renewal, lineage priming and culture condition. We leveraged publically available CAGE and ChIP-seq data and demonstrated that membrane associated elements indeed contribute to the initiation and maintenance of pluripotency and self-renewal via involvement in the key transcriptional networks. These observations support a model of the stem cell phenotype as inherently plastic, where variability in molecular profiles between individuals describes the transition from pluripotency to lineage commitment.

W-1435

NEW MONOCLONAL ANTIBODIES ENABLING VIABLE DETECTION AND ENRICHMENT OF HUMAN CELLS DISPLAYING DISTINCT STATES OF PLURIPOTENCY

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The availability of new cell surface markers to human pluripotent stem cells (hPSCs) will enable new detection and enrichment strategies for viable hPSCs. These strategies will be useful for future investigations of the pluripotent state, as inputs to clinically relevant differentiation assays, and for the stringent quality control of live cell populations following hPSC differentiation. To generate tools for detecting cell-surface proteins on viable hPSCs we selected candidate genes identified from our published immunotranscriptional profiling of hPSCs. Following immunisation with target antigens, hybridoma cultures were screened by robotic solid-state antigen array analyses for detection of the corresponding immunogen and then via high-throughput flow cytometry to confirm capability for detecting live hPSCs. Here we report the generation of a new panel of 7 monoclonal antibodies (mAbs) that efficiently detect these known proteins on the cell surface of live and fixed hPSCs derived from both the embryonic inner cell mass (hESCs) and somatic cell reprogramming (hiPSCs). We performed detailed characterisation studies using multiparameter immunostaining and FACS analyses showing the correlation of novel mAb hPSC-associated protein profiles with previously known hPSC cell surface detecting mAbs and POU5F1/ OCT4. We analysed changes in mAb profiles during 28 days of hPSC differentiation, and compared them to profiles for SSEA-3, SSEA-4, TRA-1-60 and the lectin UEA-1. Furthermore, we discovered that our new panel of antibodies can distinguish hESCs and hiPSCs cultured in a lineage primed state from hiPSCs reprogrammed and cultured in conditions supporting the naive or ground state of pluripotency. We anticipate that these novel antibodies generated to known antigenic targets will be valuable tools for enabling further investigation of human pluripotency and should also facilitate the development of strategies for quality control of hPSC-derived cell populations destined for clinical use.

W-1436

DECIPHERING THE ROLE OF HUMAN XIST IN PLURIPOTENCY

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Placental female mammals undergo X chromosome inactivation (XCI) early in embryogenesis to achieve dosage compensation of X-linked genes. XCI is initiated by the cis-acting long non-coding RNA Xist early in embryonic development, but the function of Xist appears to be regulated differently in mouse and human. In mice, expression of Xist is always associated with silencing: mouse Xist is never expressed in female pre-XCI cells such as epiblast cells of the blastocyst and embryonic stem cells (ESCs). In contrast, others and we have shown that XIST is expressed in both male and female human blastocysts without causing silencing. Moreover, our work has shown that FGF-dependent human ESCs do not capture the pre-XCI state of the blastocyst, and are typically in a post-XCI state with an epigenetically unstable inactive X chromosome. Hence, these primed pluripotent cells are of little use for studies of de novo XCI and potentially unsafe for therapeutic applications due to their variable X chromosome status. Therefore, we asked whether 'naïve' culturing conditions reset the X chromosomes to an active state with XIST expression, allowing for de novo XCI upon differentiation. We find that culturing human primed ESCs with an inactive X chromosome in naïve conditions recently published by

Theunissen et al leads to robust reactivation of the inactive X. While the reactivation of the inactive X occurs quickly upon adaptation to naïve culture conditions, only a few cells in the population recapitulate the X-state of the human blastocyst and express XIST from both X chromosomes. Moreover, the induction of XIST expression only occurs after several passages in naïve conditions. Thus, cells in these naïve conditions are very heterogeneous with respect to XIST expression. We are defining the heterogeneity of cells in molecular terms, and hypothesize that cells with different XIST expression patterns in naïve culture conditions capture different states of naïve pluripotency. Additional experiments are directed towards understanding how XIST expression is achieved without causing chromosome-wide silencing, and at improving culture conditions for the efficient establishment of the blastocyst-like X chromosome state.

W-1437

EFFECT OF TGF- AND MAPK INHIBITORS ON EXPRESSION PATTERN OF KEY REGULATORY FACTORS DURING GENERATION OF MESC

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Small molecules have wide application in order to achieve the optimal culture conditions for derivation and maintenance of pluripotent stem cells. They contribute to raise the efficiency and stability of pluripotent stem cells in vitro by affecting signaling pathways. Recently, the impact of two signaling pathway inhibitors PD0325901 and SB 431542 in R2i culture conditions simultaneous, as an optimal medium for derivation and maintenance of mouse ES cells, has been reported. In this study, expression pattern of the key pluripotency and early differentiation markers were evaluated during mESC derivation from ICM. Blastocysts (3.5-day) from the Balb/c strain were cultured in R2i+ LIF (main group) and Serum+LIF (control group) conditions on feeder cells. Then ICM outgrowths were collected on days 3, 5 and 7. At the next step, gene expression level of pluripotency and early differentiation markers were evaluated by qRT-PCR. The trophectoderm-like cells around the attached ICM outgrowths decreases significantly in R2i+LIF compared with Serum+LIF condition. qRT- PCR analysis reveals significant expression of pluripotency genes such as *Oct4, Nanog, Sox2, Rex1, Dppa3, Dax1, Nodal, Sall4, Utf1, Tcf3, β–catenin* and *Blimp1* as well as some epigenetic markers such as *Tet1, Carm1* and *Setdb1*. In addition, expression of early differentiation markers including *Cdx2, Lefty2* and *Gata6* are decreased during this course. The maximum expression level of pluripotency and epigenetic genes are observed on day 5 in R2i condition. In contrast, the minimum genes expression is detected on day 5 in Serum/LIF condition. Our results show that presence of small molecules such as PD0325901 and SB431542 in culture medium has a tremendous effect until the 5th day in the process of generating mouse embryonic stem cells. Also, it proposes that the day 5 is optimum time for ICM-outgrowth cells to pass reprogramming barrier or capturing the pluripotent cells in vitro.

W-1438

EXPRESSION OF 5-HT RECEPTORS IN HUMAN BLASTOCYSTS AND ESCS

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Serotonin (5-hydroxytryptamine, 5HT) is a major neuromodulatory neurotransmitter and hormone in nearly all animal species. The effects of serotonin are exerted via 7 subtypes of 5HT receptors (5HTRs) of which 6 are G-protein-coupled receptors (5HTR1, 2, 4, 5, 6, and 7) and one is a ligand-gated ion channel (5HTR3). The physiological effects of serotonin include regulation of subcortical sensory and motor systems and of higher cognitive functions, and dysregulation of serotonin is implicated in a myriad of neuropathologies, including pain syndromes, motor, sleep, eating and mood disorders. During mammalian development, 5HT has been implicated in regulating neurodevelopmental processes, and alterations of the fetal 5HT supply can have adverse effects on neural circuit formation. 5HT has been detected in embryos or larvae of rodent and non-mammalian species, where it is believed to have important signaling functions. Nothing is known, however, about the effects of 5HT in preimplantation human embryos. To address this question, we have assessed the expression of 5HTRs in day 5 human blastocysts and in 16 hES cell lines. Four hESC lines were then tested for 5HTR-mediated changes in cell proliferation, signaling and gene expression after exposure to 5HT. We detected transcripts of most 5HTR subtypes in blastocysts, and of all 5HTR subtypes in every hES cell line. In hES cells, 5HT exposure upregulated transcription of all 5HTRs, and of selected stemness, proneural and serotonergic specification and pathway genes. Cell cycle entry was accelerated 3h after 5HT exposure, and transcription of cyclins D1-3 and of Cdk4 and 6 was upregulated 3h after cell arrest release in the presence of 5HT. Phosphorylation of STAT3 and Erk1/2 was significantly increased by exposure to 5HT within 5 min and 10 min, respectively. These results indicate that the activation of 5HTRs stimulates hES cells towards increased proliferation and regulates the expression of a broad spectrum of stemness and differentiation genes. Current experiments are focused on dissecting the effects of specific 5HTRs.

W-1439

DYNAMIC REGULATION OF TET1 AND TET2 EXPRESSION DURING CELL STATE TRANSITIONS BETWEEN NAIVE AND PRIMED PLURIPOTENCY IN HUMAN AND MOUSE

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The recently discovered TET 5-methylcytosine dioxygenases (TET1, TET2 and TET3) catalyze DNA de-methylation. *Tet1* and *Tet2* are highly expressed in naive mouse embryonic stem cells (mESCs) and are differentially regulated in primed epiblast-like cells (EpiLCs) and upon differentiation to somatic lineages. In contrast, human ESCs (hESCs), which resemble primed epiblast-derived stem cells in the mouse, express much lower levels of *TET1*. The transcriptional mechanisms governing the cell type- and species-specific regulation of *TET1* and *TET2* during transition of pluripotency states are unclear. We have recently defined promoter and enhancer domains

in murine *Tet1* and *Tet2*. In *Tet1*, a novel distal TSS with associated promoter region is highly active in the naive state and rapidly undergoes DNA methylation and silencing upon differentiation. The proximal TSS is associated with a constitutively unmethylated weak CpG-island promoter, which is activated by an intragenic enhancer in both naive mESCs and primed mEpiLCs. *Tet2* has a dominant CpG island promoter with strong pluripotency-independent activity and an intragenic Oct4/Sox2-binding enhancer that facilitates high expression in naive ESCs but is downregulated in EpiLCs. Recent studies to convert conventional hESCs to a naive state using alternative defined conditions suggests that elevation of *TET1* expression can be among the critical hallmarks of primed-to-naïve conversion. Our comparative analysis using publicly available RNA-seq and ChIP-seq datasets indeed suggests that additional TSS and NANOG binding peaks can be detected distal to *TET1* in naïve human cells. We are currently evaluating whether this upregulation of *TET1* is mediated by a distal promoter homologous to that in mouse. New transgenic reporter systems based on *Tet1* and *Tet2* *cis* regulatory domains may be valuable tools to distinguish nuanced changes during transition of pluripotent states and to derive better quality induced pluripotent stem cells without epigenetic abnormalities

W-1440

VIABILITY, CYTOTOXICITY AND PLURIPOTENCY ANALYSIS OF MOUSE EMBRYONIC STEM CELLS CULTURED ON PLGA SCAFFOLDS

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3D scaffolds can be used to simulate an in vivo embryonic stem cell microenvironment. The aim of this study has been to cultivate mouse embryonic stem cells (mESC) on poly(lactic-co-glycolic acid) (PLGA) scaffolds to provide an efficient culture niche. The mESC (C57Bl/6) lineages were derived in the laboratory. The cells were cultured in DMEM high glucose, with 10% FCS, 50 IU/mL penicillin and 50 μg/mL streptomycin, LIF 1,000 IU/ml, 1 mM sodium piruvate, 2 mM L-glutamine, 0.1 mM b-mercaptoethanol and 0.1 mM non-essential amino acids. To produce the scaffolds, PLGA (75:25) was dissolved in dichloromethane:ethanol (8:2) hexafluoro-2-propanol to create a 20% (w/v) solution. The set-up to conduct the electrospinning included a high voltage of 14 kV, a collector to needle distance of 20 cm, and a constant flow rate of 3.0 mL/h. The scaffolds were characterized with a scanning electron microscope (SEM) and the average diameter of the fibers was calculated (ImageJ). Half of the scaffolds were hydrolyzed with NaOH (hydrophilic) and the other half were non-hydrolyzed (hydrophobic), the control group was cells cultured on a plate with a gelatin coat. A total of 1.5 x 104 cells/cm2 was seeded and the analysis was performed after 2, 7 and 14 days. Cell viability was assessed by MTT test and the cytotoxicity by measuring lactate dehydrogenase enzyme activity (LDH). The pluripotency markers OCT-4 and SOX-2 were analyzed by immune stainning on the 14th day. Statistical analyses were performed by the Student T-test (P<0,05). The average diameter of the fibers of the hydrophilic scaffolds was 3.593μm and the fibers of the hydrophobic scaffolds were 3.664μm. The contact angle was 135.8 ± 1.62 for the hydrophobic group and 118 ± 1.8 for the hydrophilic group. Cell viability showed no differences between the hydrophilic and hydrophobic scaffolds on days 2, 7 or 14 in culture. The viability was only higher for the control groups for the periods analyzed. The LDH activity showed no statistic significance between the experimental groups. The pluripotency markers were positive for all the groups. Despite the mESCs remaining pluripotent and viable after 14 days in the scaffolds, the controls showed better results. However, the scaffolds are an important tool for cell therapy and future experiments must been made to facilitate mESC adaptation in scaffolds.

W-1441

IDENTIFICATION OF A NOVEL GENE MEDIATED HUMAN EMBRYONIC STEM CELL RENEWAL THROUGH OXIDATION PATHWAY

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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 undifferentiated 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.

W-1442

NOVEL EMBRYONIC STEM CELL-LIKE CELLS WITH PLACENTA CHIMERIC ABILITY DERIVED FROM PORCINE PREIMPLANTATION EMBRYOS

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Swine is one of the most important candidates for human disease models, and establishment of bona fide porcine embryonic stem cells (pESCs) will facilitate making disease models. Since the first pESCs was reported by Strojekl in 1990, researchers had tried to establish pESCs for more than 20 years, but no pESCs comparable with mouse embryonic stem cells (mESCs) were derived. All reported pESCs could not spontaneously differentiate and contribute to the chimera in spite of expressing pluripotent markers. The culture system employed in pESCs derivation, especially the factors related with pluripotent signal regulation in the culture medium, are the most wildly discussed questions. In this study, we cultured Day 6 in vitro fertilized porcine blastocysts in a new developed culture medium, which was named MXV medium. pESCs-like cells passaged for more than 50 passages were derived, and pluripotent signal pathway in this cell line were explored. pESC-like cells colonies were flat, which were similar to human embryonic stem cells (hESCs) .They were alkaline phosphatase positive, had normal karyotypes, and expressed classic pluripotent markers of Oct4, Sox2 and Nanog, as well as the mesendoderm markers of Gata3, Gata6 and Sox17. The cell line relied on FGF pathway rather than Lif-STAT3 pathway to maintain proliferation and pluripotency, and had the ability to form embryonic bodies in vitro and teratomas in vivo with three germ layers differentiation. Furthermore, red fluorescent protein (dsred) was successfully introduced into these cells via lentiviral vectors, pluripotency and differentiation potential of transgenic cells were unimpaired. When the transgenic cell line was used for nuclear transfer (NT), the NT embryos could develop to blastocyst with 11.52%. When the transgenic cell line was injected to day 4.5 blastocyst, it could involve in the embryonic development, and after transfering into recipients, the chimeric blastocysts could develop into fetuses with cells expressing dsred in placenta. These results indicated that this pESC-like cells are pluripotent cells which depend on FGF signal pathway and have chimeric developmental potent at least in placenta. This cell line will be a precious material for exploring porcine pluripotency and will also contribute to complicated genetic editing disease models.

W-1443

IDENTIFICATION OF NANOG RNA BINDING PROTEINS

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Nanog is a well-known transcription factor that plays fundamental roles in stem cell self-renewal and maintaining pluripotent cell identity. There remains a large data gap with respect to the spectrum of the key pluripotency transcription factors' interaction partners. Limited information is available concerning Nanog-associated RNA binding proteins (RBPs), and the intrinsic protein-RNA interactions characteristic of the regulatory activities of Nanog. Herein we used an improved affinity protocol to purify Nanog-interacting RBPs from mouse embryonic stem cells (ESCs), and 2 RBPs of Nanog (YBX1 and ILF3) were identified. The interaction of these two proteins with Nanog RNA were further confirmed by in vitro and vivo assays such as Western Blot, RNA immunoprecipitation (RIP), Immunofluorescence (IF) and Fluorescent In Situ Hybridization (FISH), as well as an new RBPs isolation strategy MS2-BioTRAP. Interestingly, RNAi studies revealed that YBX1 and ILF3 positively affected the expression of Nanog and other pluripotency-related genes. Particularly, down-regulation of YBX1 or ILF3 resulted in high expression of mesoderm markers. Thus, a reduction in the expression of YBX1 and ILF3 controls the expression of pluripotency-related genes in ESCs, suggesting their roles in further regulation of the pluripotent state of ESCs.

W-1444

LARGE-SCALE COMPARATIVE ANALYSIS REVEALS CONSERVED AND DISTINCT FUNCTIONS OF SOX2 IN HUMAN EMBRYONIC STEM CELLS AND NEURAL PROGENITOR CELLS

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Transcription factor SOX2 is a master regulator of both embryonic stem cells (ESCs) and neural progenitor cells (NPCs). However, the dynamic roles of SOX2 and the underlying mechanism by which SOX2 controls different stem/progenitor cell states are not fully elucidated, especially in cells of the human origin. By comparing genome-wide DNA binding sites of SOX2 in human ESCs (hESCs) and hESC-derived NPCs (hNPCs) combined with transcriptome analysis upon SOX2 knockdown, we find that, SOX2 regulates dynamic developmental processes through context and stage dependent regulation of distinct networks. Almost all genes whose promoters are bound by SOX2 are actively expressed, and a majority of SOX2 bound promoters in hESCs overlap with those in hNPCs, with functions in basic yet important cellular processes such as metabolism, cell cycle, transcription, chromatin organization. This finding indicates that SOX2 positively regulates a fundamental network in safeguarding self-renewal by occupying a common group of promoters. Also, we identify thousands of SOX2 bound enhancers, whose annotated genes constitute cell type and cell stage dependent networks. Our analyses reveal that SOX2 regulates much broader developmental aspects through enhancer binding in hESCs, including early blastocyst and trophectoderm formation and specific tissue development, while genes with SOX2-bound enhancer in hNPCs are enriched in neuron and cell migration, manifesting SOX2's more restricted regulation in neural development from NPCs onwards. Moreover, we discover a group of genes which are preoccupied and poised by SOX2 in hESCs and later switch to an active state in hNPCs through a change in histone modifications. The functions of these genes are highly enriched in central nervous system development, and thus likely represent the pro-neural network executing SOX2's role as a pioneer for neural development. Furthermore, signaling pathways and specific components in the pathways responsible for the roles of SOX2 in hESCs and hNPCs are defined and verified functionally. Together, through large-scale comparative analyses and functional verification, we provide novel insights into the cell type dependent regulatory networks of SOX2, laying the ground work for future application of hESCs and hNPCs.

EMBRYONIC STEM CELL CLINICAL APPLICATION

W-1445

GENERATION OF HIV RESISTANT HEMATOPOIETIC LINAGE CELLS FROM HUMAN PLURIPOTENT STEM CELLS CARRYING THE NATURAL CCR5 DELTA 32 MUTATION

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The chemokine receptor 5 (CCR5) is the primary co-receptor for β-chemokines and is used by HIV for entry into macrophages. Individuals with homozygous deletion of 32 base pairs in the CCR5 gene are highly resistant to HIV infection. A patient was cured of HIV infection following allogeneic hematopoietic stem cell transplantation from a homozygous CCR5Δ32 donor. This finding suggests a promising avenue for developing stem cell therapy to treat HIV infection. Human induced pluripotent stem cells (hiPSCs) may provide a rich source of patient-specific stem cells due to their ability to differentiate into various cell lineages and lower degree of HLA-compatibility. Several studies have attempted to disrupt the CCR5 gene in $CD34^+$ hematopoietic stem progenitor cells (HSPCs), $CD4^+$ T cells, and hiPSCS. However, the effects of integration mutagenesis could potentially cause other complications and raises safety issues. In the present study, we generated multiple human iPSCs colonies (*hiPSCs-CCR5Δ32*) from donors naturally containing the CCR5Δ32 mutation in feeder free conditions. These colonies maintained a normal karyotype, exhibited features of pluripotency that showed high expression of Oct4, SOX2, TRA-1-60, Nanog, and formed teratomas when injected into immunocompromised mice. The *hiPSCs-CCR5Δ32* were cultured in our feeder free and defined induction conditions in the presence of an optimized cocktail of human hematopoietic cytokines for generation of HSCPs. The suspension cells were characteristic of HSCPs, based on colony forming assays and expression of CD34, CD38, CD45RA, CD90, and CD49f. These cells were later differentiated into mature monocytes using myeloid/monocyte lineage cytokines and expressed CD14, CD11b, CD11c, CD16. Monocytes were further differentiated into macrophages by supplementation with M-CSF or GM-CSF. The resulting macrophages have characteristic phagocytotic capabilities and expression markers, such as $CD68^+$ and $CD206^+$, with the exception of CCR5 in *hiPSCs-CCR5Δ32* that retains the genetic variant. Our system provides a highly feasible protocol to produce functional HPSCs, monocytes and macrophages from hiPSCs. Our approach offers an attractive system for investigating human macrophage biology and a novel strategy for function cure of HIV patients.

W-1446

GLUCOSAMINE-INDUCED GPAT1 EXPRESSION THROUGH O-GLCNACYLATION IS CRITICAL FOR PROTECTION AGAINST HYPOXIA-INDUCED APOPTOSIS ON MOUSE EMBRYONIC STEM CELLS

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Recent researchers reported that O-GlcNAcylation is contributed to regulation of cellular metabolism as well as cell behavior. Moreover, some studies showed glucosamine (2-amino-2-deoxy-D-glucose; GlcN) has a protective role in exposures to noxious extracellular stimuli such as hypoxic condition through the O-linked β-N-acetyl glucosamine glycosylation (O-GlcNAcylation). These findings provided evidence that the protective effect of GlcN is associated with metabolic regulation by augmented O-GlcNAc signaling and could be translated to the in vivo environment. Therefore, there is possibility that metabolic change using GlcN will be a novel approach to improve ESCs survival under hypoxic condition. Thus our study aimed to investigate the role of GlcN in protection of hypoxia-induced apoptosis on mouse embryonic stem cells (mESCs). Our results present that treatment of GlcN decreased hypoxia-induced apoptosis and increased O-GlcNAcylation (CTD110.6 and RL-2) but not N-linked glycosylation (ALG10) in mESCs. In addition, we found GlcN increased O-GlcNAcylation of SP1 and SREBP1, which subsequently leads to their nuclear translocation. Furthermore, GlcN-induced activation of SP1 and SREBP1 increased expression of glycerol-3-phophate acyltransferase 1 (GPAT1), a lipid metabolic enzyme producing lysophosphatidic acid (LPA). Silencing of GPAT1 expression by gpat1 siRNA treatment reduced GlcN-mediated anti-apoptosis on mESCs. To verify the role of GlcN-induced GPAT1 under hypoxia condition, we treated GPAT1 metabolite (LPA) in mESCs. Exogenous LPA prevented mESCs from hypoxia-induced apoptosis and increased phosphorylation of mammalinan target of rapamycin (mTOR) and its substrate (p70S6K1). Morover, inhibition of mTOR activation by rapamycin (mTOR inhibitor) decreased reduction of hypoxia-induced mESCs apoptosis by glucosamine. In conclusion, GlcN stimulates LPA production through O-GlcNAcylation-dependent GPAT1 expression, which subsequently prevents hypoxia-induced mESCs apoptosis through the mTOR pathway.

W-1447

BIOENGINEERED SCAFFOLDS ENHANCE THE FUNCTIONAL INTEGRATION OF TRANSPLANTED HUMAN EMBRYONIC STEM CELL-DERIVED CORTICAL PROGENITORS IN AN ANIMAL MODEL OF FOCAL ISCHEMIA

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Necrotic brain injury such as those caused by stroke or traumatic brain injury result in primary cell death (primary insult), surrounded by a penumbra (salvageable tissue susceptible to secondary degeneration). Current therapies fail to repair the primary injury and minimally reduce secondary damage. We believe efforts to promote repair following necrotic injuries, will depend on the capacity to: (i) restore the tissue architecture; (ii) deploy replacement neurons; (iii) provide a suitable environment for the integration of transplanted cells and (iv) support residual cells in the penumbra. Scaffold gels were bioengineered to form "bio-bridges" across the necrotic

lesion cavity that provided both physical and trophic support to grafted human embryonic stem cell-derived cortical progenitors, as well as residual host cells. Scaffolds were synthesized by the assembly of peptides for the laminin-derived epitope IKVAV-the brain's major extracellular protein. In an athymic rat model of focal ischemia, these scaffolds were shown to significantly improve the survival and integration of hESC-derived grafts as well as reduce secondary degeneration of the host cortex. Grafts, in the presence of IKVAV gels, showed increased functional maturity, as revealed by their electrophysiological profile, and resulted in enhance functional recovery, compared to cell grafts alone. This study demonstrates the ability to repair the injured brain following focal ischemia, and will also yield important knowledge for the ability to exploit such conduits in other neural injury settings.

W-1448

FILIA IS AN ESC-SPECIFIC MASTER REGULATOR OF DNA DAMAGE RESPONSE AND SAFEGUARDS GENOMIC STABILITY OF MOUSE ESCS

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Pluripotent stem cells (PSCs) possess great promise in cell-based therapy. However, genomic instability observed in them represents a major challenge for their potential applications. Understanding the mechanisms that regulate their genome stability is critical to address the tumorigenic challenge. In particular, unique factors by which PSCs regulate their genomic stability remain elusive. Here we identify an ESC specific protein, Filia (official name, KH domain containing 3; other alias Ecat1), as a master regulator of genomic stability. Filia expression is induced by various genotoxic stress. Through its coordinated cytoplasmic and nuclear functions, Filia regulates centrosome integrity and DNA damage response (DDR) at multiple levels. These include ATM/Chk2 signaling, S phase and G2/M cell cycle checkpoints, DNA damage repair, ESC differentiation and apoptosis. Thus, Filia depletion not only causes robust genomic instability, but also impedes elimination of damaged cells by ESC differentiation or apoptosis. This, in turn, increases the risk of ESC transformation and tumorigenesis. Filia carries out its multiple functions through multiple ways. It interacts with another master regulator of DDR, PARP1, and stimulates PARP1's enzymatic activity to propagate its regulations on ATM/Chk2 signaling, cell cycle checkpoints, and DNA damage repair. Filia also functions in PARP1-independent manners. It constitutively localizes on centrosomes where it interacts with Numa. Upon DNA damage, Filia is phosphorylated at S349 which allow it to enter into nucleus and translocate onto DNA damage site to facilitate DNA repair. Filia also relocates to mitochondria where it regulates ESC apoptosis in response to DNA damage. Taken together, our data demonstrate that Filia functions as a unique master regulator of genome integrity, the first of its kind, in ESCs. Of note, reprogramming somatic cells into induced PSCs (iPSCs) is characterized by large variation in induction of Filia (Ecat1) expression, although the endogenous pluripotency markers are abundantly expressed. This suggests that Filia expression could serve as a molecular marker of iPSC quality.

TISSUE ENGINEERING

W-1450

MESENCHYMAL STEM CELL INFILTRATION, PROLIFERATION AND BONE DIFFERENTIATION ON IMPROVED ELECTROSPUN SCAFFOLDS COMPRISED OF POLYCAPROLACTONE (PCL)/NANO HYDROXYAPATITE (NHA)

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Biomaterial-based constructs using mesenchymal stem cells (MSCs) offer a valuable strategy for bone tissue regeneration. Electrospinning is one of the most powerful methods for scaffold fabrication since it creates nano-fibrous structure which mimics the extracellular matrix architecture. Small pore size, however, is a major drawback of these scaffolds which limits the cellular infiltration and tissue-ingrowth. In this study, we prepared a scaffold of polycaprolactone (PCL)/nano hydroxyapatite (nHA) by electrospinning and tried to increase in the pore size of the scaffolds using several strategies including application of ultrasound (with sonotrode), performing co-electrospinning of PCL/nHA and polyethylene oxide (PEO) and application of both (combined methods). The scaffolds where then investigated in terms of human marrow-derived MSC infiltration, proliferation and bone differentiation by DAPI staining, MTT, alkaline phosphatase activity and real time RT-PCR. According to our findings, applied methods tended to improve scaffold pore size in such a way that the pore size was increased up to 1.9, 2.4 and 3.4-fold for ultrasound, co-electrospinning and combined method respectively. Based on the DAPI-stained crysections of the scaffolds, while in the control group there was no cell infiltration, at the group undergoing two modifications (sonication and co-electrospinning), there appeared to be an average of 36.51 μm cell infiltration. Moreover, SEM and MTT data from this group showed comparatively a better cell spreading and a higher cell proliferation. Interestingly we observed a significantly more bone differentiation at combined group than the others as the ALP activity appeared to be increased two-fold and the bone specific gene including osteocalcin and collagen type I tended to upregulated 2.5 and 2.9-fold respectively. Taken together it seems that at the time of scaffold fabrication using electrospinning, application of both sonication and co-electrospinning is promising strategy in order to enhance the scaffold pore size which in turn improve MSC infiltration and bone differentiation of MSCs.

W-1451

HYDROGELS TO SUPPORT 3D MICROVESSEL FORMATION BY BLOOD-DERIVED ENDOTHELIAL PROGENITOR CELLS

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Endothelial progenitor cells (EPCs) derived from umbilical cord blood are a promising cell source for vascular tissue engineering. Poly(ethylene glycol) (PEG) hydrogel systems are FDA approved, biocompatible, and can be modified to recapitulate the biochemical and biophysical microenvironment of the vascular niche. The aim of this study was to investigate the use of PEG hydrogel systems to support EPC microvessel formation for tissue engineering and regenerative medicine applications. EPCs were isolated from the mononuclear cell fraction of umbilical cord blood. The EPCs expressed the endothelial markers CD31, VE-cadherin, eNOS, vWF; and lacked expression of the monocytic markers CD45, CD115, CD14. To evaluate the ability of PEG hydrogels to support EPC network formation, we employed both 2D and 3D hydrogel systems. For the 2D system, RGDS was conjugated to acryl-PEG-succinimidyl valerate (SVA) and grafted atop diacrylate PEG (PEGDA) using 2,2-dimethoxy-2-phenylacetophenone under UV light. EPCs were plated on PEG hydrogels in coculture with vascular smooth muscle cells (SMCs) at a 1:4 ratio with a total cell number of $8x10^4$ cells/cm^2 in EBM2 media containing 10% FBS. Lentivirally transduced EPCs expressed GFP or RFP. For the 3D system, PEG hydrogels were rendered cell-degradable by incorporation of the matrix metalloproteinase (MMP) sensitive peptide, GGGPQGIWGQGK (PQ). SMCs and EPCs were combined at a 1:1 ratio using a total seeding density of $1x10^4$ cells/μl, encapsulated with 3.5 mM of RGDS, 5 wt% PEG-PQ, and crosslinked with Eosin Y, under white light, to create 380 μm-thick hydrogels. 2D PEG hydrogel systems supported EPC microvessel formation to the same extent as tissue-culture glass based upon a lack of significant differences in total tubule length, branch points, and average segment width. The 2D microvessel system was extended into 3D, where EPC-generated microvessels were stable for at least 30 days in vitro, formed lumens, deposited the basement membrane proteins Collagen IV and Laminin adjacent to microvascular structures. Moreover we observed pericyte-like wrapping of SMCs which was positive for PDGFR-β and α-SMA. This study demonstrated that EPCs can form microvessels recapitulating aspects of physiological angiogenesis within a tunable and clinically translatable 3D PEG hydrogel system.

W-1452

NEW PERSPECTIVES OF BONE TISSUE ENGINEERING FOR CLEFT LIP AND PALATE PATIENTS

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Cleft lip and palate (CLP), one of the most frequent congenital malformations, affects the alveolar bone in the great majority of the cases, and the reconstruction of this defect still represents a challenge in the rehabilitation of these patients. The gold standard in alveolar bone reconstruction is autogenous bone grafts. However, these surgical procedures may be subjected to complications such as donor area morbidity, post-surgical reabsorption and infections. To circumvent these problems, researchers have been focusing on the development of bone tissue engineering strategies and osteogenic substances that may offer alternative methods with minimal or no donor site morbidity for bone grafts. Therefore, in order to use non-invasive source of stem cells with osteogenic potential without conferring morbidity to the bone donor area , we have used dental pulp stem cell (DPSC) obtained from deciduous teeth of CLP patients to make bone tissue engineering. The cells were obtained from deciduos dental pulp using previously described pre-plating technique. These cells, through flow cytometry analysis, were mainly positively marked for five mesenchymal stem cell antigens (CD29, CD90, CD105, CD73,CD166), while negative for hematopoietic (CD45) and endothelial cell marker(CD31). After induction under appropriate cell culture conditions, these DPSC were capable to undergo chondrogenic, adipogenic and osteogenic cell differentiation, as evidenced by immunohistochemistry. We also demonstrated that DPSC together with a biomaterial composed of collagen and hydroxyapatite lead to bone tissue reconstruction in alveolar cleft defect of medium size animals (mini pig). In conclusion, we showed that DPSC of Cleft Lip and palate patients have phenotypic and behavior characteristics similar to other adult stem cells in concern to bone tissue engineering, both in vitro and in vivo. Our findings suggest that DPSC obtained from celft lip and palate patients represent a promising source of stem cells for alveolar bone grafting treatment (bone tissue engineering), particularly in young CLP patients. Recently we obtained ethical permission to start the clinical trials using DPSC to perform alveolar bone tissue engineering for cleft lip and palate patients open new avenues to perform the treatment to these patients.

W-1453

CHROMATIN REMODELING PROTEINS SMYD1 AND SMARCD1 FOR FACILITATED MATURATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED VENTRICULAR CARDIOMYOCYTES AND THEIR ENGINEERED TISSUES

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Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) can self-renew and differentiate into ventricular (V) cardiomyocytes (CMs), serving as an unlimited source for heart disease modeling, drug screening and regeneration. However, hESC/iPSC-VCMs are known to display immature structural and functional traits. We recently reported that the epigenetic state of hESC-VCMs is dynamic and primed to promote growth and development, but proper environmental stimuli with chromatin remodeling might be required to synergistically trigger maturation. In this study, we investigated the consequences of applying pro-maturation signals in a 3D niche with forced expression and knockdown of two chromatin remodeling proteins that have been implicated in transcriptional control. From a scale-free co-expression network analysis, we identified two chromatin remodeling proteins that are differentially expressed in undifferentiated hESCs, hESC-, fetal (F)- and adult (A)-VCMs. While SMYD1 is one of the most abundant transcripts in F- and A-VCMs, but not hESCs, SMARCD1 displayed the opposite expression pattern. Using lentiviral (LV)-mediated over-expression and shRNA knockdown, we modulated the expression levels of SMYD1 and SMARCD1 in hESC-VCMs. While both over-expression of SMYD1 and suppression of SMARCD1 led to changes of several genes associated with CM functions, the electrophysiological properties of the CMs were unaltered. Interestingly, in a unique 3D system of human ventricular cardiac micro-tissues (hvCMT) that we recently developed and reported, either forced expression of

SMYD1 alone or knockdown of SMARCD1 alone in hESC-VCMs led to increased contractile forces along with significant up-regulation of genes encoding for cardiac contractile proteins, Ca2+-handing proteins, and cardiac ion channels. Furthermore, hvCMTs generated from hESC-VCMs with simultaneous over-expression SMYD1 and sh-SMARCD1 knockdown displayed an even more robust contraction, indicative of a synergistic effect. This study presents a novel idea that chromatin remodeling and transcriptional regulation are dynamic, and both biological and physical cues are important in promoting growth and maturation of hESC-VCMs to a more adult-like phenotype.

W-1454

OSTEOGENIC RESPONSE OF HUMAN IPSC-DERIVED MESENCHYMAL CELLS TO MACROPOROUS CALCIUM PHOSPHATE SCAFFOLDS

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Treatment of large bone defects remains a clinical challenge worldwide. Approaches of bone engineering using human induced pluripotent stem cells (iPSC) and compliant biomaterial scaffolds open the opportunities to grow therapeutically relevant grafts for personalized applications. Among others, calcium phosphate cements create a stable bond with the host bone, are biocompatible, bioresorbable and osteotransductive (replaced by new bone tissue), thus representing suitable biomaterials for the construction of porous scaffolds for optimal skeletal reconstructions. The objective of this study was to engineer bone cement scaffolds and study their potential to support attachment, proliferation and osteogenic differentiation of human iPSC-derived mesenchymal progenitors. Macroporous synthetic cement scaffolds (8 mm Ø x 3 mm height) were fabricated using a dissolving phase approach by mixing monocalcium phosphate monohydrate and β-tricalcium phosphate with poly(ethylene glycol) particles of different size (100-600 μm) in distilled water. Fabricated scaffolds were then characterized for chemical composition, porosity and mechanical properties and select groups used for cell seeding and differentiation studies. Human iPSC lines were expanded and induced into the mesenchymal lineage for 7 days, and then cultured on bone cement scaffolds for 7 weeks. Decellularized bone scaffolds and bone marrow-derived stem cells were used as control for all experiments. Cell viability and proliferation, and osteogenic differentiation and tissue formation were investigated along the experimental period. Results demonstrate that functional and highly proliferative osteocompetent cells can be derived from iPSC lines, and their culture on bone cement scaffolds result in the increased expression of a large set of markers associated with osteogenesis and bone development, and the formation of bone-like tissue. The use of progenitor cells derived from iPSC lines in combination with clinically-compliant biomaterial scaffolds open the possibility to engineer unlimited amount of bone grafts for personalized treatments of the skeletal system.

W-1455

TRANSPLANTATION OF ENDOMETRIAL STEM CELLS WITH ARRESTED PROLIFERATION AFFECTS DECIDUA FORMATION IN PSEUDOPREGNANT RATS

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Decidualization of endometrium is an essential process for embryo implantation, placenta forming and maintenance of pregnancy. On all terms of pregnancy, insolvency of decidual reaction of endometrial cells is one of the reasons of miscarriages and fetal growth delay. The insufficient decidualization of endometrium causes infertility at such pathologies, as Asherman's syndrome and an endometrium atrophy. Earlier we showed the stimulating effect of the mesenchymal stem cells derived from human endometrium (eMSCs) for decidualization processes on the model of pseudopregnancy in rats. Pseudopregnancy exhibits many features of early pregnancy: presence of large functional corpora lutea, development of the mammary glands and progestational changes in the uterine mucosa that makes it susceptible to deciduomata formation. Injection of human eMSC into uterine lumen of pseudopregnant rats stimulates development of all decidual tissue elements compared to PBS injection in the control uterine horn. Cell therapy frequently requires substantial cell biomass. MSC content in the initial tissue usually is very low. Their accumulation may be reached by expansion of the cells in culture. However, cultivation of somatic cells may be accompanied by significant changes of their properties, including malignant transformation. In this work we tried to study the effect of the eMSCs with arrested cell proliferation for decidualization processes on model of pseudopregnancy in rats. eMSCs proliferation was blocked by mitomycin C treatment and exposure to ionizing radiation. Our study shows that human eMSC with arrested cell proliferation injected into uterine lumen of pseudopregnant rats promote development of decidua compared to PBS or ethanol-fixed eMSC. Transplantation of rat bone marrow cells gives similar result that excludes xenogenic effect of human eMSC. In conclusion, in rat model transplantation of eMSC induces development of all elements of the decidual tissue. Arrested proliferation of transplanted cells secure tumorigenesis development. Altogether, our results open the possibility of infertility treatment.

W-1456

IMMUNOSUPPRESSION EFFECT OF FETAL HUMAN PLACENTA MSCS ON TISSUE ENGINEERING SKIN TRANSPLANTATION IN MURINE MODEL

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In our previous study, we had found the number of CD200 positive placental mesenchymal stem cells of fetal origin (fPMSCs) was higher than that of maternal origin. To explore the immunosuppression effect of fPMSCs on skin allogeneic transplantation and its underlying mechanism, the tissue engineering skin constructs were generated

in vitro and transplanted onto mouse model of skin damage. Human skin keratinocytes were seeded on top of collagen I gels and cultured in a submerged state for 4 days and then at the air-liquid interface for 12 days for construction of skingrafts. fPMSCs were isolated from human placentas abutted amniotic membrane. The expressions of MSC cell surface markers were detected by flow cytometry. Sixty C57BL/6 mice were random divided into 3different treatment groups (PBS, fPMSCs, fPMSCs+Anti-CD200) after establishing the tissue engineering skin allogeneic transplantation model, the treatments were applied to different groups respectively by tail intravenous injection. In the fPMSCs+Anti-CD200 group, fPMSCs were neutralized with anti-CD200 monoclonal antibodies. The general condition and the survival time of the transplanted skin grafts were observed daily after surgery. Peripheral blood was collected at 7 days post transplantation, T cells subsets were counted by FACS analysis and immune cytokines wereanalyzed by ELISA. Skin grafts where excised under anesthetic condition when there was visual evidence of inflammation. The observation of skin xenograft survival time revealed significantly longer in fPMSCs than that in PBS and fPMSCs+Anti-CD200 groups ($P<0.05$); The levels of blood IL-17, IFN-γ, TNF-α and IL-22as well as the number of CD4 and CD8positive T cells were significantly reduced in fPMSCs group mice. Immunohistochemical staining of CD8 and CD4 confirmed that the xenografts had a diffuse lymphocytic infiltration of the epidermis and dermis while there was no evidence of an infiltrate in fPMSCs transplanted mice at the same time. The immunosuppression effect of fPMSCs on immune rejection of skin transplants was significant. These therapeutic potentials were at least in part due to the differences in CD200expression on MSCs. Thus, fPMSCs may be a suitable choice for immunosuppression on tissue engineering skin transplantation.

W-1457

A 3 DIMENSIONAL GRAFT FROM HUMAN AUTOLOGOUS ADIPOSE-DERIVED STEM CELLS FOR BONE DEFECT RE-CONSTRUCTION

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Critical size bone reconstruction using stem cells remains limited by the large size of the bone defect. We assessed the capacity of human adipose-derived stem cells (ASC) to produce, in vitro, a scaffold-free osteogenic 3D graft. The feasibility and safety of autologous 3D graft was studied to cure a bone non-union in extreme clinical and pathophysiological conditions. ASC were isolated (from human subcutaneous adipose tissue, n=10, 1.2-9.1 g)/expanded up to passage 4 to be finally incubated in osteogenic media with various concentrations (0, 1, 5, 10 mg/mL) of Demineralized Bone Matrix to determine (DBM): volumetric bone mineral density (quantitative computed tomography, X-ray microtomography); histomorphometry for osteocalcin expression and mineralization; BMP-2 content and the in vivo osteogenic efficacy (nude rats, n=20). When the optimal dose of DBM was selected, the 3D graft was transplanted in 6 patients (bone tumour n=3, bone pseudarthrosis, n=3) to follow the clinical safety and efficacy. Genetic stability was studied on ASC at both proliferation/differentiation phases before graft release. The 3D graft (only obtained with DBM 10 mg/ml as confirmed by X-ray) demonstrated a significant higher expression of osteocalcin cells (+ 456%, $p<0.005$)/positive-von Kossa area (+ 30%, $p<0.005$)/ mineralization (according to pQCT, +470%, $p<0.005$) and in vivo osteognicity at 30 days post-implantation in comparison to osteogenic ASC (alone or with DBM at 1-5 mg/ml). No native tumour anomalies (several clonal cytogenetic alterations) were found prior/after osteogenic differentiation of ASC. Although ASC expansion can induce aneuploidies, such as tri-/tetraploidies or clonal trisomy 7 in 6-20% of cells, the osteogenic differentiation significantly reduced these clonal anomalies. No acute (<3 months) or long-term side effects, such as tumour development, were associated with the graft up to a maximum of 3 years post-transplantation. Rapid bone consolidation was demonstrated after 3 months without reversibility up to 36 months. We demonstrated that autologous ASC can be fully differentiated into a scaffold-free 3D and can safely promote osteogenesis in extreme conditions of bone non-unions, leading to restoration of bone anatomy and function, with no donor site morbidity and no oncological side effects.

W-1458

A CUSTOMIZED 3D MYOCARD MODEL ENGINEERED BY SEEDING HUMAN IPS-DERIVED CARDIOMYOCYTES ON SMALL INTESTINAL SUBMUCOSA SCAFFOLDS

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In spite of recent advances in medicine cardiovascular disorders remain major causes of mortality in the world. A main shortcoming of innovative therapeutic concepts represents the lack of ideally autologous cell sources and robust functional integration into the host tissue and the vascularization of the implant. Induced pluripotent stem (iPS) cells might serve as an unrestricted cellular source of patient-specific cardiac cells for future regenerative applications. Here we used a protocol of robust cardiac differentiation of human iPS cells by systematically modulating BMP and WNT signaling and metabolic selection by lactate enrichment. We show efficient derivation of beating cardiomyocytes from multiple iPS lines. In particular we demonstrate cardiomyocyte differentiation within 15 days with an efficiency of up to 95 %. hiPS-derived cardiomyocytes (iPS-CM) were functionally validated by alpha-actinin staining, transmission electron microscopy as well as electrophysiological analysis. In order to explore the possibility to derive iPS-CM-based cardiac 3D tissues we seeded a single cell suspension of 106 iPS-CM cells onto a decellularized 1cm2 small intestinal submucosa scaffolds patch. To assess the potential beneficial impact of other cells on survival, integration and cellular function of seeded iPS-CM we co-cultured iPS-CM with mesenchymal stem cells (MSC) and fibroblasts (derived from a human heart biopsy) as well. 2 days after seeding we observed recovery of spontaneous beating in various regions on the patches. 3 days later the whole patch exhibited synchronous beating. Hematoxylin and eosin staining (HE) shows agglomerates in the mono-culture whereas the cells are well distributed all over the surface of the matrix in the co culture. In both culture conditions the expression of the cardiac specific markers cardiac Troponin T, cardiac Troponin C and MF20 can be confirmed by immunhistological staining. Moreover, we show that engineered tissue patches responded to β-adrenergic stimulation with changes in the beating rate upon β-adrenergic agonist (isoproterenol) and antagonist

(propranolol) treatment. We expect our 3D cardiac model to provide a robust basis for personalized cardiotoxicity studies, disease modeling as well as cardiac cell replacement therapies.

W-1459

IN VITRO METABOLIC ZONATION THROUGH OXYGEN GRADIENT ON A CHIP

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Developing new strategies for mimicking early organogenesis has a high scientific relevance and therapeutic potential. The role of oxygen tension as a key regulatory mechanism has not been yet well described in hepatic differentiation. The aim is to recapitulate early in vitro organogenesis in physiological conditions to efficiently derive mature hepatic cells from human pluripotent stem cells under a stable oxygen gradient, and to integrate the specific lineages into a microfluidic platform to obtain a functional liver tissue on a chip. Human embryonic stem cells and human induced pluripotent stem cells were expanded and differentiated on matrigel-coated microchannels. We developed a multi-stage microfluidic technology to derive mature cells from pluripotent cells. Microfluidic technology was implemented with 1 mm width oxygen gradient generation and stabilization. Obtained cells have been characterized both with hepatic markers (alpha-fetoprotein, cytokeratins 18, albumin, CYP3A) and functional tests (proliferation, glycogen storage, indocyanine green uptake, albumin secretion). We were able to generate a stable microfluidic O2 gradient during culture and differentiation of human pluripotent cells. Hepatocyte-like cells were obtained over a 14-day period, showing high CYP3A expression, indocyanine uptake, glycogen storage and albumin secretion. At day 8 of differentiation it resulted a higher expression of AFP in the oxygenated side of the channel (21% O2), compared to lower expression in the deoxygenated side (<5% O2). At day 15 of differentiation, glycogen resulted more abundant in the oxygenated side (corresponding to in vivo zone I hepatocytes) compared to lower glycogen storage in deoxygenated side (corresponding to in vivo zone III hepatocytes). In the control cells, cultured with no O2 gradient, glycogen storage was more homogenous. The engineerization of pluripotent cell differentiation into hepatic lineages under defined oxygen gradient will allow us to further understand the mechanisms involved in tissue development. Hypoxic condition and mitochondrial function enhancement can define successful hepatocyte generation from both human embryonic and human induced pluripotent stem cells.

W-1460

INTERPLAY OF MATRIX STIFFNESS AND PROTEIN TETHERING IN MESENCHYMAL STEM CELL DIFFERENTIATION

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Stem cells regulate their fate by binding to, and contracting against, the extracellular matrix. Recently, it has been proposed that in addition to matrix stiffness and ligand type, the degree of coupling of fibrous protein to the surface of the underlying substrate, that is, tethering and matrix porosity, also regulates stem cell differentiation. By modulating substrate porosity without altering stiffness in polyacrylamide gels, we show that varying substrate porosity did not significantly change protein tethering, substrate deformations, or the osteogenic and adipogenic differentiation of human adipose-derived stromal cells and marrow-derived mesenchymal stromal cells. Varying protein-substrate linker density up to 50-fold changed tethering, but did not affect osteogenesis, adipogenes is, surface-protein unfolding or underlying substrate deformations. Differentiation was also unaffected by the absence of protein tethering. Our findings imply that the stiffness of planar matrices regulates stem cell differentiation independently of protein tethering and porosity.

W-1461

IMMORTALIZATION AND CHARACTERIZATION OF MOUSE DENTAL PAPILLA CELLS

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Mouse dental papilla cells (MDPCs) can be differentiated into component cell types that have the ability to regenerate the pulpo-dentinal complex. At the cap stage of tooth development MDPC component cells are programmed for tooth generation. Therefore, isolated and cultivated component cells can be useful in evaluating cellular, molecular, and environmental scenarios in tooth germ formation and tooth morphogenesis. Since the programmable stage for whole organ regeneration is confined to the brief moment of the cap stage, such cells have a short lifespan in the laboratory. Increase the proliferation capacity of MDPCs and preserve its original phenotypic and genotypic characteristics for tooth organ regeneration.An immortalized mouse dental papilla cell line was created via intracellular insertion of SV40T antigens into the nucleus by lentivirus particles. The generated clonally-isolated Sv40T immortalized MDPC line was then characterised and validated for transfection success and efficiency. Detection of SV 40T cellular expression was carried out using western blotting and RT-PCR protocols. To test for multi potency the cells were forcibly induced into adipogenic, osteogenic and chondrogenic lineages. The immortalized MDPC line showed clear expression of SV40T antigen both in western blotting and RT-PCR experiments. These cells displayed a higher proliferation rate and both genotype and phenotype characteristics were similar to the original primary cell line. This was highlighted via expression of a broad array of tooth specific markers. Furthermore, the test results to show transformed cells preserved multi potency were also positive. Immortalised cells were inducted into an adipogenic phenotype, as shown by production of lipid globules and an osteogenic phenotype, by forming mineralized nodules. However, the osteogenic marker, ALP was expressed by induced cells from 2 weeks following induction. Established, stable immortalized MDPCs may be used to determine the mechanisms of an array of developmental phenomena such as, early dental cell proliferation, reconstitution of tooth germ, dentine mineralization and other significant growth factor signaling pathways influencing tooth morphogenesis.

W-1462

ESTABLISHING THREE DIMENSIONAL VASCULARIZED EPITHELIUM FROM HUMAN EMBRYONIC STEM CELLS

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Human epidermis acts a barrier between deeper tissues and environmental agents. Numerous studies have shown the interaction of human epithelium with various environmental factors. These studies have either used models of in vivo (live animals) or in vitro (immortalized cell lines) of either animal or human origin, which often poorly reflect human physiology. Primary human cells are difficult to procure in sufficient quantity and are prone to loss of functionality and inter-donor/batch variability. Additionally, regulatory authorities, academics, research institutes and health, food, pharmaceutical and cosmetic industries are presently hindered by the lack of functional, healthy and standardized human platforms, and predominantly use costly live animal models in addition to the cells of low relevance. Self-renewable, healthy and single-sourced human embryonic stem cells (hESCs) exhibit pluripotentcy, biological relevance, stable reliability and predictability. Hereby, we propose to differentiate hESCs into keratinocytes, endothelial cells, vascular smooth muscle cells and fibroblasts, and subsequently assemble the various cell types to form a vascularized epithelium. In this study, we have developed a highly-reproducible, stabilized and reliable methodology of culturing hESC-derived cells in a polyethylene glycol (PEG)-fibrin based scaffold and air-lifting the organotypic cultures for 2-3 weeks to form three-dimensional (3-D) vascularized and multi-layered stratified epithelium (3D-in-vitro HVE) under serum-free conditions. This standardized live and functional human skin model manifests great demand in academia and pharmaceutical industries, especially in drug discovery, and in toxicity testing. Additionally, our proposed model 3D-in-vitro HVE being the world's first in-vitro model derived from single-sourced hESCs has potential applications in tumour biology modelling, high throughput screening, and pre-clinical studies.

W-1463

A BIOACTIVE POLY(EPSILON-CAPROLACTONE) MEMBRANE COMPRISING CRYPTIC LAMININ INFORMATION AS A STRATEGY TO COUNTERACT TGF-BETA-1-INDUCED TISSUE FIBROSIS

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Therapeutic intervention to modulate the epithelial-to-mesenchymal transition (EMT) has been proposed as a promising strategy for the treatment of tissue fibrosis. Irreversible EMT as a hallmark of fibrogenesis leads to the accumulation of collagen producing myofibroblasts and a loss of epithelial homeostasis eventually impairing tissue function. The differentiation towards a migratory phenotype is associated with the expression of matrix metalloproteinases that degrade the underlying basement membrane. This process releases extracellular matrix (ECM) fragments that can interact with cell receptors to trigger changes in EMT-related pathways. Here, we propose a biomaterial-based strategy to interface such a "cryptic" laminin-111 fragment with epithelial tissue and mitigate the effects of EMT resulting from chronic inflammation. The recombinantly produced laminin-111 fragment β1-Ln-Le1-4 is immobilized on a poly(ε-caprolactone) (PCL) membrane via a polydopamine coating to increase the immobilization density. This bioactive membrane is interfaced with mouse mammary gland (NMuMG) epithelial cells - a well-established model for the study of EMT processes in vitro - and EMT is induced by transforming growth factor β1 (TGFβ1), to mimic post-inflammation epithelium. The functionalization of the membrane with the β1-Ln-Le1-4 fragment is shown to decrease the expression and activity of matrix metalloproteinase-2 (MMP2), a protease significantly up-regulated in fibrotic tissue and implicated in the degradation of the basement membrane. Additionally, it down-regulates the expression of a transcriptional inhibitor of E-cadherin (a key epithelial adhesion molecule), snail1. Targeting MMPs as an antifibrotic strategy has faced challenges thus far, because of the lack of specificity and has shown poor clinical outcomes due to off-target effects. Here, we propose to use a localized approach, where the bioactive β1-Ln-Le1-4 fragment of laminin-111 is directly interfaced with epithelial cells and can act on basement membrane remodeling and on the EMT directly by down-regulating the expression of MMP2 and snail1 and thereby decreasing the number of epithelial cells that undergo a change in phenotype and motility.

W-1464

TRANSIENT JUNCTION ANISOTROPIES ADJUST 3-DIMENSIONAL CELL POLARIZATION TO TISSUE GEOMETRY

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In contrast to cell sheets in vitro, the 3-dimensional (3D) shapes of tissues display characteristic anisotropic physical parameters. Current research in bioengineering and organ regeneration aims to identify the mechanisms by which the 3D tissue topography influences the biochemical activities of the constituting cells. Tubular organs exhibit a striking orientation of landmarks according to the physical anisotropy of the 3D shape, in addition to planar cell polarization. Here, we identify a regulatory network polarizing cellular biochemistry according to the physical anisotropy of the 3D tube geometry (tube cell polarization, TCP) by a genome-wide, tissue-specific RNAi screen. During Drosophila airway remodeling, each apical cellular junction is equipotent to establish perpendicular actomyosin cables, irrespective of the longitudinal or transverse tube axis. A dynamic transverse enrichment of aPKC shifts the balance and transiently targets activated small GTPase RhoA, myosin phosphorylation and Rab11-vesicle trafficking to longitudinal junctions. We propose that the PAR complex translates tube physical anisotropy into longitudinal junctional anisotropy, where cell-cell communication aligns the contractile cytoskeleton of neighboring cells. Our annular-TCP analysis provides a regulatory model on how 3D organ topography changes cell behavior during organ remodeling.

W-1465

MECHANISMS UNDERLYING ENHANCED CONTRACTILITY AND ISCHEMIC RESISTANCE OF 3D HUMAN HEART MUSCLES ENGINEERED FROM PLURIPOTENT STEM CELLS MATURED BY TRIIODOTHYROXINE AND NON-CELL AUTONOMOUS NICHE

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It is generally accepted that human pluripotent stem cell (hPSC)-derived cardiomyocytes (CMs) are structurally and functionally immature with fetal-like phenotypes. Such can be attributed to their conventional 2D culturing and experiments as single cells. In normal cardiac development, CM maturation involves various perinatal developmental stimuli acting in concert in a 3D microenvironment. Herein, we investigate the pro-maturation effects of these stimuli, in particular triiodothyroxine (T3), an important hormone in perinatal cardiac development, in engineered 3D tissues of hPSC-CMs. hPSC (hES2, in-house iPSC)-derived ventricular (V) CMs were differentiated using a highly efficient V specific protocol as we recently reported. For creating a 3D niche, ~10^3 and 10^6 hPSC-VCMs (≥70% $cTNT^+MLC2v^+$) were mixed with a collagen-based extracellular matrix in our custom designed bioreactors to construct human ventricular cardiac microtissues (hvCMT, ~0.4 mm in length) and tissue strips (hvCTS, ~1-cm), respectively. Compared to 2D cultures, qPCR analysis showed increased expression of cardiac-specific genes such as MHC-α and MHC-β in hvCMTs and even more so in hvCTSs. When exposed to T3 for 6 days, the developed tensions in hvCMTs and hvCTSs, as deduced from optically-tracked movements of the pillars between where the tissues were suspended, enhanced by up to 3-fold in comparison to their controls. Mechanistically, Ca^{2+} transient properties significantly matured, with increased expression of key Ca^{2+} handling genes such as RYR2 and SERCA2a. Interestingly, mitochondrial ATP-sensitive potassium (mKATP) channels, a known crucial player in cardioprotection robustly expressed in adult but totally absent in hPSC-VCMs, were upregulated by T3. In T3-treated hvCMTs, the mKATP opener diazoxide significantly improved tissue survival during simulated ischemia/reperfusion (I/R) by hypoxia (1%O_2)/ reoxygenation as evidenced by the increased developed tension when compared to diazoxide-free controls. We conclude that T3 and 3D niche enhance the functionality and survival during I/R insult of hPSC-VCMs and their engineered tissues by improving Ca^{2+} handling and acting on mKATP. Such results have implications for improving both the efficacy and engraftment of future transplantation constructs.

W-1466

ENHANCING NEUROGENESIS AND ANGIOGENESIS WITH TARGETED DELIVERY OF STROMAL CELL-DERIVED FACTOR-1 ALPHA BY USING A PH-SENSITIVE POLYMER IN A RAT STROKE MODEL

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Stromal cell derived factor-1 alpha (SDF-1α) is small cytokine belong to the chemokine family that is increased after cerebral ischemia in the ischemic border zone. According to previous reports, SDF-1/CXCR4 axis is induced adult neurogenesis, angiogenesis and bone marrow-derived stem cells homing to maintain homeostasis in the brain from ischemic stroke. The pH-sensitive polymer is synthetic macromolecule with the potential for acidic condition targeted drug delivery. In present study, we investigated regeneration therapeutic potential of SDF-1α in transient middle cerebral artery (MCA) occlusion animal models and conducted studies about effective delivery of SDF-1α toward ischemic region using pH-sensitive polymer. Firstly, we performed that SDF-1α were stereotaxically injected into striatum at 24 hours after operation, and verified effect of neurogenesis, angiogenesis and cell survival. Secondly, ischemic region-targeted delivery effect of pH-sensitive polymer was identified in transient and permanent MCA occlusion model using lysozyme (alternative protein) and near infrared fluorescence (NIRF) imaging. Lastly, SDF-1α-Cy5.5 loaded pH-sensitive polymer was systemically administered at 3 hours after permanent MCA occlusion. As a result, we observed that SDF-1α increased BrdU/DCX double positive cells in subventricular zone (SVZ) and Von Willebrand factor (vWF) positive microvessels in ischemic border zone (IBZ) at 7 days after transient MCA occlusion. Ischemic regiontargeted delivery effect was better in permanent model than transient, and that effect was further improved in SDF-1α. In present study, we demonstrated that SDF-1α could facilitate brain remodeling after ischemic stroke. Furthermore, ischemic stroke therapy could be maximized by pH-sensitive polymer.

W-1467

TONSIL DERIVED STEM CELL: NEW CANDIDATE FOR HUMAN STEM CELL SOURCE

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Stem cell serves as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person is still alive. Recently stem cells are getting the spotlight in not only the cell therapy research but also almost field of medicine. The source of stem cell is roughly divided into the embryonic stem cells (ESCs) and the adult stem cells (ASCs). ESCs are derived from embryos. Therefore there are ethical problems with getting and researching ESCs. ASCs have several advantages. Firstly they are free from an ethical issue. Secondly the sources of ASCs are various. ASCs have been isolated from a number of adult tissues. However, mentioned ASCs have some drawbacks; donor morbidities, low cell yields, and

limitation of differentiation property. The tonsils are lymphoid tissues located in the pharynx. Tonsillar epithelium is derived from the second pharyngeal pouch (of endodermal origin) and during fetal development is invaded by lymphoid tissue (of mesodermal origin). Therefore, embryologically, tonsils could be a source of MPCs. Our results show that MPCs exist in the stroma of palatine tonsils and can be isolated and expanded in culture. These tonsil-derived stem cells (TSC) show multipotent differentiation properties and share similar immunosuppressive characteristics as BM-MPCs in mixed lymphocyte reaction. TSC differentiated into parathyroid tissue, one of the endocrine organ. Differentiated parathyroid tissue secreted parathyroid hormone and it was regulated by external cellular calcium level.

W-1468

3D BIOSCAFFOLD BASED RE-ENGINEERING OF THE HUMAN STEM CELL DERIVED-RETINA

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Current investigations into gene, cell, and pharmaceutical therapies to treat retinal degenerations are limited by fundamental gaps in our understanding of the establishment, maintenance, and deterioration of retinal architecture during the degeneration process. The advent of human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC) technology promises the possibility of screening patient-specific retinal cells in culture; however, the creation of valid disease models for many of these retinal degenerations requires highly differentiated aspects of the retina be recapitulated in vitro. These characteristics require an organized 3-dimensional (3D) state as in the eye. The work presented here tests this hypothesis. First, we tested the optimal well diameters needed to promote columnar retinal lamination as in the eye instead of random cell alignments. We micro-fabricated microwells with well diameters ranging from 20-40um. Upon culturing human iPSC-derived retinal cells over the micro-fabricated scaffolds, we found that well diameters ranging from 20-25um was optimal for the differentiated cells to migrate and align along the wall while wider diameters promoted random cell assembly. Next, we focused on microfabrication techniques to generate high-aspect ratio microwells which was a significant challenge. We developed specialized approaches for SU-8 resist photolithography and by optimizing UV exposure time, refractive index matching, and photomask pattern dimensions. Using these techniques, we obtained microwells approximately 20 µm in diameter and 115 µm in depth using either poly (lactic-co-glycolic acid) (PLGA) and poly-capro-lactone (PCL). Using these stereotypical scaffolds, we have been able to show hiPSC-derived retinal cells can be cultured in a 3D context to allow for lamination and morphological maturation. In summary, 3D contextual information is critical of cellular maturation and providing such information will be key for understanding tissue development and for creating accurate disease models.

W-1469

STEM CELL-DERIVED MATRICES PROVIDE IMPROVED MAINTENANCE OF HMSC CHARACTERISTICS IN VITRO

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Adult stem cells from bone marrow stroma (hMSCs) are isolated by their adherence to tissue-culture treated plastic (polystyrene) and passaged multiple times on the same plastics until they are able to produce enough cells to be useful for research or clinical therapeutic trials. These stem cells that reside in the bone marrow have the capacity to persist throughout the normal lifespan of the individual, cycling through proliferation and quiescence, as needed. Unlike their microenvironments in vivo, there has been an increasing body of evidence to suggest that an ex vivo, artificial plastic environment may cause accumulating damage to the hMSCs and limits their utility to a finite number of passages after their initial isolation from bone marrow. Our aim is to gain a better understanding of how these environmental factors can negatively affect adult stem cell behavior during ex vivo expansion. We used a system to examine the hMSCs' response to plastic (synthetic) versus hMSC-derived (natural) matrix. Each time we passaged the hMSCs, the cells were required to repopulate the synthetic polymer surface with natural proteins like Fibronectin, Vimentin, Collagen I, VI, and XII before they were able to begin their normal processes of division and motility. This is partially responsible for the cells' initial lag phase of growth that was observed here as well as in other reports. The stem cells grown on plastic were less able to proliferate and differentiate into the three mesenchymal lineages of bone, fat, and cartilage. Reducing this environmental stress on the cells resulted in smaller cells that showed improvements in markers for early, less mature stem cells. These desirable traits were also maintained in cells serially cultured on the natural matrix. In addition, we found evidence that the natural matrix supported hMSCs that could be more effective in repairing critical sized calvarial defects in nude rats. Taken together, the data suggest an alternative strategy for the maintenance of hMSCs under decreased stress, with increased proliferation, and improved in vivo performance. These studies add to our understanding of how different types of growth substrates can provide signals to hMSCs in vitro and offer pathways to manipulate these inputs with the ultimate goal of benefitting hMSC efficacy in clinical cell therapies.

W-1470

MESENCHYMAL STEM CELLS PROTECT CO-GRAFT IN KIDNEY CAPSULE AGAINST IMMUNOREJECTION BY IMMUNOSUPPRESSIVE FACTORS TGF-BETA, IL-10, AND GALECTIN-3 SECRETION

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Mesenchymal stem cells (MSCs) represent a promising tool for new clinical concepts in repair and regeneration of tissue. MSCs are used as seed cells for nature-based tissue engineered biomaterials

or transplanted to the lesion site for cell therapy. However, the high demand and the lackness of MSCs limit the clinical use of MSCs. Endothelial cells are important element of blood vessels. To study the relationship between MSCs and biocompatibility, we co-transplanted MSCs derived from induced pluripotent cell (iPS-MSCs) and human umbilical vein endothelial cells (HUVECs) into the kidney capsule of C57BL/6 mice as a vessel graft model in vivo. We found that the graft iPS-MSCs group showed lower level of lymphocytes infiltration than graft HUVECs group and co-transplanted group. We revealed that iPS-MSCs reduced the proportion of IFN-γ+ and IL4+ T cells, inhibited the proliferation of splenocytes and T cells, down-regulated IL-2, IL-4 and IFN-γ mRNA level and up-regulating TGF-β mRNA level both in vivo and ex vivo. Furthermore, HUVECs had trophic effects on promoting iPS-MSCs self-renewal partly via FAM3B secretion, but no similar effect was observed in contrast. To further illustrate the mechanism of immunosuppressive effect of iPS-MSCs, RayBio® human label-based antibody array was performed to analyze the cytokines secretion in the supernatant of HUVECs and iPS-MSCs culture. Comparing with the control group HUVECs, iPS-MSCs got an extremely strong ability to secrete 198 cytokines 2-fold than HUVECs supernatant, in which included a number of immunosuppressive factors like TGF-β, IL-10 and galectin-3. Conversely, HUVECs expressed only 32 cytokines 2 fold than iPS-MSCs supernatant. These works partly explain the mechanism of MSCs suppressing immunorejection and imply that co-seeding MSCs and HUVECs for tissue engineering blood vessel will be more stable than seeding a single one.

W-1471

NANOSCALE SPATIAL REGULATION OF NOTCH SIGNALING IN NEURAL STEM CELLS

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The canonical Notch signaling pathway is a conserved cell-cell communication system with fundamental roles in normal development, particularly in the development of the nervous system. We have previously shown that the spatial organization of membrane bound ligands is an important regulator of signaling mediated by Eph receptors. Here we explore the hypothesis that the spatial organization of Notch ligands contributes to fine-tuning canonical Notch signaling activity. We developed a DNA origami-based method that can be used to tailor the spatial distribution of protein assemblies. DNA origami is a nanofabrication technology that that uses DNA self-assembly to drive the precise formation of 3D nanostructures. This novel tool allows for display of well-defined protein nanoclusters in solution and is therefore uniquely suited for investigating the roles of the biophysical properties of ligand/receptor interactions on downstream signalling and cellular outcomes. To investigate the roles of spatial regulation of Notch signalling in neural stem cells, we treat Neuroepithelial stem like (NES) cells derived from induced pluripotent stem cells with DNA origami/Notch ligand nanoclusters with various spatial configurations. The resulting canonical Notch signaling activity at single cell resolution as well as cellular functional outcomes are analyzed. This work has the potential to provide new insights into the fundamental mechanisms of the roles of biophysical properties in Notch signaling activation during neural development.

W-1472

BLASTOCYST COMPLEMENTATION RESTORES APANCREATIC PHENOTYPE OF PDX1-/- PIGS

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We have demonstrated that overexpression of the *Pdx1-Hes1* transgene induced a pancreatogenesis-disabled phenotype in the pig. The present study aimed at (i) examining whether *PDX1* knockout (*PDX1-/-*) can induce apancreatic phenotype in the pig and (ii) whether the apancreatic phenotype can be restored by exogenous cells using blastocyst complementation. *PDX1* of the porcine fetal fibroblasts were homozygously knocked out using mRNA of transcription activator-like effector nucleases (TALENs) targeting the exon 1 of the gene. The cloned cells carrying induced mutations were selected using limiting dilution. The targeted portion of the *PDX1* gene was amplified using PCR, followed by sequencing and mutation analysis. Among the collected KO cell colonies, cells showing good proliferation and morphology were selected and used for somatic cell nuclear transfer (SCNT). SCNT was conducted as described in a previous study [1]. Cloned embryos were transplanted into the oviduct of estrus-synchronized recipient gilts. Efficiency of the homozygous KO was 4.6 % (5/109). Two lines of the PDX1$^{-/-}$ cells (1218 del / 61 del; 4bp del / 195 ins) were used for SCNT, and transfer of 101 SCNT embryos gave rise to 4 fetuses (day-56) showing apancreatic phenotype. Blastocyst complementation was performed as reported previously using the SCNT embryos derived from the PDX1$^{-/-}$ cells as the host embryos and normal cloned embryos expressing Kusabira-Orange as the donor. Transfer of 120 chimeric blastocysts to 2 recipients produced 3 full-term chimeric fetuses. Generation of normal pancreas was confirmed in the chimeric fetuses. These results demonstrate that PDX1$^{-/-}$ pigs exhibit apancreatic phenotype which can be restored by blastocyst complementation. This study was supported by JST, ERATO, Nakauchi Stem Cell and Organ Regeneration Project and MUIIBR.

W-1473

REGULATORY MECHANISMS OF PITUITARY STEM/PROGENITOR CELL SPECIFIC TRANSCRIPTION FACTOR PROP1

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The anterior pituitary lobe is a key endocrine tissue composed of five types of endocrine and non-endocrine cells. Among non-endocrine cells, SOX2-positive cells exist as pituitary stem/progenitor cells and play important roles in pituitary organogenesis and regeneration of endocrine cells. We have reported that a pituitary-specific transcription factor PROP1 (Prophet of PIT1) is present in SOX2-positive stem/progenitor cells in the pituitary primordium, Rathke's pouch, on rat embryonic day (E) 13.5, and consistently co-localizes with SOX2 throughout life. Moreover, during differentiation from stem/progenitor cells into endocrine cells, the Prop1-expression rapidly disappears before terminal differentiation, but mechanism of Prop1-regulation is not yet revealed. In the present study, to examine Prop1-regulation, we analyzed regulatory activity of various transcription factors using 5'-upstream region of Prop1 by reporter assay and DNA methylation pattern in the Prop1 locus. Reporter assay using 5 cell lines revealed that promoter activity of the 5'-upstream region of mouse Prop1 is cell-type specific and that SOX2 is able to regulate Prop1-expression. In addition, we found that 18 factors, which are expressed during early pituitary organogenesis, have SOX2-dependent or -independent regulatory activity on the Prop1-expression and that putative responsive elements of some factors are present within 50 base regions including 5 putative SOX2-binding elements in the 5'-upstream region of mouse Prop1. Next, we analyzed the DNA methylation status of Prop1 locus by bisulfite sequencing method using genomic DNAs prepared from rat pituitaries of E13.5, E14.5, postnatal day (P) 4 and P30, and the liver of P30 as a control. Although some Prop1 locus in pituitaries were more hypomethylated than that of liver, significant difference in pituitaries was not observed among all stages tested and between the anterior and intermediate/posterior lobes, indicating the presence of epigenetic modulation in tissue specificity. In consequence, we suggest that expression of Prop1 is regulated by combination of SOX2 and multiple transcription factors during early pituitary organogenesis and that subtle DNA methylation concern to tissue specificity.

W-1474

FUNCTIONALIZED ELECTROSPUN SCAFFOLDS FOR USE AS VASCULAR SUBSTITUTES

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In severe cases of peripheral arterial diseases, tissue loss can occur. In these situations, implantation of a vascular graft may be required. Scaffolds produced by electrospinning (ES) exhibit adequate properties for use as vascular substitutes. These scaffolds can be functionalized with specific molecules, such as anticoagulants and growth factors, to improve vessel regeneration. The aim of this study has been to evaluate the interaction between mesenchymal stem cells (MSCs) and scaffolds functionalized with heparin and vascular endothelial growth factor (VEGF). The scaffolds were produced by ES using poly(caprolactone) (PCL). The scaffolds were then hydrolyzed and immersed in 1% heparin solution (overnight). Following this, they were immersed in 1 μg/mL VEGF solution for 2h. The interaction between the scaffolds and the MSCs was analyzed for cellular adhesion and viability and cytotoxicity. Four groups of scaffolds were evaluated: (I) PCL, (II) hydrolyzed PCL, (III) hydrolyzed PCL, functionalized with heparin and (IV) hydrolyzed PCL, functionalized with heparin and VEGF. MSCs from dental pulp were seeded onto the scaffolds (5 x 105 cells/sample). To evaluate adhesion, the cells were stained with DAPI and counted. Cytotoxicity and cellular viability were evaluated through lactate dehydrogenase (LDH) enzyme dosage and WST8 test, respectively. All groups of the scaffolds were good substrates to sustain cellular adhesion. The number of adhered cells after 3h of cultivation was similar between the four groups. The LDH dosage was similar for all the groups, showing that no type of scaffold was toxic for MSCs. WST8 test showed that the number of viable cells increased over 15 days cultivation in all the samples. However, groups III and IV exhibited a higher number of cells than the other groups. The functionalization creates functional groups on the scaffold surfaces improving cell proliferation. These results demonstrate that the functionalization of the scaffolds can favor cellular development. In addition, these modifications assist with the success of the scaffolds as a vascular substitute. Heparin prevents thrombosis and VEGF makes the scaffolds more conducive for vasculogenisis. These functionalized scaffolds present great potential for application in vascular tissue engineering.

W-1475

CHARACTERIZING THE EXTRACELLULAR MATRIX PROTEOME TO UNDERSTAND ITS ROLE IN THE REGULATION OF STEM CELL FUNCTION

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The extracellular matrix (ECM) is a complex three-dimensional network of proteins and growth factors that provide stem cells with the appropriate environment and signals to regulate cell functions, such as proliferation, migration, differentiation and survival. 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 scaffolds for tissue engineering and regenerative medicine. However, precise characterization of the ECM remains challenging because of the biochemical intractability of its components, which are predominantly highly cross-linked and insoluble proteins. Further, the complexity and diversity of ECM components (e.g., core proteins, ECM-affiliated proteins, ECM regulators and secreted factors) adds to the difficulty of its characterization. To address these challenges, we have applied a whole proteome approach to characterize the molecular composition of cell-derived ECM produced by human mesenchymal stem cells (hMSCs). The proteomic method consists of a sequential digestion of the ECM followed by mass spectrometry and bioinformatic analyses. In parallel, we cultured different human cell types (hMSCs, human dermal fibroblasts, human cancer cells) on the cell-derived ECM and compared their proliferation or stemness to the same cells cultured on tissue culture polystyrene (TCPS). We observe that the ECM leads to differential response across the various cell types as compared to standard TCPS culture, indicating that the components of the ECM influence each cell type in a unique

manner. As an example, the ECM improved the growth of hMSCs compared to TCPS but decreased the proliferation of other cell types. By identifying the key components of the ECM, our approach will inform the design of defined systems that recapitulate critical ECM effects for therapeutic applications.

W-1476

TISSUE ENGINEERING OF HUMAN CORNEAL ENDOTHELIUM FOR FUNCTIONAL TRANSPLANTATION IN CLINICS

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We propose to obtain differentiated human corneal endothelial cells for engineering them towards developing therapies against the corneal endothelium disorders which affect vision in humans. We have isolated and selected an adult stem cell population from fat by our protocol that express linage specific corneal endothelial and stemness. we have examined multipotent differentiation capability of selected population of toward adipo-, osteo-, chondro-, and cornea endothelogenic directions. The adult stem cell population from fat express linage specific corneal endothelial and stemness *NGFR, E-CADHERIN, N-CADHERIN, BMI, NANOG, NESTIN, NOTCH, OCT4, SOX2, WNT1 and WNT4* cell markers. During cornea endothelogenic differentiation the selected stem cell population expressed some corneal endothelial cell specific gene markers related to gap and tight junctions such as *GJA1 (CONNEXIN43), TJP1(ZO1) and ATP1A1 (Na+/K+-ATPase)*. This adult stem cell population cab be used toward cornea endothelial differentiation.

W-1477

GROWTH OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS ON MECHANICALLY STIMULATED POLYURETHANE-BASED SCAFFOLDS HIGHLY INCREASES EXPRESSION OF CARDIOMYOGENIC-RELATED DIFFERENTIATION GENES

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A successful approach in tissue engineering medicine consists of a porous scaffold with mechanical properties similar to those of the native tissue, an easily available autologous cell source and a bioreactor to in vitro expand and eventually differentiate cells. A further step was taken toward the differentiation of adult human adipose-derived multipotent stem cells (ASCs) into cardiomyocytes by submitting differentiating ASCs to mechanical stimulation. For this purpose we developed a new bioreactor mimicking the physiological environment of heart cells. Cells were grown in a defined serum-free culture medium (Patent pending) seeded onto a biomimetic polyurethane-based scaffold and mounted in the bioreactor allowing the application of longitudinal mechanical stimulus to the scaffold. ASCs were subjected to mechanical stress in presence of a new cardiogenic serum-free differentiating medium (Patent pending) for up to 12 days and then analysed for the expression of cardiogenic-related genes. Scaffolds with the required structural properties were successfully produced via thermally induced phase separation. The PUR used in this study was synthesized starting from poly(ε-caprolactone) diol, 1,4-budandiisocyanate and L-lysine ethyl ester. The scaffolds were characterized by scanning electron microscopy (SEM), tensile tests, contact angle and fluid uptake tests. Durability tests were conducted to evaluate scaffold capability to withstand cyclic mechanical stress (0.4±0.2N, 1Hz) in the bioreactor. When seeded on the scaffold and run in the bioreactor, ASCs presented a high viability up to 15 days. Cell viability, morphology and cardiogenic differentiation were evaluated on cells in 2D culture conditions and on scaffolds under mechanical stimulation (3D-m), whereas gene expression were evaluated on hearth biopsies, 2D and 3D-m cultures. A 120-fold, 8-fold, 4-fold and 2-fold increase in mRNA expression of respectively NKX2.5, MEF2C, HAND2 and C-KIT were observed in comparison to non-induced cells.

W-1478

MESENCHYMAL STEM CELL SHEETS AS SCAFFOLD FREE CONSTRUCTS FOR CORNEAL SURFACE THERAPY

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Globally, corneal disorders are the fourth leading cause of blindness. In India alone, around 6.8 million are affected by blindness due to corneal surface damages. Corneal epithelium, is continuously replenished by limbal stem cells residing at the corneo-scleral junction. Limbal stem cell deficiency due to thermal or chemical burns, Stevens-Johnson syndrome etc., prevent the corneal epithelium from self repair. In such cases allogeneic or cadaveric tissues are the options currently in practice, with associated risks of immune suppression and graft rejection. Here we propose to use mesenchymal stem cells (MSC) from adipose tissue as an alternate autologous cell source instead of limbal stem cells. Adipose MSC collection is non-invasive and is an abundant source of stem cells. We have developed a technology to retrieve in vitro expanded MSC as a cell sheet for use in corneal surface reconstruction therapy. Poly-(N-isopropylacrylamide-co-glycidylmethacrylate) (NGMA) is an in house developed thermo-responsive polymer characterized for its Physico-chemical properties using FTIR, NMR and DSC. Utilizing the thermo responsive property of NGMA, a scaffold-free tissue construct was developed using adipose MSC's from rabbit models. Isolated cells were positive for MSC markers CD105, CD44 and CD90 (flow cytometry). The specific cytocompatability of NGMA was assayed using MSC's by cell proliferation (MTT assay), viability (FDA/PI), adhesion (scanning electron microscopy) and morphology (actin cytoskeleton staining). MSC's grown on NGMA was also shown to preserve the stem cells markers confirmed by immunocytochemistry. The presence of ABCG2 (limbal epithelial marker) in MSC indicated the trans differentiation potential to corneal linage. MSC's cultured with limbal stem cell condition medium showed the trans differentiation potential as evidenced by CK3/12 (corneal epithelial marker) staining and quantitative PCR. Identifying MSC's as an alternate autologous cell source along with cell sheet technology for scaffold free tissue transplantation, will be a novel step in the field of corneal reconstruction therapy. Moreover this technology once adopted will be a new ray of hope for patients with bilateral limbal stem cell deficiency.

W-1479

APPLICATION OF IPS TECHNOLOGY FOR NATURAL KILLER T CELL-MEDIATED TUMOR IMMUNOTHERAPY

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Invariant natural killer T (*i*NKT) cells are characterized by the expression of an invariant Vα14-Jα18 paired with Vβ8.2 in mice and Vα24-Jα18 paired with Vβ11 in humans, that recognizes glycolipids, such as α-galactosylceramide (α-GalCer), presented on the MHC class I-like molecule, CD1d. *i*NKT cells act as innate T lymphocytes and serve as a bridge between the innate and acquired immune systems. *i*NKT cells augment anti-tumor responses by producing IFNγ, which acts on NK cells to eliminate MHC-non-restricted (MHC^-) target tumor cells, and on $CD8^+$ cytotoxic T lymphocytes to directly kill MHC-restricted (MHC^+) tumor cells. Both of these tumor cell types are simultaneously present in cancer patients, and at present *i*NKT cells are only the cell type capable of eliminating them. Based on these findings, it had developed *i*NKT cell-targeted adjuvant immunotherapies with strong anti-tumor activity in humans. However, two-thirds of patients were ineligible for this therapy due to the limited numbers of *i*NKT cells in their bodies. In order to overcome this problem, we applied iPS technology to regenerate *i*NKT cells. In this study, we succeeded in establishing iPSCs from human *i*NKT cells (NKT-iPSCs) using Sendai virus vector. The Vα24-Jα18 paired with Vβ11 genomic rearrangement were confirmed by conventional PCR and direct sequencing. We succeeded NKT cell induction from NKT-iPSCs (iPS-NKT) by co-culture with OP9/OP9DLL1. Furthermore, iPS-NKT produced IFNγ and showed anti-tumor activity upon stimulation in vitro. Finally, we observed iPS-NKT mediated NK cell activation in vivo (adjuvant activity). We will discuss the characterization of iPS-NKT and the potential for iPS-NKT-mediated tumor immunotherapy.

W-1480

ACELLULAR HUMAN AMNIOTIC MEMBRANE AS A MATRIX FOR THE FUNCTIONAL THREE-DIMENSIONAL CULTURE OF HUMAN ADIPOSE STEM CELL DERIVED HEPATOCYTE-LIKE CELLS FOR TRANSPLANTATION

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Adult stem cell-derived hepatocyte transplantation holds considerable promise for future individual clinical therapy of liver failure or dysfunction. The efficient engraftment and survival of transplanted cells in the liver disease microenvironment are important for therapeutic effectiveness. In the present study, we report that human adipose stem cell-derived hepatocyte-like cells (hASC-HLCs) may form a functional three-dimensional (3D) hepatic construct on an acellular human amniotic membrane (AHAM) in vitro. To assess the effects of engraftment under injured liver conditions, the constructs were implanted into the livers of immunodeficient mouse hosts treated with carbon tetrachloride (CCL4). At 24 hours post-implantation, the degree of liver injury in the hASC-HLC-AHAM group significantly decreased compared with the AHAM group, and the grafts localized to site of the surgical incision in livers. Three days later, most of the hASC-HLCs formed a net structure between the AHAM and the recipients' livers and maintained the expression of the human hepatocyte-specific markers albumin and HNF4α. Two weeks later, the grafted cells generated cords that were similar to the ductal plates in the liver. In conclusion, AHAM may be a potential matrix for the functional 3D culture of hepatocytes. The hASC-HLC-AHAM graft transplantation corrected acute liver injury in a mouse model, highlighting the potential use of these grafts as a viable alternative for individual clinical therapy of liver disease.

REGENERATION MECHANISMS

W-1481

THE SYSTEMATIC REVIEW AND META-ANALYSIS OF STEM CELL TRANSPLANTATION IN EXPERIMENTAL MODELS OF ACUTE NEUROLOGICAL INJURY

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The purpose of this study was to assess the efficacy of stem cell therapy on the improvement of neurological function in two different acute neurological ischaemic diseases: stroke and (SCI). We have performed DerSimmonian and Laird random effects meta-analysis and meta-regression on the combined data for motor outcome in experimental models of stroke and SCI, currently held in the CAMARADES database. Combining both stroke and SCI datasets yielded 527 comparisons, involving 8640 animals where stem cell implantation improved motor outcome by 31.2% (95% CI 29.6 - 34%). There was greater improvement in models of ischaemic stroke 39.9% (95%CI 35.4-44.4%) than SCI 27.3% (95% CI 24.6-29.9%). Heterogeneity was explained by both stem cell biology and the experimental modelling paradigm with method of injury induction and method of injury assessment having the greatest impact. A large and relatively homogeneous portion of the combined dataset consisted of rats implanted with stem cells after an injury induced by either an impactor or intraluminal suture. In this data subset, with injury assessed by either the Basso, Beattie, and Bresnahan (BBB) or Neurological severity score (NSS) tests, the efficacy of stem cell transplantation was reduced to 29.5% (95% CI 26.4-32.6%). However, stem cell biological variables accounted for more of the heterogeneity seen, including the effects of dose and the time of assessment. Stem cells appear to improve neurobehavioural outcome but do so less effectively following SCI than stroke. This might reflect a greater potential for salvage of injured tissue after stroke models which most often induce transient rather than permanent MCAo. Potential neuroprotective effects of stem cells in these acute neurological injuries seem confounded by large heterogeneity in the behavioural testing methodologies used, suggesting that application of a core assessment protocol might be beneficial. More preclinical work would still seem to be required to assess the impact of stem cell transplantation on these two acute neurological injuries before developing clinical trial protocols for testing efficacy of this potential treatment in humans.

W-1482

INTERLEUKIN-3 HAS A POTENTIAL TO ENHANCE THE MIGRATION OF HUMAN MESENCHYMAL STEM CELLS

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Human mesenchymal stem cells (MSCs) differentiate into cells of adipose tissue, cartilage and bone; and are being used as a therapy for enhancing hematopoietic stem cell transplantation, modulating graft-versus-host disease, treating neonatal bone disorders and delivering therapeutic genes in various animal models of human diseases. However, these clinical trials of human MSCs did not show the long-term retention and homing of cells after transplantation. Thus, the major barrier to the successful implementation of MSCs-based therapies could be lack of both specific homing and directed migration of exogenously infused cells to the site of injury. Although genetic modification may be a viable approach to improve cell homing and/or survival, current methods raise potential safety concerns and are technically convoluted. In the present study we propose interleukin-3 (IL-3) pre-treatment as a novel strategy for the better survival and homing of human MSCs using unconditioned NOD/SCID animals. We observed that IL-3 increased the cell motility, migration and wound healing abilities of human MSCs in vitro. Increased cell motility by IL-3 was consistent in human MSCs derived from different sources such as bone marrow, adipose tissue and gingiva as evident by time-lapse microscopy. IL-3 increased the surface expression of various chemokine receptors involved in cell migration such as CCR1, CCR7, CCR9, CX3CR1 and CXCR4. Moreover, the expression of CXCR4- an important receptor for cell migration, was consistently increased at both surface and intracellular levels in all MSCs. This was confirmed by increased migration of IL-3 treated cells towards SDF-1. In the preliminary studies of xenogeneic transplantation of human MSCs we observed increased biodistribution of IL-3 treated MSCs towards bone marrow, heart, kidney and brain. Thus, our results indicate the potential of IL-3 to enhance the migration and homing of transplanted human MSCs.

W-1483

MESENCHYMAL STEM CELLS - ENDOTHELIAL CELLS INTERACTION IN ANGIOGENESIS, IMPACT OF TNF-ALPHA

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Mesenchymal stem cells (MSC) play an important role in homeostasis support. MSC are present in all tissues and remain quiescent until injury occurs, so they act to induce regeneration and promote angiogenesis. Inflammatory microenvironment can regulate MSC potency to interact with immune and vascular cells. This regulation relies on paracrine modulation of MSC function via inflammatory cytokines including TNF-α. In this work we studied interaction between human umbilical vein endothelial cells (HUVEC) and adipose tissue-derived mesenchymal stem cells (ADSC) and its role in formation of new blood vessels under inflammatory conditions created by TNF-α treatment. Cell-to-cell interactions play an important role in tissue repair and angiogenesis. We have found that ADSC activates self-assembly of network by HUVEC only when cells were co-cultured in direct contact. uPAR expression was upregulated on the surface of HUVEC in this co-culture model, and uPAR inhibition with uPAR-blocking antibody inhibited network formation in a dose-dependent manner. Immunocytochemistry revealed that Cx43, the major gap junction protein, was expressed not only in HUVEC but also in human ADSC so we suggested that these two cell types can communicate via Cx43. We have shown that blockade of gap junctions by carbenoxolone and heptanol-1 significantly suppresses self-assembly of network by HUVEC. In ADSC TNF-α caused production of MMP-9 and activation of MMP-2, doubled expression level of intercellular molecule of adhesion ICAM-1 and at the same time did not significantly influence other adhesion molecules and growth factor receptors. In a fibrin gel bead assay model ADSC supported and potentiated the formation of vessel-like structures by endothelial cells. Moreover, the pre-treatment of ADSC with TNF-α significantly stimulated microvessels elongation and branching. In adhesion tests TNF-α enhanced ADSC interaction with monocytes and endothelial cells. In a mouse model of hindlimb ischemia we have shown significant increase of perfusion after delivery of ADSC pre-incubated with TNF-α compared to untreated cells delivery. We demonstrated the crucial role of direct ADSC - endothelial cells interaction in the formation of blood vessels and the significance of inflammatory conditions for ADSC pro-angiogenic properties.

W-1484

LACK OF ENDOGENOUS NEUROGENESIS IN THE INJURED NEONATAL SPINAL CORD AND ENGRAFTMENT OF FETAL NEURAL PROGENITORS AS POTENTIAL COMPENSATION FOR CELL LOSS

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In the adult spinal cord, injury can activate endogenous neural stem and/or progenitor cells, which are believed to generate primarily glia. To assess whether the reaction is the same or different in the neonatal spinal cord, we have generated compression injuries in approximately a single segment of the thoracic spinal cord of newborn (P1) mice, and assessed the cellular reactions that occur afterwards using EdU labeling to reveal newly generated cells. Within 24 hours of injury, nearly all the neurons present in the injured segment, both motoneurons and interneurons, have disappeared. EdU labeling over the subsequent week demonstrate a moderate cellular proliferation, but the newly generated cells are microglia and astroglial, with no indication of newly generated neurons. Thus, even in neonates, there is no de novo neurogenesis that is triggered by spinal cord injury. To test the potential for cell implantation to replace neurons that are lost following a neonatal spinal cord injury, we injected human fetal neural progenitor cells into the injured spinal cord, after transducing them with GFP so that they could be easily identified. We found that the human progenitor cells survived for at least 7 weeks, differentiated morphologically to extend neurites over substantial distances, and thus showed clear signs of anatomical, and likely functional integration into the injured spinal cord. Ongoing experiments aim to assess directly the degree of functional integration and whether this contributes to functional recovery in the injured neonatal spinal cord. In conclusion, it appears

that endogenous neurogenesis does not contribute to recovery after spinal cord injury even in the neonate, but that cell implantation can be a promising approach.

W-1485

ORGAN-LEVEL QUORUM SENSING DURING STEM CELL ACTIVATION: REVELATION OF NOVEL COLLECTIVE REGENERATIVE BEHAVIOR IN HAIR FOLLICLE POPULATIONS

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Coordinated organ behavior is crucial for an effective response to environmental stimuli. Here we show that, in a field of resting telogen follicles, plucking a few properly arranged hairs can trigger the regeneration of up to 5 times more neighboring, unplucked resting hairs. Such collective regeneration is threshold dependent and provides an example of quorum sensing at the organ-level., a form of social behavior through which populations make collective decisions. Through mathematical modeling of experimental data, the range of action of the quorum signal was estimated to be on the order of 1 mm, greater than what is usually expected from diffusible signaling molecules. Using a combination of molecular profiling, in situ expression, and analyses with transgenic mice, we discovered a two-step mechanism. The release of CCL2 from injured hair keratinocytes of plucked follicles leads to the recruitment of TNF-α secreting macrophages, which accumulate and signal to both plucked and unplucked follicles. Thus a chemical cytokine is combined with a mobile cellular vector to mediate the long distance spreading of the quorum signaling. By topological positioning of plucked and unplucked follicles (what matters is the density and the shape of the plucked field, not the absolute number of plucked follicles), we can enhance the spreading of quorum sensing signals and maximize the activation of stem cells in the hair follicle population. Thus, by coupling immune response with regeneration, this mechanism allows skin to respond predictively to distress, disregarding mild injury, while meeting stronger injury with full-scale cooperative activation of stem cells. We contemplate such organ level quorum sensing behavior principle to be present in the regeneration of tissue and organs beyond the skin.

W-1486

STEM CELL SEEDED BIOARTIFICIAL TRACHEAL TRANSPLANTATIONS IN RATS - A CELL FATE STUDY

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Tissue engineering combines scaffolds with cells to create functional tissue and organs. The transplantation of a such-created tracheal graft has been recently proved to be clinically feasible. Experimental studies elucidated the necessity of cell seeding prior implantation to yield a successful clinical outcome. The immune response towards the implanted tissue is mitigated by unknown biological processes and actual cell fate and contribution to tissue regeneration are still poorly understood. A novel electrospun synthetic tracheal graft made from polyethylene terephthalate (PET) was seeded with GFP-labeled mesenchymal stem cells (MSCs) and in vivo cell fate investigated in an orthotopic transplant model in rodents. Besides, we investigated secreted signal molecules from these cells in order to elucidate potential underlying processes. We used two-photon microscopy for live imaging of interaction between MSCs and the electrospun synthetic scaffolds in vitro and calcium imaging for cell functionality. Combined with in vivo and postmortem studies including immunohistochemistry and scanning electron microscopy. After 72 hours seeded GFP positive cells were no longer present on the implanted scaffolds. However, scanning electron microscopy combined with immunohistochemistry reveals native-like ciliated epithelial cells on the internal surface of the scaffold 30 days post transplantation. In contrast, the implantation of non-cell seeded scaffolds let to graft failure and recipient´s health impairment within 2 ± 2 days post-implantation. The present findings suggest the crucial value of cell seeding for tissue regeneration during the early post-operative phase and their potential immuno-modulatory role.

W-1487

STEM CELL ACTIVATION AND QUIESCENCE IN THE ZEBRAFISH BRAIN

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It is well established that non-mammalian vertebrates such as zebrafish and amphibians display a robust potential to regenerate lost tissue after injury. Furthermore, across the evolutionary spectrum of organisms including mammals the juvenile phase has a superior capacity to repair tissues. A key question waiting for answer in stem cell and regenerative biology is how stem cell quiescence and activation is controlled. Recent studies suggest that an altered metabolic state can prime quiescent stem cells and improve regeneration of multiple adult mammalian tissues. This is at least in part regulated by growth factor signalling and the mTOR pathway. We have taken advantage of the highly regenerative genetic model zebrafish and identified modifiers and inducers of the genetic programme regulating metabolism and growth signals that are needed to activate quiescent stem cells and modulate tissue growth during homeostasis and after injury. These findings open up new avenues to improve tissue regeneration in vertebrates.

W-1488

MIR-20A INCREASES CELL PROLIFERATION OF MSC CULTURED WITH ISCHEMIC SERUM CONDITIONED MEDIA BY TARGETING CDKN1A

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The differences in property bone-marrow derived mesenchymal stem cells (BM-MSC) 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. However, whether miRNAs regulate ischemic rMSCs induced proliferation remains unclear. In this study, we assessed whether MSCs preconditioned by growth in ischemic serum derived from a stroke model increase the proliferation compared to that cultured rMSCs in FBS. Using miRNA microarray analysis, we identified a miRNA expression profile cultured MSCs in FBS and ischemic serum contained media. We observed that among miRNAs associated with proliferation, miRNA-20a was increased the most significantly. Further, the deregulated miR-20a was significantly correlated with cell number of cultured rMSCs in ischemic serum conditioned media. The results of MTT assay and ki-67 staining showed that Overexpression of miR-20a of cultured rMSCs in FBS improved the proliferation and Knockdown of endogenous miR-20a decreased proliferation of cultured rMSCs in FBS. In addition, miR-20a positively regulates proliferation by suppressing the expression of cyclin-dependent kinase inhibitor 1 (CDKN1A). We validated that CDKN1A is a target of miR-20a using dual-luciferase reporter assay. We conclude that the upregulated miR-20a can be regulate CDKN1A and promote to cell growth in rMSC. These findings indicate that MSCs may be able to adjust characteristics itself by controlling expression of miRNA in MSCs through process such as ischemic serum preconditioning.

W-1489

AMYLOID-BETA-42 AGGREGATION CAUSES NEURODEGENERATION AND INDUCES NEURAL STEM CELL PLASTICITY AND REGENERATIVE NEUROGENESIS IN ADULT ZEBRAFISH BRAIN

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Alzheimer's Disease (AD), the most prevalent neurodegenerative disease in humans, manifests as toxic protein aggregates of Amyloid-beta-42 (Ab42) peptide that ultimately kills neurons. As a consequence of this neurodegeneration, we cannot replenish lost neurons with concerted activity of neural stem/progenitor cells (NSPCs). Thus, a plausible therapeutic way might be to induce the plasticity of our existing NSPCs to for regenerating the lost neurons. Such a regenerative therapy needs understanding the molecular basis of how we could elicit that regenerative response. Unlike humans, zebrafish can effectively regenerate its brain due to the neuro-regenerative potential of NSPCs. Therefore, zebrafish offers a unique opportunity to elucidate how vertebrate brain can regenerate. To investigate whether Ab42 could cause neurodegeneration in adult zebrafish brain, and whether zebrafish could respond to this neuronal loss by regeneration, we synthesized the short Ab42 peptide by a specific surface chemistry, coupled it to a cell-penetrating peptide we have recently optimized for deep penetration and injected it into adult zebrafish brains. We observed that significant intracellular Ab42 deposition occurs throughout the zebrafish brain as early as 2 days after injection. This accumulation causes significantly elevated cell death, inflammation and degeneration of synapses, phenotypes reminiscent of human pathology of AD. Furthermore, unlike mammalian brain, Ab42-mediated neurodegeneration induces the proliferation of NSPCs in zebrafish brain. These stem cells also form neurons fulfilling a regenerative neurogenesis, highlighting a fundamental superiority of fish brain to cope with neurodegeneration. By blocking the activity of microglia by Clodronate, we found that inflammation is required for Ab42-mediated NSPC plasticity. Transcriptome sequencing identified 386 genes differentially expressed in NSPCs upon Ab42-aggregation. We will present our functional studies with a gene required for Ab42-induced NSPC plasticity. To our knowledge this first Ab42 model in adult zebrafish brain led us to identify a molecular program of neurodegeneration-induced regenerative neurogenesis, which could be instrumental to impose a regenerative ability to the NSPCs if harnessed in mammalian models.

W-1490

PRIMING WITH EXOGENOUS MIR-1 INCREASED POST TRANSPLANTATION SURVIVAL OF MESENCHYMAL STEM CELLS IN INFARCTED MYOCARDIUM BY SUPPRESSING PYROPTOSIS

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Preventing post-transplantational cell death is one of the crucial requirements for a successful cell therapy for damaged hearts. High death rate of transplanted cells has hampered the efficacy of stem cell-based cell therapy. Thus, a long-term survival of the transplanted cells should be achieved to develop an optimized cell-based therapeutic strategy. Since the transplanted cells will be directly and immediately exposed to the harsh microenvironment that caused initial damage to myocardium, the cell death mechanism of the transplanted cells would be similar to that of host cells. Among various cell death mechanisms observed in myocardium, pyroptosis is the most recently recognized form of programmed cell death. Pyroptosis is characterized by cell lysis and release of inflammatory cytokine such as interleukin 1β (IL-1β). Differ from apoptosis, pyroptosis does not show the typical membrane blebbing of apoptosis, but it induces cell lysis, swelling and pore formation that are not observed in apoptosis. Furthermore, the type of cysteine-dependent aspartate-specific proteases (caspases) that orchestrates disassembly of the cells in pyroptosis is caspase-1

as opposed to the caspase-3 of apoptosis. To our best knowledge, there is no previous study examined the feasibility of improving the survival of transplanted mesenchymal stem cells (MSCs) into infarcted myocardium by suppressing pyroptosis. Thus, we examined whether down-regulation of pyroptosis-regulating proteins (we refer as priming) prior to transplantation could improve the survival of transplanted cells into damaged myocardium. In the present study, we identified microRNA-1 (miR-1) as miRNA targeting multiple components of pyroptosis signaling cascade, and demonstrate that suppressing these pyroptosis-regulating proteins using miR-1 effectively improved the survival of MSCs exposed to hypoxia in vitro. Furthermore, priming MSCs with miR-1 prior to the transplantation into infarcted heart significantly attenuated death of transplanted MSCs and improved cardiac function in vivo. The result of this study strongly suggests that pyroptosis significantly contributes to the post-transplantational death of MSCs, and it can be a potential target in improving survival of transplanted cells, especially into infarcted myocardium.

W-1491

IDENTIFICATION OF A NEW CELL POPULATION WITH A HEALING CAPACITY CIRCULATING IN HEALTHY CONDITIONS

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Stem/progenitor cells are the critical units affecting tissue maintenance, regeneration, and repair. The activation of the endogenous regenerative events in response to tissue injury correlates with the functional mobilization of progenitor cells contributing to the healing process. However, until now there has been no evidence for the presence of pluripotent cells with a healing capacity circulating in healthy conditions. Here we define the regenerative role of a rare population of cells present in the peripheral blood of healthy mice. We named these cells Circulating Healing (CH) cells. Using an innovative and functional flow-cytometry strategy, we identified small cells (2-6 m), not expressing CD45 and lineage markers, and with a stemness mRNA profile (expression of Sox-2, Oct-4, Nanog, and Klf4). The percentage of CH cells in the blood of mice decreased in a time-dependent manner after a femoral fracture. Freshly isolated CH cells systemically injected in syngenic fractured mice homed and engrafted in wounded tissues (bone, articular cartilage, skeletal muscles), and spontaneously differentiated into tissue-specific cells. The transcriptome analysis of CH cells revealed their uniqueness when compared to other cells characterized by varying stemness degree. Moreover, CH cells presented a high expression of key pluripotency-associated genes and, positive selective cell markers of the epiblast developmental stage, as well as of the downstream primitive streak, anterior primitive streak, and mesoderm stages. We anticipate that this rare and undifferentiated cell population may be used in the future as effector of therapeutic tissue regeneration.

W-1492

REGENERATIVE CAPACITY AND CELLULAR PLASTICITY OF PANCREATIC PROGENITORS

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While the adult pancreas is unable to fully restore following injury, it possesses some regenerative capacity. This capacity is contingent upon age, extent of loss, injury method and cell type affected. It was previously shown that the adult pancreas size is limited by the number of embryonic progenitor cells in the primordium. However, the regenerative capacity and plasticity of pancreatic progenitor cells (PPCs) with varying injury and developmental stage, has never been addressed. Indeed, around embryonic day 12.5 (E12.5), extensive changes take place in the structure of the epithelium, its gene expression profile and potency of progenitors comprising it. We hypothesize that there exists a stage-dependent plasticity and regeneration during pancreas organogenesis, after the progenitors organize into a single polarized layer at around E12.5. With the overarching goal of understanding the regenerative capacity and cellular plasticity of the developing pancreas, we explore the extent of tissue recovery, revealing the origin of the regenerating pancreatic cells and analyzing the signaling pathways involved in this process. We are using a genetic ablation approach which is implemented on transgenic mouse strains enabling lineage tracing of PPCs. We exploit innovative, live imaging methods to monitor the healing process with a cellular resolution. Together, these approaches will serve as a solid platform for identifying genes and signaling pathways affecting PPC regeneration and plasticity, which will inform new strategies for regenerating β-cells to treat diabetes mellitus and augment our very limited understanding of the mechanisms governing regeneration during pancreatic morphogenesis.

W-1493

HAIR GROWTH PROMOTING EFFECT OF HUMAN DERMAL STEM/PROGENITOR CELL DERIVED CONDITIONED MEDIUM IS ENHANCED BY THE ACTIVATION OF WNT/BETTA CATENIN SIGNALING

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We previously reported that human dermal stem/progenitor cell-derived conditioned medium (hDSPC-CM) mediates skin regeneration effects, such as wound healing and reducing the number of UVA-irradiated apoptotic cell population. Here we demonstrate the effects of hDSPC-CM on the hair re-growth. hDSPC-CM was used for study regarding the activation of alkaline phosphatase (ALP) activity and the up-regulation of mRNA expression for dermal papilla cell markers, such as SOX2, S100β, VCAN and CORIN. And the secretion of Wnt3a protein from hDSPC-CM was significantly increased compared to non hDSPC-CM. Furthermore we investigated the effect of hDSPC-CM on hair regeneration of C57BL/6 mice by considering the relationship between Wnt/β-catenin pathway and hair formation. Taken together, these data suggest that hDSPC-CM can exert some beneficial effects on hair regeneration and may be used as a therapeutic agent for curing hair loss.

W-1494

REGENERATION IN THE MOUSE PITUITARY AFTER CELL-ABLATION INJURY: TIME FACETS AND MOLECULAR MECHANISMS

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The pituitary is the central gland in the endocrine system controlling, amongst others, growth, metabolism, fertility and stress. We recently showed that the adult mouse pituitary is capable of regenerating hormonal cells after diphtheria toxin (DT)-induced ablation, and that the pituitary's stem cells promptly react to participate in this restoration process. Here, we further characterized the pituitary's regenerative capacity in relation to age and recovery period, and started to search for underlying molecular mechanisms. Young-adult GHCre/iDTR mice (2-3 months old), expressing the DT receptor upon expression of Cre in growth hormone (GH)-producing cells and treated with DT for 3 days (3DT), regenerate the ablated cells up to 60% after 5 months. Extending the recovery period (to 21 months) does not result in higher regeneration levels. In addition, the pituitary's regenerative competence disappears when mice are older (7-8 months). Surprisingly, prolonging the DT treatment period in young-adult GHCre/iDTR mice from 3 to 10 days (10DT) blocks the regenerative capacity although the ablation grade obtained is similar (80-90%). The stem cell compartment still expands promptly after 10DT and retains intrinsic stem-cell functionality as assessed in vitro. To search for underlying molecular grounds of reparative failure, the stem cell-clustering side population (SC-SP) of the non-regenerating (10DT) pituitary was compared to the SC-SP of the regenerating (3DT) pituitary using whole-genome expression analysis. A number of stemness factors and components of the Notch, Shh and epithelial-mesenchymal transition (EMT) pathways are higher expressed in the SC-SP of the regenerating pituitary. These factors are also upregulated when compared to the basal, non-injured pituitary. In conclusion, the regenerative capacity of the pituitary appears to rely on activation of specific stem-cell pathways but is limited both in age and final efficacy. Identifying the molecular repair mechanisms may eventually be instrumental to induce or boost regeneration in situations of pituitary deficiency due to damage by tumor growth, surgery or traumatic brain injury.

W-1495

BONE MARROW DERIVED MESENCHYMAL STEM CELLS TREATED AORTIC ANEURYSM VIA NF-KAPPA B AND AKT SIGNAL PATHWAY IN MICE

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The pathogenesis of aortic aneurysm (AA) is chronic inflammation caused by atherosclerosis. We have reported that intravenous injection of mesenchymal stem cells (MSCs) could reduce the morbidity rate of aortic aneurysm by anti-inflammatory and tissue repair properties of MSCs. However, the mechanisms of MSCs-mediated AA regression are still unknown. In this study, we investigated the signal pathways in AA treated with MSCs. The AA induced mice (continuous angiotensin II administrated aged apolipoprotein E knockout mice) were intravenously injected 1 million bone marrow derived-MSCs (BM-MSCs) with 0.2 mL saline (n=6, BM-MSCs group) or 0.2 mL saline without BM-MSCs (n=5, control group). Two weeks after, intra aortic phosphorylation of JNK, NF-kB, ERK1/2, Akt, Smad2 and RhoA were assessed by western blotting. In BM-MSCs group, phosphorylated (p)-NF-kB was significantly decreased (MSC vs Control = 0.21 ± 0.13 vs 0.41 ± 0.11, $p < 0.05$) and p-Akt was significantly increased (MSC vs Control = 0.12 ± 0.04 vs 0.06 ± 0.03, $p < 0.05$) compared with the control group. There were no differences in levels of JNK, ERK, RhoA and Smad2 between MSC and the control group. These results suggest that the attenuation of NF-kB and acceleration of Akt phosphorylation may be induced by injection of BM-MSCs in AA.

W-1496

MSX2-DRIVEN DEDIFFERENTIATION OF MAMMALIAN MYOTUBES

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In contrast to several non-mammalian vertebrates, mammals lack the regenerative responses to replace large body parts. Amphibian and fish regeneration uses dedifferentiation, i.e. reversal of differentiated state, as a means to produce progenitor cells to replace damaged tissues. Therefore, activation of dedifferentiation response in mammalian tissues holds an immense promise for human regenerative medicine. Although mammals do not exhibit dedifferentiation as a regenerative response, many of the signaling pathways and the molecules involved in salamander regeneration are conserved, raising the possibility that dedifferentiation can be recapitulated in mammalian tissue. We demonstrate that ectopic expression of the transcription factor Msx2 in cultured mouse myotubes recapitulates several aspects of amphibian muscle dedifferentiation. Msx2, but not its homolog Msx1, leads to cellularization of myotubes and downregulates expression of myotube markers, such as MHC, Mrf4 and myogenin. Transcriptome analysis of myotubes ectopically expressing Msx2 shows downregulation of >500 myotube-enriched transcripts and upregulation of >300 myoblast-enriched transcripts. Msx2 selectively downregulates expression of Ptgs2 and Ptger4, two key members of the prostaglandin pathway with important roles in myoblast fusion during muscle differentiation. Ectopic Msx2 expression leads the myotubes to a partial cell cycle re-entry by upregulating CyclinD1 expression without initiation of S-phase. Finally, we show that Msx2-induced dedifferentiation in mouse myotubes can be recapitulated by a pharmacological treatment with Trichostatin A (TSA), Bone Morphogenic Protein 4 (BMP4) and Fibroblast Growth Factor 1 (FGF1). These studies establish that Msx2 is a major driver of dedifferentiation in mammalian muscle cells.

W-1497

IMPACT OF CARTILAGE PASTE IMPREGNATED WITH MESENCHYMAL STEM CELLS ON REGENERATION THE FOCAL ARTICULAR CARTILAGE DEFECTS IN RABBITS

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Chronic arthritis is one of widespread disease. Several therapies have been attempted to treat focal cartilage defects, but none of them resulted in full repair with formation of typical hyaline cartilage. To identify an effective treatment is essential to minimize the side effects of conventional therapy. However, stem cell therapy may establish a prospective therapy for OA in the future. There is a requirement for further new efficient therapy options. On the femoral condyle right Knee of all rabbit groups the Focal cartilage defect was created. First group the defect left without treatment. The second group the Human umbilical cord MSCs was infused in the defect. A third group received MSCs on a fibrin glue scaffold. A fourth group received minced cartilage impregnated with MSCs. Healing was assessed clinically and radiologically in post-mortem samples. The surgical technique proved to be safe. After eight weeks no infection was reported and the animals were able to move normally. Ex-vivo examination of the knee joints showed better healing of the defect in rabbits that received cartilage paste compared to those receiving MSCs+ fibrin glue followed by those receiving MSCs only. MRI showed persistent osteochondral defect in rabbits treated with MSCs, without and with fibrin glue, mean MOCART score was 10 points. However. The defect was smaller with fibrin glue. Complete defect fill, intact cartilage surface, and complete integration with adjacent cartilage were observed in rabbits treated with MSCs and Cartilage, mean MOCART score was 85 points. Pathologic examination revealed that MSCs, alone, induced chondrocyte proliferation and stimulated cartilage repair. A better result was obtained with MSCs + fibrin glue. The best repair was obtained when autologous cartilage paste was used. This is in agreement with MRI findings. HUC-MSCs with autologous cartilage paste shows a best healing in focal articular cartilage defects. This effective result was proven clinically and radiologically.

W-1498

INFLUENCING THE FATE DECISION OF CARDIAC PROGENITOR CELLS WITH NOVEL SMALL MOLECULES

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Several progenitor cell populations have been reported to exist in the heart which can play a role in normal turnover and/or repair. Despite the presence of cardiac stem and progenitor cells within the myocardium, functional repair of the heart after injury is inadequate. Identification of signaling pathways involved in the differentiation of cardiac progenitor cells (CPCs) will broaden insight into the fundamental mechanisms playing a role in homeostasis and disease and may provide strategies for in vivo regenerative therapies. We have isolated an iPSC-derived NKX2.5+ CPC population that has multilineage differentiation capacity. In order to facilitate the identification of pathways and targets involved in differentiation of resident CPCs, we have developed phenotypic screening assays using this iPSC-derived model. Screening paradigms for therapeutic applications requires a robust, scalable, and consistent methodology, and here we have demonstrated suitability of these cells for medium to high-throughput screens in a 384-well format. We have focused on assessing multi-lineage differentiation with an emphasis on cardiac and endothelial differentiation as these cell types are critical for functional repair. Using a small directed compound set, differentiation screening has led to the discovery of compounds, including inhibitors of TGFβ pathway signaling and inhibitors of members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) protein family, which can induce differentiation towards the cardiac or endothelial lineages as well as molecules that can induce differentiation of both lineages simultaneously. The mechanisms and signaling pathways of these compounds are currently under investigation. The identification of compounds that can influence the fate decision of cardiac progenitor cells is a significant step towards developing potential therapies that have the potential to result in true cardiac regeneration.

W-1499

THE ROLE OF REACTIVE OXYGEN SPECIES IN IMFLAMMATION-INDUCED ACTIVITY OF ADIPOSE TISSUE DERIVED MESENCHIMAL STEM CELLS

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MSC behavior strongly depends on cellular microenvironment defining their state of dormancy or activity. It is suggested that they reside in a perivascular niche where hypoxia promotes their dormant state, and that low oxygen tensions maintain undifferentiated states of stem cells; moreover, stem cells residing in niches characterized by low levels of reactive oxygen species (ROS). Quiescent MSC can be mobilized in response to some stimuli to promote tissue regeneration. One of these stimuli is inflammation, which accompanies any tissue lesion and is a natural response to damage or infection. ROS generation is a major factor mediating cellular actions of inflammatory cytokines. Therefore, in our study, we have address the question, if ROS generation in adipose tissue derived MSC (ASC) is the important physiological regulators of cellular activity triggered by inflammation. Here we used TNF-α treatment to model inflammatory microenvironment and studied ROS involvement into migratory, proliferative and secretory responses of ASC. We have shown that both physiological (5ng/ml) and supraphysiological (100 ng/ml) concentrations of TNF-α rapidly induce ROS generation in ASC as was determined using either DCF-DA or HE-staining. Furthermore, TNF-α triggers some key intracellular signaling pathways leading to ROS generation: Akt, small GTPase Rac1, p38 MAP-kinase. We have observed using MTT assay that TNF-α enhances ASC proliferation as well as promotes entering into G2 phase as was tested by flow cytometry. This effect was abolished by ASC treatment with synthetic antioxidants. TNF-α-induced enhancement of ASC chemotaxis in transwell was significantly reduced by pretreatment of cells with the antioxidant ebselen suggesting that TNF-α-induced increase in proliferation and chemotaxis of ASC is ROS-dependent. TNF-α exerted expression and secretion of several inflammatory and angiogenic factors in ASC, like IL-1β, IL-8, MCP-1 and VEGFA. In our real-time PCR experiments, MCP-1 mRNA expression triggered by TNF-α was dramatically inhibited while VEGF expression was increased by antioxidants. These data suggest that ROS are involved in cytokine and growth factor gene expression in ASC. These results allow us to conclude that ROS generation play a significant role in the ASC response to inflammatory stimuli.

TECHNOLOGIES FOR STEM CELL RESEARCH

W-1500

HEART, BRAIN, EYE AND PANCREAS CELLS THRIVE ON BIOLOGICALLY RELEVANT DEFINED AND XENO-FREE LAMININS

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Laminins are a group of 16 heterotrimeric glycoprotein isoforms found in the basement membrane in the extracellular matrix and are composed of α, β and γ chains, and are the only tissue-specific proteins in the basement membrane. They are thus critical factors differentiating one cell niche from another and influence the behavior of associated cells, such as adhesion, differentiation, migration, phenotype stability, and resistance to anoikis. The use of specific laminins for tissue culture and cell therapy applications have been hampered by lack of access to most laminin isoforms. Tissue-purified laminins have been available for years but often result in poor quality due to protein degradation, lot-to-lot variation and impurities, resulting in variable and unreliable research results. We have now solved these problems by successful production of human recombinant laminins and have shown that individual laminin isoforms drastically improve the functional properties of different cells. Pluripotent stem cells: By using LN-521, which is naturally expressed by human PSCs, we can culture stem cells for over 130 single cell passages at split ratios of 1:10-1:30, without any abnormal genetic aberrations and with maintained expression of pluripotency markers. Cardiomyocytes: By using heart specific laminins, LN-211, LN-221 and LN-521 in the natural combination of adult heart cell expression, the differentiation can now be fully controlled in a defined and xeno-free context and the number of hPSC-derived beating cardiomyocytes is significantly increased. Neurons and glia: Neural stem cells prefer LN-521, different neuronal subtypes require primarily LN-511/521 and LN-111 for full maturation, and glia cells express LN-111, LN-211 and LN521. RPE cells: Robust retinal pigmented epithelium cell culture has been reported on LN-521/511, LN-111 and LN-332. In conclusion, cell culture of primary cells and stem cells is reliable and robust when growing cells on the natural human recombinant laminin that match the in vivo niche. Almost all cells grow on specific laminins in the human body and as they now are available as recombinant laminins it makes cell culture in a physiologically relevant environment possible, making production of clinically relevant cells possible.

W-1501

ANALYSIS TOOL FOR HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTE BEATING DYNAMICS USING VIDEO IMAGING

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Cardiac side effects remain one of the most common reasons for withdrawal of drugs from market. Human induced pluripotent stem cells (hiPSCs) have provided a novel platform for drug development and testing, due to availability of cardiomyocytes (CMs) with various genetic profiles. Until recently, the means to analyse hiPSC derived CMs have been limited to electrophysiology, such as patch clamp and microelectrode array measurements. In our previous studies, we have shown the video analysis of these cells is a feasible addition to standard electrophysiological studies. It can provide a detailed analysis of the beating dynamics of single dissociated CMs using digital image correlation (DIC). Here we present a semiautomatic analysis tool designed for studying the biomechanics of hiPSC derived CMs. The tool is based on the method presented by Ahola et al. in 2014. DIC is used for calculating velocity vector fields between subsequent video frames across single dissociated hiPSCs. The beating focus point is selected, and the cell is divided to 8 sectors providing means to quantify the directional motion. The motion vectors of each segment are divided into radial and tangential components, creating two beating signals for each sector. Average waveforms are calculated using cross-correlation templates, similarly as by Pradhapan et al. in 2013. Our video motion analysis tool can detect CMs, and suggest their beating focus point and region of interest to the user. For a selected sector, the software calculates a one-beat average movement waveform, which represents the beating characteristics of the cell. Further, the software calculates parameters describing the beating from the average waveform. The results of the analysis can be uploaded to a database, and statistical parameters on the analysis results of multiple analyses can thus be calculated. We have shown that our tool is capable of detecting differences in mechanical beating characteristics between different areas of a single CM. The method is non-invasive and non-toxic, allowing for repeated analysis of the same cells, without introducing foreign agents to the analysis. The tool, along with the collected data from all analysed cells makes this a valuable asset for different CM studies, including drug screening applications.

W-1502

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

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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.

W-1503

ENHANCED USAGE OF CRISPR/CAS9 SYSTEMS IN STEM CELLS UTILIZING PIGGYBAC™ STABLE INTEGRATION AND SEAMLESS REMOVAL

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The development of targeted double-strand nucleases such as zinc finger nucleases, TALENs and CRISPR/Cas9 has dramatically improved the ease and efficiency of precise genome modification, leading to increased focus on the modification of cell lines for gene therapy. Despite the continued success of these technologies, there are still technical hurdles that need to be addressed in order to make them a feasible solution. One important challenge is overcoming the low efficiency rates in cells that are less amenable to gene editing. Here we describe methods for the unique combination of Transposagen's piggyBac™ (PB) Genetic Modification System and CRISPR/Cas9 to overcome lower gene editing efficiencies in different cell types. Previous demonstrations of piggyBac™ footprint-free gene editing have proven useful due to the ability to positive/negative screen and, unlike other similar technologies, seamlessly remove the selectable markers. Applying this approach to CRISPR/Cas9, we quickly and efficiently introduced stable copies of CRISPR/Cas9 along with a positive and a negative selection marker, into the cell line via piggyBac™ and were able to boost the efficiency of the system. After achieving the desired edits, we seamlessly removed the entire cassette using a modified excision-only piggyBac™ (PBx). Since this system may be used for drug discovery or gene therapy purposes, the removal of the entire cassette, including the selectable markers, cleanly and without mutations is critical. As gene editing technologies move closer to the clinic, safe and effective solutions will be needed to overcome low gene editing rates while at the same time leaving behind no changes to the target cells. The approach detailed here using piggyBac™ is applicable to any cell type, and is particularly useful for gene therapy applications where higher efficiencies are needed.

W-1504

WHERE'S WALLY? FINDING AND TRACING HUMAN MSC IN 3D MICROENVIRONMENTS WITH THE PHOTOCONVERTIBLE PROTEIN DENDRA2

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To improve the effectiveness of current tissue engineering strategies it is critical to understand the determinants of stem cell migration in 3D microenvironments. One of the most promising cell types for cell-based therapies - from tissue regeneration to treatment of autoimmune diseases - are Mesenchymal Stem/Stromal Cells (MSC). Here, a simple and reliable imaging technique was developed to study MSC dynamical behavior in natural and bioengineered 3D matrices. Human bone marrow MSC were transfected to express a fluorescent photoswitchable protein, Dendra2, which was used to stably highlight and follow the same group of cells for long periods of time. Transfection did not affect the phenotype or differentiation potential of cells and photoswitching did not impact on the cells motility. Tracing green-to-red converted MSC provided reliable tracking in 3D for more than seven days and allowed quantification of differences in stem cell migration in real time. It was possible to track cells in microenvironments with different properties, including the hydrogels Matrigel and alginate as well as chitosan porous scaffolds. As a proof-of-concept, comparison of cells mobility within matrices with tuned physicochemical properties revealed that MSC embedded in RGD-alginate with 1% polymer concentration migrated 51% faster than in 2% RGD-alginate. This platform thus provides a novel and straightforward approach to characterize MSC dynamics in 3D microenvironments and has applications in the field of stem cell biology and for the development of biomaterials for tissue regeneration. *We would like to thank FEDER -Programa Operacional Factores de Competitividade - COMPETE and FCT - Fundação para a Ciência e a Tecnologia (project EXPL/BIM-MED/0022/2013).*

W-1505

CRISPR BASED GENOME EDITING AND ITS APPLICATIONS IN GENOME AND STEM CELL ENGINEERING

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Thermo Fisher is dedicated to providing researchers with a robust, efficient, and less labor-intensive genome editing workflow. Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR), a bacterial adaptive immune system from *Streptococcus pyogenes* has been recently engineered and demonstrated to function as a genome editing tool that can rapidly generate engineered cell lines, model organisms and perform large scale gene modifications in a wide variety of hosts. This system is an attractive tool for genome engineering due to its simple design including Cas9 nuclease and a non-coding guide RNA (gRNA) with target specificity defined by only a short 17-20 base nucleotide region. Embryonic and induced pluripotent stem cells (ESC/iPSCs) hold great promise in regenerative medicine and in studying the mechanics of human diseases. To maximize the potential of these stem cells for research and therapy, efficient and precise genetic engineering techniques are warranted. Discussed here is application data comparing the gene editing efficiency using plasmid DNA, Cas9 nuclease mRNA and Cas9 protein formats of the CRISPR/Cas9 system in human embryonic and induced pluripotent stem cells. Previous work with plasmid-based CRISPR systems in stem cells produced relatively low genomic cleavage efficiency. Using the optimized methods and newly developed CRISPR formats described in this work we

have demonstrated greater than 50% genome editing efficiency in human ESC and iPSCs with much lower cell toxicity. In addition, we demonstrate the use of these new tools in a broad variety of cell types including human hematopoietic stem cells and primary human keratinocytes. The gene editing tools and workflows described here greatly facilitate efficient disease model generation, thereby paving the way for new therapies.

W-1506

OPTIKD: AN INDUCIBLE GENE KNOCKDOWN SYSTEM TO STUDY HUMAN DEVELOPMENT USING PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) represent an invaluable in vitro system to model human development. However, detailed analyses of complex molecular mechanisms are hindered by the lack of efficient methods to manipulate gene expression during hPSCs differentiation. We developed a new method to conditionally knockdown gene expression in hPSCs with an optimized tetracycline-responsive inducible system (OPTiKD). OPTiKD allows tightly controlled, dose dependent, and reversible knockdown in hPSCs. Generation of OPTiKD hPSCs is simple (lipofection-based), rapid (2 weeks), and efficient (>95%). Moreover, OPTiKD is stably maintained during differentiation of hPSCs into lineages from all the three germ layers. To further demonstrate the usefulness of OPTiKD, we investigated the role of H3K4me3 deposition during hPSCs differentiation into multiple cell types by knocking down the expression of DPY30, a common subunit of MLL/SET methyltransferse complexes. These analyses revealed divergent functions for DPY30 in multipotent progenitors and in terminally differentiated cells, thereby suggesting different requirements for H3K4me3 during development. Overall, OPTiKD represents a unique novel tool to probe and to dissect the mechanisms involved in human development using hPSCs-derived cells.

W-1507

GENERATION OF KIDNEY LINEAGE-SPECIFIC PLURIPOTENT REPORTER CELL LINES BY CAS9/CRISPR MEDIATED TARGE-TED INSERTION IN HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS)

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Chronic kidney disease is a major global health problem causing significant morbidity and mortality. Therapeutic options for end-stage kidney disease, specifically dialysis and transplantation, improve patient survival but remain inadequate due to insufficient access and secondary complications. Dysfunction of the podocyte cell of the glomerulus accounts for approximately two-thirds of all kidney diseases leading to end-stage renal disease. In order to mechanistically study the functional role of this cell type in both health and disease it is necessary to have relevant in vitro cell models to complement studies in animals and humans. Since the podocyte is a highly differentiated cell that undergoes limited replication, it represents a difficult cell type to grow in culture and there are no adequate podocyte models that faithfully recapitulate the in vivo situation. The iPSC-technology has made it possible to access a broader range of target cell types, however, an optimized protocol for the derivation kidney cell lineages remains to be established. Toward this end, we have generated two GFP-reporter cell lines from hiPSCs using CRISPR/Cas9 technology targeting the nephron progenitor marker SIX2 and the terminal podocyte-specific marker Nephrin (NPHS1). These cell lines will facilitate high throughput cell based optimization of differentiation protocols and culture conditions, and can also be used for monitoring transplanted cells as well as for early stage drug discovery.

W-1508

HYALURONIC ACID COATINGS AS A SIMPLE AND EFFICIENT APPROACH TO IMPROVE MSC HOMING TOWARD THE SITE OF INFLAMMATION

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A major challenge in mesenchymal stem cell (MSC) therapy is to deliver therapeutic cells to a target location efficiently and with minimal invasion, as less than 1% of culture expanded MSC home to the tissue of interest. The aim of this work was to develop a system that is able to promote the transient expression of CD44, the hyaluronic acid (HA) receptor, on MSC membranes, which ultimately leads to an improved cell migration toward the site of inflammation. We studied a different coating system of HA on tissue culture plates (TCP). Atomic Force Microscopy demonstrated that we were able to deposit a compact and uniform coating of HA on TCP. Murine bone marrow-derived MSC (BM-MSC) were seeded onto coatings with HA at concentrations ranging from 1mg/ml to 0.01 mg/ml. CD44 expression was evaluated at 1 and 3 day time points. At day 1, a significant increase (74.60 ± 5.5) of CD44 expression was observed at the mRNA level in cells treated with the highest concentration of HA in comparison to untreated cells. Flow cytometry analysis demonstrated a 4-fold increase in the mean fluorescence intensity of CD44 at day 3 in the same experimental group when compared to the controls. No significant differences were observed among cells treated with lower concentrations of HA at any time point. To determine whether the overexpression of CD44 could lead to an enhanced homing potential, a migration assay was performed in vitro. Data showed a 2.87-increase in migratory potential compared to untreated cells. To prove the efficacy of the system in vivo, we used the LPS-induced inflamed ear murine model to determine whether HA-treated MSC displayed

enhanced homing to other distant sites of inflammation following systemic administration. 24 hours after cell injection, mouse ears were imaged using an IVIS system for the presence of injected cells. Preliminary data demonstrated that BM-MSC treated with HA (1mg/ml) exhibited enhanced homing to the inflamed ear 24 hours after injection with a 2-fold increase in the number of homing MSC compared to the controls. These results suggest that the HA-coating represent a simple and versatile method to transiently overexpress CD44 on the MSC surface and potentially target systemically administered cells to the site of inflammation without affecting MSC morphology, proliferation, and MSC-associated markers expression.

W-1509

INTEGRATED STRATEGIES FOR THE PRODUCTION AND STORAGE OF FUNCTIONAL CARDIOMYOCYTES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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The ability of human pluripotent stem cells (hPSC) to self-renew indefinitely in culture and to differentiate into any somatic cell type makes them a powerful unlimited source of cardiomyocytes (CM) suitable for regeneration therapies and cardiotoxicity testing. However, the complex nets of signaling pathways involved in cardiomyogenesis and the line-to-line variability compromise the effectiveness of the existing differentiation protocols to reproducibly produce high-quality CM from multiple hPSC lines. Moreover, the applicability of these cells in the clinical/industrial settings is highly dependent on the development of efficient methods that allow worldwide shipment and long-term banking of CM. In this study we aim to overcome these hurdles by devising an integrated strategy for scalable production and storage of functional CM derived from hPSC lines. hPSC were cultured as aggregates in environmentally controlled bioreactors, where the necessary conditions to control stem cell fate are tightly tuned. By combining a hypoxia culture (4% O2 tension) with a specific hydrodynamic environment we were able to improve hPSC differentiation towards CM by enhancing CM purity and yields. Cell characterization and functional analysis confirmed that the generated CM presented typical cardiac morphology, calcium transients, electrophysiological profiles and drug responsiveness. Metabolomic and transcriptomic analyses are being applied along the differentiation process to disclose which pathways are differentially activated/repressed in low versus high yielding bioprocess conditions. Efficient methods for cryopreservation of pure monolayers of CM or 3D cardiospheres were developed. Using xeno-free cryopreservation formulations and a controlled slow freezing rate protocol, significantly higher cell viabilities and recovery yields (>80%) were obtained after thawing. We also showed that CM can be stored for up to 7 days in hypothermic conditions without compromising cell viability, ultrastructure and functionality. This work describes significant advances towards mass production of hPSC-derived CM and their short- and long- term storage, meeting some of the current needs of the cardiac regenerative medicine market and industrial field.

W-1510

MULTIWELL MICROELECTRODE ARRAYS FOR HIGH-THROUGHPUT SCREENING AND DRUG DISCOVERY USING IPS CELLS

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Multi-well microelectrode array (MEA) systems provide simultaneous measurements of extracellular electrophysiological activity over long periods of time. By measuring functional end points from cellular networks, MEAs offer an unbiased phenotypic assay that is independent of the target. Each electrode is capable of capturing extracellular action potentials of excitable cells in ultra-high resolution (millisecond events with microvolt amplitudes), while multiple recording sites within each well allow population network activity measurements. MEAs offer label free, non-invasive recordings of natural cell functions, in a regulated physiological environment. Together with induced pluripotent stem cell derived (iPSC) cardiomyocytes and neurons, MEA technology has recently become a method of choice for high-throughput screening and risk-assessment of new drug candidates. Here, we present evaluation data of different iPSC cardiomyocytes and neurons, performed on high-throughput multi-well MEA system. For each cell type, the network activity was monitored and analyzed in response to application of standard reference compounds. For cardiomyocytes, the field potential duration, a measure of cardiac action potential repolarization, and arrhythmia incidence were analyzed for specific blockers of sodium, potassium, and calcium ion channels. For neural cultures, advanced metrics describing the degree of bursting and synchrony of the network activity were computed in response to ion channel blockers and compounds altering synaptic activity. In each case, the network activity from the iPSC derived cardiac and neural cultures produced expected functional responses, supporting the continued development and use of multi-well MEA systems for high throughput screening in drug discovery and safety, and for the development of phenotypic disease-in-a-dish cellular models.

W-1511

OPTIMIZATION OF EMBRYOID BODY FORMATION FROM SINGLE CELL SUSPENSIONS OF HUMAN EMBRYONIC STEM CELLS CULTURED ON LAMININ-521

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Embryoid body (EB) formation is a standard approach to assess differentiation potential of human pluripotent stem cells (hPSC). However, EB size has been shown to influence the differentiation course of the cells. In order to compare functional variability between individual hPSC lines it is therefore mandatory to use equal-sized EBs, using a standardized EB protocol. Our cells are routinely cultured on laminin-521™ (LN521) in Nutristem™ (NS) medium and passaged as single cells, providing feeder- and xeno-free conditions. In this system, hPSC expansion depends more on cell adhesion to the surface than

on cell-to-cell contacts, leading to less efficient EB formation when starting from a single cell suspension. As previously published and commercially available protocols were therefore not applicable, we developed a new single cell-based EB protocol optimized for this culture system. We aimed to obtain equal-sized EBs after spinning the single cell suspension in ultra-low attachment round bottom 96-well plates (5000 cells seeded per well) in the presence of ROCK inhibitor. In a first approach, cells were pre-differentiated for 2 days before harvesting. Results were highly variable, with cells either aggregating, partially aggregating or completely failing to form EBs. We did not observe significant differences between enzymatic vs non-enzymatic cell harvest approaches, but we noticed that differentiation in the presence of serum caused poor aggregation of EBs and early disaggregation, whereas aggregate formation and subsequent growth for 21 days was much better in Knockout™ Serum Replacement (KOSR)-based medium. Finally, we observed that the crucial factor influencing the aggregation of single hPSC was the growth phase of the cells. Only cells that were freshly passaged 2 days before EB formation were consistently forming aggregates, not only in KOSR-based medium but also in commercially available xeno-free APEL™ medium and even in NS medium. Our optimized EB formation protocol has until now been successful on 6 different hESC lines and 12 hiPSC lines. Our next step is to quantify the size of EBs formed by different numbers of cells during a 21-day differentiation course.

W-1512

THE STEMCELLFACTORY - AUTOMATED PRODUCTION OF INDUCED PLURIPOTENT STEM CELLS

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Reprogramming of patient cells to human induced pluripotent stem cells (hiPSCs), has tremendously facilitated the study of the molecular and cellular mechanisms underlying human disease pathogenesis and progression. The field of stem cell-based disease modeling is increasingly moving from monogenic diseases to complex disorders, creating an urgent need for standardized and automated processes for reprogramming and expansion of hiPSC lines from large patient cohorts. Industrialization of iPSC generation demands a broad set of expertise. We tackled this challenge by combining know-how in stem cell biology and process automation to establish a large system integration for the automated production of patient-specific hiPSC lines (www.StemCellFactory.de). The StemCellFactory provides automation and standardization of all required cell culture steps, ranging from adult human dermal fibroblast (HF) expansion via feeder-free Sendai virus-based reprogramming to clonal selection and expansion of the obtained hiPSCs. Measurement technologies for quality control, including high speed microscopy, have been implemented to ensure high fidelity performance via in-process data generation. We developed a fully automated, feeder free, Sendai virus-mediated, E8-based reprogramming protocol that delivers footprint-free hiPSC within 3 weeks with state-of-the-art efficiencies. Evolving hiPSC are automatically detected, harvested and clonally propagated in 24-well plates (CellCelector, ALS). Automatically propagated hiPSCs exhibit normal growth characteristics and pluripotency-associated marker expression profiles. Fully automated well- and plate-based splitting processes are scheduled via an image-based confluence measurement. FACS analysis shows sustained Tra1-60 expression across 34 days (10 passages) of automated cultivation (automated 94.4% ± 4.3% vs. manual 96.2% ± 3.5%). We expect the StemCellFactory to advance medical research by providing large numbers of hiPSC lines for disease modeling and drug screening at industrial scale and quality.

W-1513

EXPANSION AND NEURAL COMMITMENT OF HUMAN PLURIPOTENT STEM CELLS AS 3D SUSPENSION AGGREGATES

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3D suspension conditions are starting to emerge as a promising alternative to perform the efficient in vitro expansion and controlled differentiation of human Pluripotent Stem Cells (hPSCs). To facilitate the study of the effect of aggregate size in the expansion of hPSCs, aggregates ranging from 200 to 5,000 cells per aggregate were formed by forcing the cells to aggregate in microwells. After one week in culture, the fold increase in total cell number changed in an inverse proportion to the aggregate size and, therefore, 200 initial cells per aggregate reached the highest value (16.0 ± 2.7). Manually dissociated aggregates achieved intermediate fold increase values (3.8 ± 1.7), possibly due to aggregate heterogeneity. After three weeks in culture, at least 90% of the cells exposed to the different conditions were found to retain the expression of OCT4 and TRA-1-60. Also, several methodologies have been applied to induce the neural commitment of hPSC, including the Dual Smad Inhibition protocol. This method appears to require a higher initial cell density, attaining higher cell yields when using 5,000 or 10,000 initial cells per aggregate (5.0±0.4 and 6.6±0.5, respectively). Also, when using N2B27 chemically-defined neural induction media supplemented with LDN193189 and SB431542 small molecules, it was possible to decrease the percentage of OCT4-expressing cells from >95% at day 0 to ~3%, after nine days of neural commitment. Moreover, after replating the cells it was possible to give rise to neural rosette structures that were tested positive for the expression of the neural markers NESTIN, PAX6, FoxG1 and Sox2, confirming that this process is also suitable to efficiently induce hPSC neural commitment in a 3D suspension culture.

W-1514

CHARACTERIZATION OF HUMAN IPSC-DERIVED NEURONS IN 3D CULTURES

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Neuronal maturation and synaptic integration critically depend on extrinsic cues provided by the cellular and extracellular environment and therefore rely on three-dimensional organization. 3D rat primary brain cell cultures contain most of the brain cell types (i.e. neurons, astrocytes, oligodendrocytes and microglial cells). The high level of cell-cell interactions and the formation of extracellular matrix allow the cells to reach a high level of maturation, as evidenced by the formation of neurites, axons, synapses and compact myelin. In this study, we took advantage of these well-established 3D rat primary brain cell cultures to examine the development of reprogrammed human neurons in vitro. Human iPSCs were stably transduced with fluorescent proteins. These cells or the neuronal progenitors differentiated from them were integrated into the 3D rat cultures. The developmental time course of the human cells was then studied using immunostaining. We observed the establishment of neuronal morphology and the expression of dendritic, axonal and synaptic markers, indicating the establishment of polarization and synaptic connectivity. The coupling of 3D rat cultures with human iPSCs will enable the assessment of gene/environment interactions in the context of individual genetic background. Furthermore, this new model represents an interesting paradigm to investigate the role of cellular environment during brain development and is a promising tool to investigate neurodevelopmental disorders.

W-1515

LINCPINT KNOCKOUT MICE DISPLAY AGING-ASSOCIATED PHENOTYPES

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Large intergenic non-coding RNAs (lincRNAs) encodes for transcripts that resemble protein coding mRNAs in structure but do not code for proteins. To elucidate the biological functions of this new class of molecules, we employed VelociGene® technology to create knockout mouse lines with a LacZ reporter for 20 selected lincRNAs. The targeted lincRNA genes showed unique and diverse lacZ expression profile in both embryos and adults and of the 20 lincRNA genes targeted, the most remarkable expression pattern we observed was for Lincpint, which exhibited an increase in the extent and intensity as the mice aged. This striking age-associated pattern prompted us to conduct a longitudinal analysis for growth and any overt signs of health abnormality. The Lincpint−/− mice appeared healthy and normal at birth but as the mice aged, they exhibited progressive hair loss and sign of muscle weakening, severe lordokyphosis, reduced body fat, bone mineral density, and a significant decline in body weight and survival in comparison to wild-type littermates. Interestingly, Lincpint heterozygous mice exhibited an intermediate or a delayed onset of phenotype. This spectrum of age-associated pathologies in the Lincpint knockout mice, along with the unusual increase in gene expression with age, implies that mice may require a critical dose of Lincpint for the maintenance of health and tissue function in vivo, and points to potential role of Lincpint in physiological aging process with potential implications in human diseases. Our aim in initiating this work was not only to shed light on the functions of the 20 particular lincRNAs whose genes we chose to mutate, but also to obtain a better understanding of the general properties of lincRNAs as a class. This study reveals that lincRNAs, like proteins, serve diverse roles in development, physiology, and homeostasis. This collection could serve as a seed for a larger-scale effort to mutate many more members of the lincRNA gene family.

W-1516

COLLECTIVE AND EMERGENT BEHAVIORS OF SINGLE HUMAN STEM CELL DERIVED COLONIES

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Our view of clonal growth initiated by single stem cells is biased by a hierarchical model of cell differentiation, in which the transcriptional control of genes dictates growth and specialization in stem cell derived colonies. Populations, however, can display a second type of collective behavior, which is referred to as emergent, and is characterized by simple elementary interactions among population members. Collective emergent behavior can be measured, and separated from the consequences of gene-programmed behavior. What proportion of tissue growth remodeling and organization indeed reflects an emergent rather than a gene-induced behavior, is not understood. Nevertheless, biological examples of emergent behavior range from bacterial growth to the flocking of birds, with only few studies on eukaryotic cells and none on stem cell populations. To begin understanding emergent behavior in human cell populations, we acquired high-resolution time-lapse sequences capturing the evolution of a clonal colony starting from a single human bone marrow stromal cell over a period of 2-3 weeks. We employed a phase contrast microscope with a 5x lens, equipped with an on-stage CO2 incubator, and a 36 MP full-frame CMOS sensor camera, Nikon D800E. This set up results in a fieldwidth of almost 4 mm, and a 2 micron resolution, which allowed us to capture the entire colony at an appropriate resolution for image analysis. In the analysis phase, first a segmentation algorithm detected the cells in each image. Then the tracking algorithm linked the segments from successive pictures that belong to the same cell and constructed the cell trajectories that are used to determine the cell lineage tree. Next, we augmented a cell motility and morphology database. In this way, we were able to document that collective migration occurs within a growing colony in a density-dependent fashion as an emergent behavior, and critically contributes to generate a pattern within the colony. Second, we obtained preliminary evidence indicating that colony growth can be modeled as a special case of nematic particle morphogenesis, provided that data on cell division and migration are integrated in time. Finally, the resulting direct visualization of a colony as an observed lineage tree revealed the coexistence of symmetric and asymmetric patterns of growth.

W-1517

ROCK INHIBITOR PROMOTES THE PROLIFERATION OF MOUSE SALIVARY GLAND STEM CELLS

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Hyposalivation caused by curative radiation therapy is still a major clinical problem in head and neck cancers. Cell replacement therapy using functional salivary gland cells may improve this condition, but the culture method should be improved to obtain enough number of primary salivary gland cells. In the present study, we investigated the effect of Y-27632, a rho-associated coiled kinase (ROCK) inhibitor on the proliferation, survival, and phenotype of the salivary gland cells cultured in vitro. Here we show that Y-27632 significantly and efficiently promoted the proliferation of salivary gland cells. A time course EdU incorporation assay showed that Y-27632 substantially increased both the total cell number and the percentage of EdU-positive cells after 48 hours of the treatment. The viability of primary salivary gland cells was also increased by the treatment with Y-27632 as determined by calcein AM and propidium iodide staining. In vitro wound scratch assay demonstrated that both migration and proliferation of salivary cells were highly enhanced by Y-27632. Importantly, immunocytochemistry and western blot analyses revealed that Y-27632 markedly increased the number of cells expressing c-met, a marker of salivary gland stem cells but did not affect the number of both amylase-positive acinar cells and alpha-smooth muscle actin-positive myoepithelial cells. In summary, these data suggest that Y-27632 may selectively induce the proliferation of salivary gland stem cells.

W-1518

CONTROLLED CONDITIONS THROUGHOUT CELL HANDLING STEPS INCREASES CELL CULTURE YIELDS AT PHYSIOLOGICALLY RELEVANT IN VITRO OXYGEN LEVELS

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Awareness of the sensitivity of induced pluripotent stem cells to oxygen has driven use of oxygen-controlled incubators for more physiologically relevant in vitro conditions. Oxygen levels have a profound effect on stem cell fate. The timescale of cellular responses to oxygen control disruptions makes cell handling steps performed in the HEPA-filtered room air of a standard biological safety cabinet a potential risk to consistent cell culture performance. Since Hypoxia-Induced Factor - alpha proteins are down-regulated at the protein level within minutes of higher oxygen exposure and take hours to return to active levels upon return to the incubator, we hypothesized that even brief disruption of optimal conditions during routine handling would affect cell culture expansion. We tested this hypothesis using K562, a multipotential hematopoietic cell line often used for T cell production process development like CAR-T cell production. K562 cultures were split into six identical flasks and their growth in 5% oxygen/5% carbon dioxide monitored for 14 days. Three cultures were housed in an external incubator fitted with an oxygen-controlled subchamber. Processing steps were performed in a HEPA-filtered room-air laminar flow hood (Room-Air Hood). The other three cultures were grown in an incubation chamber installed within a closed processing chamber (Hypoxia Hood™), so that during all handling steps, conditions remained constant. All media for both groups were pre-equilibrated and pre-warmed before use. Statistically higher cumulative cell yields (p=0.003956, two-tailed T test, unequal variances) were seen under full-time oxygen control (Hypoxia Hood™) than in conditions that were broken briefly for routine cell sampling and passaging (Room-Air Hood). Cell viability was greater than 98% at all times in both groups, suggesting a cytostatic rather than cytotoxic effect on cell populations. We conclude that even without a highly visible effect on cell viability, cell cultures kept in physiologically relevant oxygen conditions are sensitive to brief disruptions in conditions. Full-time protection of cell cultures from room air, even during handling steps, may prevent detrimental long-term effects and increase in vitro cellular production yields.

W-1519

IN SITU LABEL-FREE ELECTROCHEMICAL MONITORING OF HUMAN PLURIPOTENT STEM CELLS ON CHIPS

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After differentiation, the precise quantification of undifferentiated pluripotent stem cells (PSCs) and a subsequent procedure to eliminate residual PSCs from a mixed population are critical for teratoma (or tumor)-free stem cell therapy. However, conventional methods such as fluorescence-activated cell sorting and real-time PCR analysis have technical limitations in terms of their sensitivity and recyclability. Herein, we took advantage of 'cell-chip' technology and designed a simple *in situ* label-free monitoring system on the basis of the unique electrochemical cathodic peak potential (E_{pc}) of human pluripotent stem cells (hPSCs) in vitro. The unique E_{pc} of both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) at -0.077 V disappeared after their spontaneous differentiation. The intensity of the cathodic peak current (i_{pc}) of hPSCs highly corresponded to the number of hPSCs and was detectable in a mixed population with differentiated cells. The electrical charge used for monitoring did not markedly affect the proliferation rate or molecular characteristics of human aortic smooth muscle cells, the differentiated counterparts of hiPSCs. After YM155 treatment to ablate undifferentiated hPSCs, their unique i_{pc} was significantly reduced. This suggests that detection of the unique i_{pc} of hPSCs is a valid approach to monitor potential contamination of undifferentiated hPSCs, which can assess the risk of teratoma formation efficiently and economically.

W-1520

RAPID EXPANSION OF HESCS AS AGGREGATES IN SUSPENSION BIOREACTORS

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A significant challenge in the application of human pluripotent stem cells (hPSC) is the generation of large numbers of highly pure undifferentiated cells prior to differentiation. To expand cells in a cost effective manner it is essential that a scale-up approach is taken. Previous work has typically employed a media designed for adherent 2D cultures yielding suboptimal growth rates and cell densities. To overcome these limitations we are developing defined medium optimized for suspension culture of hPSC aggregates. Two hESC cell lines (H1, H9) and 2 hiPSC lines maintained using standard 2D culture protocols in mTeSR™1 medium on Corning® Matrigel® were used for suspension culture experiments. Suspension cultures were performed in shake flask (~30 mL) and spinner bioreactors (~50 mL) with either a novel Bioreactor Medium (BM), a novel Fedbatch Medium (FbM) or mTeSR™1. Growth and seeding efficiencies were analyzed over several passages for each medium. Aggregates rapidly formed in suspension with up to 80% of cells seeded forming aggregates. Following seeding, aggregates were ~75µm in diameter by 6 hours and grew to ~400µm after 4 days (image analysis). Passaging was performed by decanting spent media and resuspending cell aggregates in the chemically defined, Gentle Cell Dissociation Reagent (GCDR) for 10 minutes at 37°C for dissociation to single cells. Following dissociation, cells were washed and resuspended in fresh media. Daily media feeding with 70% BM replacement was compared to the novel FbM strategy. Expansion rates were similar with both batch and fedbatch protocols using the new optimized medium formulations, with greatly simplified operation at larger volumes with fedbatch feeding. New media formulations enabled final cell densities up to 10-fold higher and growth rates 20% greater than standard 2D culture in mTeSR™1. Analysis of aggregates revealed uniform staining of pluipotency markers (Oct4, Tra-160) and no accumulation of necrotic cells within aggregate cores (PI staining). Cells grown as 3D aggregates were able to rapidly transition into 2D cultures in mTeSR™1 and generate colonies with high quality hPSC colony morphology and normal karyotype. Furthermore, 3D culture expanded hPSC readily differentiated into all 3 germ layers with high efficiency indicating that they retained pluripotency.

W-1521

STREAMLINING THE PGE IPSC LINE GENERATION - ESTABLISHING AN EFFICIENT AND QUALITATIVE PLATFORM FROM TRANSFECTION TO TARGET CLONE

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We have generated a streamlined process to produce Precise Genome Editing (PGE) hiPSC lines efficient and at a high quality. An in-house process has been developed to increases the throughput and efficiency of this workflow by optimizing each step of the process. 1. Transfection - establishing a reverse transfection method in 24 well scale 2. Cloning - depending on cloning strategy, FACS sort on GFP and clone by either single cell sorting or limiting dilution in 384 wp format. Comparing culturing systems and optimising culture conditions to obtain cloning efficiencies of 20-40% (depending on PGE line) 3. Clone maintenance - adapting the clone maintenance to an automated 384wp format 4. Clone split - adaption to automated 384wp format 5. Clone screening - combining automated plate screening with Echo dosing to pick and screen clones (fragment based screening, digital droplet PCR, NGS) To maintain flexibility in the workflow, several culture systems have been evaluated to full fill our defined criteria; robust, reliable, keeps the cells in a pluripotent state and leaves the cells transferable to most commonly used culturing systems available worldwide. We have chosen to proceed with three culturing systems, LN521 (recombinant laminin from BioLamina) in combination with NutriStem (Biological industries), DEF-CS (a complete system from Takara Bio Europe) and Geltrex in combination with E8 medium (Life technologies). All three systems have their own uses and advantages. LN521+NutriStem give us a very reliable, robust and homogenous pluripotent iPSC population that very easily differentiates to most cell types of interest and that gives us the highest yield of cells per passage and using Rock-inhibitor for passaging is unnecessary. DEF-CS has shown to be superior for the initial steps where we in this system, are able to get high transfection efficiency (up to 40%) and high clonal cell line generation both using FACS and limiting dilution in 384 well plates (10-40% survival).

W-1522

IMPACT OF FEEDING STRATEGIES ON SCALABLE EXPANSION OF HUMAN PLURIPOTENT STEM CELLS IN GMP-COMPLIANT SINGLE-USE STIRRED TANK BIOREACTORS

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The application of human pluripotent stem cells (hPSCs) and their derivatives in regenerative medicine and drug discovery will require the constant supply of high cell numbers generated by robust and well defined processes. This cannot be achieved by adherent 2D culture systems. Previous studies revealed the expansion of 3D aggregates in suspension as the most promising alternative. Stirred tank bioreactors are well monitored and controlled systems to optimize and up-scale suspension cultures and are widely used in pharmaceutical biotechnology for the production of recombinant proteins with mammalian cell lines. Recently, the expansion of single cell inoculated hPSCs as cell-only-aggregates in suspension, as well as the transfer of this technology into stirred tank bioreactors was shown by us. Thereby the culture medium was manually replaced daily ("batch-feeding"). Process analysis, however, showed that these culture conditions resulted in linear growth kinetics rather than aspired exponential cell growth. Perfusion mode, an alternative feeding strategy, is characterized by continuous exchange of depleted medium by fresh medium using a cell retention device to maintain cells inside the bioreactor. This feeding strategy supports better automation and consequently improved control of the culture environment including physiological parameters such as pO2, pH, and nutrient concentrations, and was technically established in GMP-compliant single-use stirred tank bioreactors. Here we present that perfusion mode results in more homogenous process characteristics, thereby yielding ~3 x 10^8 cells in 100 mL culture in 7 days. This represents an ~50% improvement of the cell yield compared to a parallel batch-feeding approach specified by the same culture medium requirements. Cells generated by both process strategies retained expression of pluripotency-associated markers as revealed by flow cytometry and global gene expression analysis, as well as

the ability to differentiate into derivatives of all three germ layers. Notably, protein-free and chemically defined culture media such as E8 are shown to be compatible with our stirred tank bioreactor approach, supporting clinical translation at reduced cost.

W-1523

GENERATION OF HEPATOCYTES ON A CHIP FROM HUMAN PLURIPOTENT STEM CELLS FOR DRUG SCREENING APPLICATIONS

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The combination of microscale engineering technologies with culture of human cells allows the development of new in vitro models for high-throughput drug screening applications. In this perspective, direct generation of functional hepatic cells from human pluripotent stem cells (hPSCs) on a chip would provide an attractive tool for multi-parametric studies in human development and diseases, overcoming poor availability of human primary cells. In this study, we employed discontinuous periodic medium delivery in microfluidic channels, with stage-dependent frequency, that is number of medium changes/day, as an effective strategy for modulating stem cell niche in vitro. We derived functional hepatic cells on a chip from hPSCs through optimal stage-dependent frequency, as revealed by qPCR analysis of endoderm and hepatic progenitors stages. Differentiated cells displayed high expression of CK-18, CYP-3A and ALBUMIN, Indocyanine Green digestion, glycogen storage capacity (75% of total cells) and albumin secretion. Moreover, compared to static conditions, we obtained higher hepatic markers expression, shortening of the time required for differentiation and 40% increase of albumin secretion. HPSCs-derived hepatic cells integrated in the microfluidic channels were directly used for temporal-defined acetaminophen stimulation in order to assess hepatotoxicity. A higher dose-dependent acetaminophen-induced toxicity was observed in static compared to microfluidic culture, consistently with a higher CYP-3A-mediated drug metabolism. The tight temporal control of medium delivery achievable by microfluidic technology also allowed new cytotoxicity assays, based on different posologies of acetaminophen administration in a 24 h timespan. Results showed that, even at low dose, repeated drug administration has a significantly higher cytotoxic impact than a one-shot at high dosage, regardless of the higher overall amount of drug in the single-administration case. Taken together, these results showed that the microfluidic environment together with optimized periodic perfusion frequency provides an effective methodology for generating hepatic-like tissues on a chip with remarkable functional differentiation, suitable for high-throughput drug testing in terms of drug concentration and posology.

W-1524

DEVELOPING DMSO-FREE CRYOPROTECTANTS FOR CELLS AND TISSUES: ONE SIZE DOESN'T FIT ALL

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Developing strategies for cryopreservation that advance our understanding of the effects of preservation media and freezing conditions on the viability, functionality and differentiation potential of stem cells is of critical importance for the development of new cell therapies and transplantation. Recently, cryoprotectant formulations have emerged that aim to reduce toxicity associated with the presence of DMSO in traditional media. The use of these requires the removal of DMSO following thawing and further washes to eliminate or reduce DMSO presence. In the search for clinically-friendlier CPAs it has recently emerged, however, that not all cells behave in the same way and different cells require stabilizers that act in different ways intra and extracellularly, which 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: mesenchymal stem cells, red blood cells and NK cells as well as complex tissues, by optimizing the medium composition and the freezing procedure. Preliminary studies have shown distinct cryoprotective behavior that is strongly dependent on the composition of the medium and the cell type. These studies have shed more light on the behavior of different, including traditionally hard-to-preserve, cell types during freeze/thaw processes. Finally, while cryopreservation of stem cells is not defined by any solid standards, novel formulations must not only ensure that cell functionality and viability is maintained after thawing, but must provide a methodology that can be routinely and consistently implemented by different end users with reproducible results. Moreover they must show low toxicity, and rapid clearance in vivo to be a suitable candidate for cell therapy products which are delivered at bedside.

W-1525

EFFICIENT METHODS FOR EDITING THE GENOMES OF HUMAN PLURIPOTENT STEM CELLS WHILE MINIMIZING UNWANTED MUTATIONS

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The CRISPR-Cas9 system has the potential to revolutionize genome editing in human pluripotent stem cells (hPSCs), but its advantages and pitfalls are still poorly understood. We therefore systematically tested the ability of CRISPR-Cas9 to mediate reporter gene knock-in at 16 distinct genomic sites in hPSCs. We optimized the assembly and delivery of targeting vectors and CRISPR-Cas9 and observed efficient gene targeting, in some cases approaching 100%. However, we found that targeted clones often carried an unexpectedly high frequency of insertion and deletion (indel) mutations at both alleles of the targeted gene. To overcome this problem, we designed strategies to physically destroy or separate CRISPR target sites at the targeted allele and to limit the temporal duration of Cas9 activity. Furthermore, we developed a bioinformatic pipeline to identify and eliminate clones harboring deleterious indels at the targeted locus. We then adapted these strategies to efficiently introduce disease-causing point mutations in hPSCs. Overall, the three-pronged approach we developed enables the reliable generation of knock-in hPSC reporter cell lines and isogenic point mutants free of unwanted mutations at the targeted locus. These methods for efficient genome editing dramatically extend the utility of human pluripotent stem cells and other stem cell types.

W-1526

CYTOTOXICITY OF HEAVY METAL IONS IN CULTURED NEURAL STEM/PROGENITOR CELLS AND DIFFERENTIATED NEURONAL/GLIAL CELLS

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We investigated cytotoxicity of zinc, copper, cadmium and mercury ions in mouse neural stem/progenitor cells (NSPCs) and differentiated progeny (neuronal/glial cells). IC50 values for cell-viability test of NSPCs following incubation with ZnCl2, CuCl2, CdCl2, and HgCl2 were 0.07, 0.03, 0.02, and 0.04 mM, respectively. Differentiated cells were less sensitive than NSPCs to ZnCl2 and CdCl2. Moreover, the gene expressions of metallothioneins (MTs) that are involved in heavy metal detoxification were measured. Induction of differentiation led to an increase in MT family gene expression (Mt1, Mt2, Mt3, and Mt4). Zinc exposure induced a dose-dependent increase in Mt1 and Mt2 expression levels in both NSPCs and differentiated cells. Our results suggest that the change in heavy metal sensitivity associated with differentiation from NSPCs to mature neurons and glia is related to the upregulation of MTs.

W-1527

DAS GRAPHENE BASED FEEDER-FREE CULTURE SYSTEM FOR HUMAN INDUCED PLURIPOTENT STEM CELLS

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Graphene has been selected as a candidate for synthetic feeder free culture substrate in the stem cell research field. However, conventional graphene grown via chemical vapor deposit (CVD) process is not an ideal material to maintain the pluriptency of human pluripotenct stem cells (hPSCs) including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) due to its intrinsic hydrophobicity and relatively flat surface topography. Here, we applied morphology-controlled nanographene (NG) coating onto the culture substrates via diffusion-assisted synthesis (DAS) process and cultivated hPSCs. It is found that enhanced hydrophilicity and controlled surface roughness of nanographene grown via DAS process (DAS-NG) enabled tight adhesion of human pluripotent stem cells onto the DAS-NG coated culture substrate and retained pluripotency for over 2 weeks. It is also found hPSCs grown on DAS-NG (hPSCs-DAS) shared comparable global gene expression profile with hPSCs co-cultured with mouse embryonic fibroblast (MEF). Importantly, the similarities in cell adhesion gene expression between hPSCs-DAS and hPSCs on MEF suggest DAS-NG may provide comparable physical cues with MEF for sustaining pluripotency. Taken together, our findings show a DAS-NG based non-xenogeneic feeder free-culture platform for long-term hPSC culture.

W-1528

ISOLATION AND BIOBANKING OF ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS WITH SEPAX

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Stromal vascular fraction (SVF) including mesenchymal stem cells could be isolated from fat tissue. Manual isolation is long, non-automated, non-reproducible technique for cellular therapy products. The closed GMP grade SEPAX widely used to isolate cells from umbilical cord blood, but never tested for the isolation of MSCs from adipose tissue. Fat tissue obtained from 101 donors after liposuction. Adipose tissue was digested with collagenase and SEPAX2 was used to separate the SVF cells from the cell suspension. For control experiments the well-known manual methods was used. The number and the viability measured with Trypan-blue exclusion assay, cell culture morphology screened with bright microscopy. Multicolour FACS was used to determine the phenotype and conventional differentiation assays were performed to test multipotency of the isolated cells. Statistical analysis was used to analyse and compare the results of the two methods. The same amount of nucleated cells could be isolated with the manual (18.215.733 $\pm$ 16.852.945) and SEPAX (20.669.770 $\pm$ 24.349.902) method from similar amount of fat tissue and no significant difference was found in the proliferation of the cells when cultured in vitro as well. Cells isolated with the conventional manual method were positive for CD105 (65.05 $\pm$ 21.95%), CD90 (92.98 + 22.77%), CD73 (85.76 $\pm$ 22.77%) and CD44 (63.40 $\pm$ 25.20%) and were negative for hematopoietic markers respectively (CD34 and CD45). In the case of SEPAX isolation the phenotype was the same whereas CD105 (66.84 $\pm$ 21.91%), CD90 (94.42 $\pm$ 19.00%), CD73 (87.84 + 20.49%), and CD44 (68.55 + 22.76%) expressed by the cells but CD34 and CD45 was missing. Cells isolated with both methods were able to differentiate in vitro to bone, cartilage and fat respectively and no difference observed between the differentiation capabilities before freezing after freezing. The isolation of SVF cells such as MSCs with SEPAX has the same results as the conventional methods, but the process is faster, automated, standardized and GMP comfortable as well. Based on the criteria of the ISCTE (2006) cells separated with SEPAX were fit to the requirements and could be expanded in vitro more than 100 days respectively as for cellular therapeutic products respectively.

W-1529

MODELING SYNTHETIC CHROMOSOME DELIVERY OF ANTI-CANCER FACTORS FROM MURINE MESENCHYMAL STEM CELLS

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Bioengineered, autologous, adult-derived stem cells represent

a unique cell-based therapeutic approach to deliver localized tumoricidal factors to inhibit cancer growth and proliferation. While in its technological infancy, bioengineered adult-derived mesenchymal stem cells (MSCs) show early promise as a delivery system of therapeutic cytokines in a variety of clinical applications. With the ease of clinical isolation of autologous MSCs and their demonstrated robust tropism for a variety of tumors in vitro and in vivo, engineered MSCs have been proposed as therapeutic anticancer vehicles. Our long-range goal is to develop a tractable, versatile, synthetic chromosome-based platform (termed ACE chromosome), onto which multiple anti-cancer factors may be placed for directing a multi-targeted approach to cancer therapy. The ACE chromosome is an autonomous synthetic chromosome "circuit board" designed to contain approximately 70, site-specific, recombination acceptor sites that can carry single or multiple copies of genes of interest. Bioengineered ACE chromosomes are readily purified by flow cytometry and chromosome sorting making the ACE system amenable to stem cell bioengineering. To date, the ACE system has been used to bioengineer a variety of stem cell types including human mesenchymal stem cells (hMSC), CD34+ human hematopoietic stem cells (hHSC), murine embryonic stem cells and other primary and transformed cells. As an initial proof-of-principle of our synthetic chromosome system's ability to produce multiple anti-cancer agents, we have utilized the ACE system to produce decorin (DCN), a small pericellular matrix proteoglycan that modulates collagen fibrillogenesis and tumor cell proliferation and interferon-beta (IFN-beta), a cytokine with anti-proliferative and immunomodulatory properties. Based upon our earlier demonstration of ACE platform delivery to hMSCs, our long-range goal is to generate DCN and IFN-beta producing murine MSCs for localized delivery of this tumoricidal factor to inhibit cancer growth. Results from our efforts indicate that in vitro production of these anti-cancer factors from murine MSCs provide a model for human therapeutics using mesenchymal stem cells.

W-1530

DECODING TOPOGRAPHIC SURFACES FOR INDUCED PLURIPOTENT STEM CELL GROWTH

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For the survival, growth and self-renewal of human induced pluripotent stem cells (hiPSCs) physical attachment to extracellular matrix (ECM) components is indispensable. It has been indicated that topographic surfaces may lead to a more reproducible regulation of stem cell fate. However, the current view on topographies lacks the understanding of multiple topographical cues including size, spacing and shape. We made use of the high-throughput screening platform Topochip which was designed using algorithmically arranged triangles, circles and rectangles to investigate multiple parameters simultaneously. Topographies were prepared by hot embossing of a polystyrene film to improve the optical properties for microscopy and reduce the cost of fabrication. To best model the ability to grow as single cell, topographies were seeded at low density (100 cells/mm^2). No coating prior to cell seeding was required. We selected topography hits with the aim of comparing topographies that sustain pluripotency to those that prevent hiPSC expansion. Top hits and bottom hits were defined as 100 topographies with the highest and lowest number of Oct4 positive cells, respectively. Standard tissue culture polystyrene was used as a control. 24 hours after cell seeding, top hits gave rise to three-fold higher Oct4 and EDU positive cells compared to the control. Significantly less Oct4 positive cells were found on the bottom hits compared to the control. Further analysis revealed that more cell clusters and a higher number of cells per cluster were found on top hits compared to the control and bottom hits. We next developed a mathematical model by comparing the top hits with the bottom hits to enable the prediction of hiPSC growth on any topographical surface. Furthermore, we were able to identify crucial topographical parameters that support the growth of dissociated hiPSCs in a xeno-free and fully defined environment. To confirm that the predicted responses reflect those from experiments we tested our model on novel topographies. In conclusion, we have gained an important insight from this screen that could be adapted for a more reproducible regulation of stem cell fate for the development of effective stem cell-derived therapies.

W-1531

SCALABLE EXPANSION OF NEURAL STEM CELLS SUPPORTED ON ELECTROSPUN NANOFIBER SCAFFOLDS: MODELING AND EXPERIMENTAL APPROACHES

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Therapeutic strategies based on stem cell therapies require large cell numbers, however these are found in low numbers in the human body. Therefore, there is a call for ex-vivo scaled-up processes in order to overcome the limited availability of stem cells. When envisaging regeneration of neural tissue, cultivation of neural stem cells (NSC) on aligned nanofiber scaffolds is particularly attractive. Such substrates actually provide physical guidance with high surface-to-volume ratio, contributing to cell attachment and orientation, in structures mimicking the natural extracellular matrix environment. Culture of NSC within nanofiber scaffolds is usually performed in static systems, often neglecting the scalability required for the systematic production of tissue transplants, or tissue engineered constructs. Effective supply of adequate factors (nutrients, growth factors, oxygen), cell interactions and shear forces are also critical. Therefore a novel scalable system, inspired by the "plate-and-frame" geometry, for culture of stem cells combining cues from controlled hydrodynamic conditions with electrospun scaffolds for guidance of human NSC culture is presented. In this work, the flow structure and shear stress in the bioreactor was investigated by computational fluid dynamics (CFD). Analysis of the CFD model results demonstrated that a bioreactor working volume of 30 mL and agitation rates above 45 rpm are necessary to promote efficient mixing in the vessel. The system was evaluated for the expansion of a NSC line derived from the ventral mesencephalon region of human fetal brain (ReNcell VM) in the bioreactor, containing 6 frames with aligned polycaprolactone nanofibers functionalized with RGD motifs. The CFD predictions were validated and, after 8 days, a 7-fold increase in cell number was achieved, with a uniform expansion of the cells

along the nanofibers. The maximum concentration of lactate in the culture medium throughout culture time was 5mM, which is not inhibitory for NSC growth. Cells expanded in the bioreactor could be successfully differentiated into neurons and glial cells as evaluated by immunocytochemistry. These results are promising for scaled up production of aligned cells for stem cell biology research, regenerative medicine or other biomedical applications.

W-1532

HIGH-THROUGHPUT MICROFLUIDIC SCREENING OF HUMAN MESENCHYMAL STEM CELL PROLIFERATIVE RESPONSES TO FGF-2 AND HEPARAN SULFATES

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Human mesenchymal stem cells (hMSCs) are currently pursued as a cell therapy for both regenerative and immunosuppressive applications. Cost-effective scale-up of quality hMSCs remains a key challenge prior to clinical deployment. FGF-2 is a key mitogen for hMSC growth, and can be supplemented exogenously in addition to autocrine production by hMSCs themselves. A critical component for FGF-2 and signalling through its receptor FGFR1 is cell surface heparan sulfate (HS), being involved both in associating ligand with receptor, and in facilitating export of autocrine FGF-2. In this work, we utilise a HS fraction affinity-selected for FGF-2-binding capacity ($HS8^{+ve}$), and deliver it to bone marrow-derived hMSCs in combination with exogenous FGF-2, whilst tracking growth responses. To gain further insight into autocrine effects under different FGF-2/$HS8^{+ve}$ stimulation regimes, we utilise continuous-flow, high-throughput microfluidic cell culture arrays (microbioreactor arrays). This platform streamlines high-throughput combinatorial analysis of the various treatments, exploiting image cytometry to offer single-cell resolution data. Microfluidic screening revealed $HS8^{+ve}$ cooperates with both exogenous and endogenous FGF-2 to increase $Ki67^{+}$ proliferating hMSCs in early phases of a passage of culture, leading to dramatic and enhanced expansion of hMSC numbers throughout a 6-day culture cycle. We present evidence for $HS8^{+ve}$ altering the autocrine/paracrine responses of the cells under FGF-2 co-stimulation, likely by increasing FGF-2 availability in the bulk culture medium. Controlling and exploiting the bioactivity of exogenous and endogenous FGF-2 will be beneficial in optimising hMSC expansion strategies for clinical scale-up.

W-1533

EXPERIENCE WITH ISOCELLPRO WORKSTATION FOR CELL THERAPY PRODUCT: A PILOT CLINICAL APPLICATION FOR BURN PATIENT.

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Good Manufacturing Practice (GMP) compliant procedures are a prerequisite for cell production in clinical application and clean rooms are ideal zones for cell therapies production. The clean rooms useful for clinical application require high running and maintenance costs and need trained operators and strict procedures to prepare the rooms and the people involved in the processes. This requires huge efforts in terms of facilities, personnel training and quality control. To provide a streamlined workflow environment reducing the set up and running costs of cell therapy products we have used ISOCell PRO, a Cell Therapy Grade A Isolator alternative to the use of GMP class A Biological Safety Cabinet in a class B clean room environment. Indeed, ISOCell PRO is a Closed System that requires Grade D surrounding environment. The Positive Pressure Isolator guarantees Grade A environment in the working area with aseptic conditions according to GMP. A CO2 incubator is integrated, what makes the system easily validated at affordable costs. Decontamination process is automatic, fast, safe and economically affordable and no need for special operators' clothes. Actually, SwissMedic completed the validation of a GMP process involving the use of ISOCell PRO units settled at the Centre de Production Cellulaire (CPC-CHUV - Lausanne). The CPC in compliance with GMP produces tissues and cells necessary to the treatment of burn patients ensuring the production of autologous skin (the patient is the donor and the recipient of its own cells). With these treatments, CHUV is for many years a center of reference at Swiss, European and international levels for the care of burn patients. The CPC are ongoing the validation of other cell type productions for clinical trial in cardiology and neurosurgery. Our experience suggests this workstation as a possible alternative to the classic clean room due to its small size and the simplification of the working and maintenance operative procedures. In conclusion this kind of isolators may represent an interesting solution in the perspective of the more and more strong request for costs reduction of GMP in clinical application.

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

W-1537

QUALITY CONTROL AT STEM CELL DRIVES: A CHECKLIST-BASED APPROACH

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Checklists are important tools in error management, and their use improves best practice adherence. Checklists are being increasingly implemented to multiple domains of healthcare; however, no published checklists exist which outline a process for stem cell donor recruitment onto donor-databases at stem cell drives. We have

previously described a model of stem cell drive design including five stations: prescreening, informed consent, registration, swabbing, and reconciliation (Fingrut, 2015). Here, we describe the design and suggested implementation of a checklist-based approach to ensuring quality control at stem cell drives. We have constructed checklists for each station of the stem cell drive, striving to include relevant quality control aspects. The prescreening station checklist reviews donor eligibility criteria, characteristics of the most-needed donors, and suggests strategies to redirect different categories of ineligible donors to help out as they are able. The informed consent checklist lists specific information that recruiters need to share with registrants to secure informed consent. The registration checklist outlines common and important registration form errors that recruiters can identify and correct, and reminds recruiters to discuss details of data collection, storage, and usage with registrants. The swabbing checklist outlines a process for recruiters to guide registrants to provide a buccal swab using the correct technique. Finally, the reconciliation checklist lists possible errors throughout the previous stations that must be corrected if present. This checklist also includes a set of final information that should be delivered to the registrant. Implementation of checklists at stem cell drives provides recruiters with a memory recall tool, allowing them to review information they need to deliver to registrants and to screen for common errors. Additionally, they allow for standardization across stem cell drives organized and staffed by different teams. In summary, we propose that these checklists be made available at stem cell drives, with stem cell drive staff instructed to refer to them for guidance during their shifts. Further, we propose that stem cell drive training materials and competency assessments be updated to incorporate these checklists.

W-1538

IDENTIFYING BARRIERS TO OBTAINING A TRULY INFORMED CONSENT IN FIRST IN HUMAN STEM CELL TRANSPLANT TRIALS IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is a progressive and disabling neurodegenerative disorder that may be amenable to neurorestoration using cell transplantation. First-in-humans clinical trials testing intracerebral transplantation of stem cell based therapies in PD are imminent. Attention to ethical issues such as developing parameters to ensure that research participants give a genuinely free informed consent is thus critical. We performed a systematic review of the literature using search terms: "informed consent", "therapeutic misconception", "Parkinson's disease", "bioethics", "stem cell therapy", "cell transplant", and identified the following themes. (1) Barriers to adequate disclosure (investigator-related). In first-in-human trials, risks and benefits are incompletely defined. The substantial literature in fetal tissue transplant in PD has only partial applicability, and serious risks remain to be determined, including tumor formation, graft rejection, and risks of associated immunosuppression if administered. Limitations in animal models and complexities of making inferences from these models to human clinical trials need to be addressed. (2) Barriers to understanding clinical research methodology (participant-related). Research participants may have difficulty understanding concepts integral to clinical research, such as equipoise. There is also strong evidence that in previous first-in-human surgical trials in PD the decision to participate was influenced by therapeutic misconception. Moreover, impaired understanding of relevant trial information may be compounded by cognitive dysfunction common in PD. (3) Need for extended consent process (investigator- and participant-related). A single visit to obtain informed consent, appears suboptimal, and an extended process would be more appropriate. In summary, overcoming these barriers is imperative, but may place additional burden on study personnel and participants. Possible solutions involve use of multimedia educational materials, research advocates, and multiple disclosure sessions. Studies will therefore be needed to ascertain their efficacy in meeting specific requirements to improve informed consent for first-in-human stem cell transplant trials in PD.

W-1539

THE ANDALUSIAN INITIATIVE FOR ADVANCED THERAPIES: A PIONEER MODEL TO FOSTER THE INCORPORATION OF INNOVATIVE THERAPIES IN THE PUBLIC HEALTHCARE SYSTEM

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Andalucia, with more than 500 patients treated with different investigational advanced therapies, is one of the leading regions in Europe in this field. This outstanding result has been possible because of a pioneer organizational model developed only few years ago. The Andalusian Initiative for Advanced Therapies (IATA) is a publicly funded organization created by the Regional Government of Andalusia to promote activities of research and development in the fields of Cell and Gene Therapy, Regenerative Medicine and Nanomedicine. The IATA aim is to develop safe and efficient treatments to be offered to the population by incorporating innovative advanced therapies in the public healthcare system of our region. The IATA is part of the Andalusian Public Healthcare System (APHS) that offers complete health services to about 9 million people. The APHS comprises: 47 hospitals, 1,500 primary care centres, several research centres and institutes, a network of 10 GMP facilities to manufacture gene and cell based therapies, a Genomics and Bioinformatics Platform and a Biobank storing more than 750.000 samples including patients and normal hiPS and hES cells lines. Being part of such a multidisciplinary system makes the IATA an ideal partner offering comprehensive support for translational research. The IATA fosters national as well as international alliances between the academic world, research institutions, health centres, SMEs and the pharmaceutical industry. The IATA acts as promoter of 24 clinical trials with different gene and cell-based therapies. We offer regulatory and scientific advice from the preclinical to the clinical phase of research. Our specialized knowledge allows the IATA also to offer, among other training programmes, an International Master's programme in manufacturing of advanced therapy medicinal products, unique in Europe, organized together with the University of Granada

W-1540

UNPROVEN STEM CELL TREATMENTS AND RE-EVALUATING THE ROLE OF THE US FDA: BRINGING THE LOST AND DESPERATE BACK HOME

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In 2004, patient advocate groups were major players in helping implement and pass significant public policy and funding initiatives in stem cells and regenerative medicine. In the following years, advocates were also actively engaged in Washington DC, encouraging policymakers to broaden stem cell research funding, which was ultimately passed after President Barack Obama came into office. Advocates did this because they were told stem cell research would lead to cures. After waiting more than 10 years, many of these same patients are now approaching clinics around the world offering experimental stem cell therapies instead of waiting for scientists in the United States to go through clinical trials. How did the same groups who were once (and often still are) the strongest supporters of stem cell research now become the opponent? And how can scientists work to bring them back home? In this paper, we will argue that this situation in which these experimental interventions continue to be marketed and provided is problematic. Public policy should be developed to correct the current situation. Central problems include patients lack protection of US liability standards, regulation of clinical sites, and clinician licensing. In addition there is inconsistent or non-existent follow up care and no assurance for patients that they are receiving the intervention promised or of what dosage they are receiving. Furthermore, these experimental interventions have insufficient evidence of safety and efficacy; patients may be wasting money and time and forgoing other opportunities for an intervention that has not been shown to be safe and effective. Finally, current practices do not contribute to scientific progress because the information from patients is not suitable for follow up research to measure outcomes. Using the HIV/AIDS and breast cancer advocate cases as examples, we will identify key priorities and goals for this policy effort. The current landscape of cell-based interventions and stem cell tourism should prompt a similar re-evaluation of current approaches with respect to the design, initiation, and conduct of US clinical trials.

W-1541

HOW SHOULD WE PROVIDE ETHICAL SUPPORT FOR THE NATIONWIDE STEM CELL RESEARCH PROGRAM?

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This poster discusses effective ethical support for the nationwide stem cell research program in Japan. It is based on our continuing experience with this issue in a governmental project, the "Highway Program for Realization of Regenerative Medicine". Stem cell research is growing rapidly around the globe. Japanese ministries and Japan Science and Technology Agency have supported such research and promoted its clinical application. This Program includes an ethical support team to deal with ethical issues in stem cell research. As a member of this team, we have developed our support strategy year by year. This presentation involves analysis of our group's consultation experience in light of its results. A comparison of consultations from 2011 to 2014 shows a correlation between their contents, our supporting activities and the entire program's progress. In 2011, we started our involvement with this program. The following year we started to promote what kind of ethical support we could provide for the researchers. In 2013, we received additional requests based on our promotion, such as collaboration with patient associations and the creation of documents for informed consent. We started providing consultations and education services, and conducted research. Following these results, in 2014 we reconstructed our support system in three main ways: providing ethical advice to researchers, offering education opportunities to researchers and the members of the ethics committee, and conducting research to support this program. As a result, half of the consultations carried out fall into this new category and in one-third of cases, researchers might acknowledged the ethical issue based on our education services. Since establishing the ethical support team, we have formed one type of ethics support system for this program and it seems to be making progress. However, we must admit the limitations of the issues that we have handled, including site-specific subjects and ethical matters outside the program. Thus, we should continuously consider what constitutes appropriate ethical support for large-scale research projects in advanced technologies.

W-1542

THE ETHıCS OF HUMAN EMBRYONıC STEM CELLS RESEARCH (SAMPLE OF TURKEY).

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The major obstacles to widespread application of a cell based therapy for uncurable diseases. Stem cells act as natural units of embryonic development and tissue regrow. Than human embryonic stem cells (HESc) are a potential source of tissue for regenerative therapies and hold huge research areas and therapies. The aim of this study was to investigate describing views of physicians about ethical principles in the embryonic stem cell research/therapies in Turkey. We planned a multicultural health center research. It contained university hospitals, private hospitals and state hospital's IVF center, hematology and oncology departments in Turkey. This centers consisted of the study's sample. Besides study contained voluntary participant physicians only. We analyzed the outcome of 123 doctors asked forms. On the basis of the results of the study, the mean age of the study group was 38.8 years in this study. Forty physicians (35%) were female, eighty three physicians (65%) were male and ninety six physicians (78%) were married, physicians twenty seven physicians (22%) were single. Eighty one participants of this study (66%) were studied in university hospitals and seven two physicians (62%) were more than nine years experimentations. Opinions of the participants about ethic on HESc research weren't different according to gender ($t=0.435$, $p>0.05$), age groups ($F=0.585$, $p>0.05$), working organisation ($F=0.202$, $p>0.05$) and state of transplantating stem cell in their departments ($t=0.277$, $p>0.05$). To data on this point in study, there isn't a significant relationship between views of participants on ethical principals of HESc researches and about clinical aplications.

W-1543

UNCERTAINTY AND INNOVATION: ASSESSING THE ROLE OF CELL-BASED MANUFACTURING FACILITIES IN SHAPING REGULATORY AND COMMERCIALIZATION ENVIRONMENTS

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Creating a regulatory and commercialization environment that is propitious for innovation in regenerative medicine (RM) remains a priority. Several jurisdictions are in the process of updating their regulatory frameworks to integrate increasingly novel and complex technologies, which do not fit into existing frameworks designed for pharmaceuticals and medical devices. Others, such as Canada, still has limited regulatory guidance, relying on an informal, case-by-case approach. Yet, the Canadian model is perceived as favorable, due to their accelerated licensing system which facilitated the regulatory approval of the first stem cell product in the world. The RM translational process is characterized by scientific, technical, regulatory and commercialization challenges. However, the relationship between these factors and innovation is not well understood. To provide a better understanding of these issues, we conducted a qualitative descriptive study in order to understand how Canadian cell manufacturing GMP facilities manage within the current regulatory and commercial environment. Data was collected by conducting semi-structured interviews with investigators, facility employees and technology transfer officers, as well as officers of a regulatory authority (N=27). Thematic analysis was used to analyze the data. The major themes that were generated were the following: 1) managing uncertainty, 2) the challenges of manufacturing and, 3) the role of networking in shaping the regulatory and commercial landscape. This presentation focuses on the findings arising in this study. From the unpredictable behavior of the cells to the fast-changing nature of translational technologies, and an evolving regulatory and commercialization environment, we consider how these facilities manage scientific and regulatory uncertainty. Finally, we consider the role of networking in redefining the regulatory and commercial landscape by addressing various manufacturing, regulatory and commercialization challenges and, in turn, standardizing the field.

CANCER CELLS

W-1545

THE EXPRESSION OF CD49 IN THE CONTEXT OF CD66+ CELLS, MARKS THE CELLULAR PHENOTYPES LINKED TO HUMAN INVASIVE CERVICAL CANCER PROGRESSION

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Cervical cancer is the second most common cancer among women worldwide. It's driven by highly oncogenic human papilloma virus sub types which intercalate into the human genome. The viral oncogenes deregulate key cellular functions and are complemented by persistent activation of pathways such as "Notch". Both the basic biology and therapeutic management of recurrent/progressive cervical cancers is poorly understood. We are interested in the nature of distinct cellular sub-sets that may be critical to cervical cancer progression and our recent work has delineated a sub-set of CD66+ with unusual properties in both invasive and early cancers. In this study, we are focusing on i) Using other markers such as CD49f, can we better understand transitions from invasive cervical cancer to metastasis? ii) Is there a segregation of cellular behaviour such as migration, proliferation etc in this transition which correlates with the pattern of CD66, CD49f expression and will give us insights into how these tumours progress? iii) Can we integrate clinical information with the use of established cell lines to make a coherent model? Spheroids of cervical cancer cell line CaSki when embedded in matrigel forms collectively invading protrusions. The invading cells are differentiated CD66+CD49f- cells. TGF beta, an EMT inducing growth factor increases the CD66+CD49f- subset. This subset of cells is also non-proliferating and Cisplatin resistant. The cells at the outer edge of the invasive protrusions dedifferentiate to more basal CD66-CD49f+ proliferative cells after migration. In cervical cancer clinical samples, CD66+CD49f- cells have differentiated phenotype and they overlap with the sarcomatoid cells which have undergone partial EMT. We have combined flow cytometry with immunostaining of key regulators and find that high levels of TGF beta 1 and nuclear pSmad3 are observed in patients with high percentage of CD66+CD49f- cells. We suggest that progression of human cervical cancer invasive tumour to metastasis involves various cellular states like migration (CD66+CD49f-) and proliferation (CD66-CD49f+) which is accomplished by switching cellular states. In conclusion we have identified CD66+CD49f- as a differentiated sarcomatoid cellular state with features of non proliferation, cisplatin resistance and invasion.

W-1546

THE EPHA2 RECEPTOR IN STEM-LIKE TUMOR-PROPAGATING CELLS FROM HUMAN GLIOBLASTOMAS AND IN NEURO-GENIC NICHE: SIGNIFICANCE AND ROLE IN THERAPEUTIC TARGETING.

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Human glioblastomas (hGBMs) have now been shown to contain a minor subset of cells bearing the defining features of somatic stem cells and the ability to establish, expand and perpetuate these tumors. They are defined stem-like tumor propagating cells (TPCs). Here we report that hGBM TPCs overexpress Ephrin receptor type A2 (EphA2). Cytofluorimetric sorting into $EphA2^{High}$ and $EphA2^{Low}$ populations demonstrates that EphA2 abundance provides a measure of the stem-like potential, invasiveness and tumor-propagating ability of these cells, correlates with both the size and tumor-propagating ability of the TPC pool in hGBMs, can be used to enrich for TPCs. Notably, $EphA2^{High}$ expression profiles impact on several signaling pahways involved in the regulation of proliferation,

migration and neurogenesis. Moreover, we show that EphA2 is also involved in the architecture of the adult mouse and human SVZ. Most important, we found that EphA2 knockdown suppress TPC self-renewal ex vivo and intracranial tumorigenicity in hGBM bearing mice. This points to EphA2 downregulation as a causal event in the loss of TPC tumorigenicity. Finally, we also analyzed the TCGA data set for relative EphA2 expression in distinct hGBM sub-categories and the correlation with TPCs key pathways and patient survival. Our work identifies TPCs as a major target of EphA2 overexpression in hGBM, which is shown to drive their self-renewal and tumorigenicity. It proposes EphA2 receptor as a specific marker to enrich and distinguish, in combination with others already identified, a neural stem cell (NSC) from a neural progenitor. It also suggests TPCs as a specific cellular target in which the enforced down-regulation of a selective molecular target, such as EphA2, may be exploited for therapeutic purposes.

W-1547

TARGETING THE MET ONCOGENE OVERCOMES GLIOBLASTOMA STEM-LIKE CELL RADIORESISTANCE

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Glioblastoma is a relatively rare but virtually incurable brain tumor, with an estimated median survival of 12-15 months, in spite of an aggressive treatment protocol including surgery, radiotherapy, and chemotherapy. Primary resistance to DNA-damaging agents, mostly ionizing radiation, has been associated with inherent properties of the glioblastoma stem-like cell (GSC) subpopulation, but it relies on molecular mechanisms still poorly understood. We show that radioresistance of GSCs, isolated in culture as neurospheres, is extensive, and independent of GSC mutational status and gene expression profile. In a subset of neurospheres, characterized by low EGFR expression, ionizing radiation positively selects a subpopulation of cells that express high levels of the HGF receptor MET, and retain stem-like properties. The MET tyrosine kinase actively sustains GSC radioresistance, by promoting the DNA repair pathway. Conversely, pharmacological inhibition of MET impairs the mechanisms of GSC radioresistance and helps ionizing radiation to deplete the GSC subpopulation. Treatment of tumors generated by neurosphere transplantation shows that MET inhibitors cooperate with radiotherapy in reducing tumor volume and prolonging mouse survival. Serial transplantation of GSCs isolated from treated tumors shows that, while radiotherapy alone selects for more aggressive GSCs, association with MET inhibitors significantly reduces the GSC frequency, exhausting the tumorigenic potential. These findings provide preclinical evidence that MET inhibitors can radiosensitize the cancer stem cell component of glioblastoma.

W-1548

ADHESIVE SIGNATURE TECHNOLOGY FOR TUMOR INITIATING CELL PURIFICATION IN CANCER RESEARCH

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The strength with which a cell binds to a surface is dependent, among other things, on both the cell's lineage and differentiation state. Our lab has recently developed a methodology to isolate cells based on their unique adhesion binding strength to the matrix. The novel technology (micro-Stem cell High- Efficiency Adhesion based Recovery [µSHEAR]) consists of a microfluidic device that applies varying degrees of shear force on cells. Using this device, human pluripotent stem cells (both hiPSCs and ESCs) have been isolated from their parental cells, spontaneously differentiated cells, and partially reprogrammed cells with high reproducibility, yield (>97%), purity (95-99%), and survival (>95%) rates. The process is fast (10 min) , label free, and scalable. In spite of the major advances in cancer research, many patients still fail to appropriately respond to therapy and develop cancer relapse or metastasis. It has been suggested that this is in part due to a small subpopulation of tumor initiating cells with stem cell like properties that are responsible for the growth of the tumor and the progression of metastasis. There is currently no efficient and reliable method to isolate these tumor initiating cells in order to study them. The objective of this research is to isolate the rare tumor initiating cells from the general cancer cell population by exploiting differences in adhesion strength. The research is innovative because it focuses on developing a novel method of tumor-initiating cell purification. The proposed purification strategy has been proven to work for pluripotent stem cell purification, offering higher yield, purity, efficiency, and survival than other methods. Preliminary data in the lab shows that different cancer cell lines have distinct adhesion strength signatures. The lab intends to study these differences in adhesion strength and investigate the possibility of separating different cancer populations by using the µSHEAR technology. Furthermore, other groups have reported the development of cancer cell lines that are stuck in this stem-like state. We intend to develop these lines and study their adhesion properties as well.

W-1549

EPIGENETIC REGULATION INVOLVED IN OBESITY INDUCED CANCER STEM CELL TRAITS

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Key mechanism involved in obesity-related breast cancer progression still remains elusive. Here, using genome-wide ChIP-seq and RNA expression profiling, we discover that leptin, an obesity associated adipokine, activates a novel STAT3-histone methyltransferase G9a complex to epigenetically silence a cohort of targeted genes and non-coding RNAs involved in regulation of cell differentiation and epithelial homeostasis. Activation of STAT3-G9a signaling promotes the gain of cancer stem cell (CSC) traits to induce a subset of highly tumorigenic breast CSCs enriched by the cell surface marker leptin receptor (OBR^{hi}). In contrast, inhibition of STAT3/G9a converts the CSC phenotype to a differentiated epithelial phenotype. Consistently, in vivo treatment of a diet-induced obesity rat model

of breast cancer with STAT3 inhibitor significantly suppresses the CSC-like OBR^{hi} population and abrogates tumor progression. Together, targeting STAT3-G9a signaling to regulate the stemness-differentiation plasticity presents a new and promising therapeutic paradigm for treatment of obesity-related breast cancer.

W-1550

IN VIVO STUDY ON THE ROLES OF HBX AND HCP IN HEPATOCARCINOGENESIS BY CONDITIONALLY ACTIVATED TRANSGENIC MOUSE MODELS

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Liver cancer is the second leading cause of cancer-related death in Taiwan. Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are the major risk factors for hepatocarcinogenesis. Co-infection with HBV and HCV is found highly associated with more severe liver disease and higher risk to develop hepatocellular carcinoma (HCC). Among the hepatitis virus-encoded proteins, HBV X protein (HBx) and HCV core protein (HCP) are indicated to be important in hepatocarcinogenesis in transgenic studies. There are several HBx- and HCP-interacting proteins identified by in vitro studies, but lack in vivo evidence to verify. Hepatic progenitor cells (also called oval cells in rodents) activation is shown in the majority of chronic liver disease and is associated with severity of liver disease. It is still not clear that if hepatic progenitor cells can be the liver cancer initiating cells. Here, we want to generate mice conditionally overexpress HBx and/or HCP in either liver or oval cells to facilitate research on hepatitis virus-induced hepatocarcinogenesis. Our mouse models may help to uncover the mechanism of hepatitis virus-related hepatocarcinogenesis and may serve as a new platform for preclinical therapeutic treatment evaluation in the future.

W-1551

PUF-A, A NOVEL GENE HIGHLY EXPRESSED IN CANCER-INITIATING BRONCHIOLAR CELLS, IS MODULATED BY P53 AND C-MYC AND REQUIRED FOR TUMORIGENESIS IN KRASG12D-INDUCED LUNG ADENOCARCINOMAS

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Through comparative genomic analysis, we identified a novel gene Puf-A, which belongs to Puf-family of RNA-binding proteins expressed in many species, including mammals. Here, we delineated an important role of Puf-A in lung cancer. Previously, we have established an inducible lung adenocarcinoma model in CCSP-rtTA/TetO-Cre/ LSL-KrasG12D mice and identified bronchiolar Clara cells as the cancer-initiating cells giving rise to Kras-induced tumor in the lung. We found greater expression of Puf-A in the cancer-initiating cells of bronchiolar origin of KrasG12D-driven lung adenocarcinomas than the non-cancer-initiating cells of alveolar origin. We also demonstrated that over-expression of oncogenic KrasG12D induced Puf-A expression, whereas depletion of KrasG12D reduced Puf-A expression. In addition, KrasG12D induced Puf-A gene expression was mediated through c-Myc binding to Puf-A promoter. On the other hand, the tumor suppressor p53 was found to directly suppress Puf-A expression by recruiting Sin3A/HDAC complex to Puf-A locus. Depletion of p53 in p53 wild type cancers enhanced Puf-A expression, whereas over-expression of p53 in p53 defective cancers repressed Puf-A expression. Furthermore, in p53 wild type cancer cells, depletion of Puf-A caused up-regulation of p53/p21 pathway, leading to cell cycle arrest. On the other hand, in p53 defective cancer cells, Puf-A depletion impaired autophagic flux culminating in aberrant LC3II accumulation and autophagic cell death. Moreover, through intranasal administration of lentivirus to silence Puf-A expression in CCSP-rtTA/TetO-Cre/LSL-KrasG12D/p53flox mice, progression of adenoma to adenocarcinoma in the lung was abrogated after KrasG12D activation and p53 deletion. Notably, Puf-A expression in human lung cancer was markedly elevated as compared to normal lung tissues, and its expression increased with advancing stages of patients with lung adenocarcinomas. Data mining revealed a correlation of greater Puf-A expression with shortened overall survival of patients with lung adenocarcinoma. Taken together, our results support an essential role of Puf-A in the development of lung adenocarcinoma and imply a link between the regulation of Puf-A in the lung cancer-initiating cells and the cell fate outcomes according to the status of p53 in cancers.

W-1552

A RED-SHIFTED FLUORESCENT SUBSTRATE FOR ALDEHYDE DEHYDROGENASE, ALDERED 588-A FOR LABELING VIABLE ALDH-POSITIVE CELLS

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Normal and cancer stem cells can be isolated based upon the enzymatic activity of aldehyde dehydrogenase 1 (ALDH1), a detoxifying enzyme responsible for oxidation of hazardous aldehyde byproducts. ALDH1 has been used to isolate cancer stem cells of various human malignancies including bladder, breast, cervical, colon, head and neck, liver, lung, pancreas, prostate and ovary. Currently, the ALDEFLUOR™ assay which uses a fluorescent substrate is the only commercially available reagent for ALDH detection. The substrate used in this assay primarily emits in the green region of the electromagnetic spectrum (512nm). For researchers with valuable cell and transgenic animal models in which the target gene of interest has been tagged with eGFP, ALDEFLUOR therefore cannot be used. Selection of cells positive for aldehyde dehydrogenase (ALDH) activity from a green fluorescent background is thus difficult with existing reagents. We now describe a red-shifted fluorescent

substrate for ALDH, AldeRed 588-A, that provides additional flexibility for utilizing ALDH as a marker for stem cell and cancer stem cell isolation. The activity of AldRed 588-A was compared with the ALDEFLUOR reagent and demonstrated similar ability to fractionate ALDH^{pos} cells in a number of cell lines tested.

W-1553

PRIMARY CILIA REGULATE INSULIN/PI3K/AKT AND WNT/BETA-CATENIN CROSSTALK TO PREVENT PREMALIGNANCY IN THE AIRWAY EPITHELIUM.

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The tracheobronchial epithelium is constantly exposed to environmental injury and thus requires a tightly controlled mechanism for repair by resident Airway Basal Stem Cells (ABSCs). After severe airway epithelial injury the repair process involves a proliferation phase during which the lost ABSCs are replaced followed by a differentiation phase which allows the functional mucociliary epithelium to form. Stem cell homeostasis is vital for maintenance of airway health and excessive proliferation of ABSCs leads to airway diseases such as COPD, premalignant lesions and lung cancer. Insulin-Insulin-like growth factors (IGFs) and Wnt signaling pathways have been reported to regulate stem cell proliferation in many organs. However, the role of the Insulin-IGFs and Wnt signaling pathways in ABSC proliferation and cell fate regulation after injury and the downstream signaling events leading to airway epithelial cell homeostasis have not been studied. Here, we show in mouse and human ABSCs that it is the IGF-PI3K-AKT and canonical Wnt pathway interaction through GSK3β that is required for regulation of ABSC self-renewal and onset of differentiation. IGF-PI3K-AKT and Wnt interactions effect proliferation and self-renewal while activation of GSK3β promotes ciliated cell differentiation. This interplay of signaling that centers around GSK3β is at the heart of maintaining homeostatic balance during repair after injury. We further show that GSK3β promotes development of primary cilia on ABSCs by inhibiting Kif24, Cep97 and Cep110 and activating Kif3a. The formation of primary cilia on ABSCs is responsible for preventing further Wnt-β-catenin mediated cell proliferation and instead promoting differentiation to a ciliated cell fate through increased expression of Myb and Foxj1. This study has identified a new signaling interaction that regulates lung stem cell fate that has significant implications for stem cell biology, lung injury and repair and diseases such as lung cancer.

W-1554

A NOVEL TECHNOLOGY FOR NEAR-IMMEDIATE DETECTION OF GLIOBLASTOMA-DERIVED STEM CELL-LIKE CELLS

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Glioblastoma multiforme (GBM) is one of the most aggressive brain tumor types. The median survival is 14.8 months with the best possible treatment including surgery, chemotherapy and radiation. One of the reasons for this poor prognosis is believed to be the existence of cancer stem cells. The GBM-derived stem cell-like cells (GSCs) have features of tumor-initiating cells (TICS) and can self-renew, leading to tumor formation. Previous studies from our lab have introduced a luminescent conjugated oligothiophene (LCO) named p-HTMI that specifically detects rat embryonic neural stem cells, but not adult neural stem cells, differentiated cells of any kind, or other stem or cancer cells. By mere application to the cell culture dish, p-HTMI however labels more than 70% of the cells in populations of GSCs derived from three patients, within 10 minutes. The detection is made easily by fluorescence microscopy or FACS, and as the fluorescent light is green, no modification of the detection tool is required. The detection of human GSCs with p-HTMI occurred without triggering apoptosis or necrosis. Besides its ease of use compared to existing stem cell markers, p-HTMI displayed a higher specificity than CD44, higher sensitivity than CD133, and overlapped to a large extent with cells labeled with CD271 (NGF receptor). p-HTMI labeling was mostly cytoplasmic in embryonic neural stem cells as well as GSCs. The side chain moiety is essential for its functionalization, and additional LCOs have been generated producing red fluorescence that currently is under verification for specificity and function. With regards to mechanism of uptake, our results indicated a passive uptake of p-HTMI that required an intact cell membrane. We are currently performing in vivo studies to verify p-HTMI selectivity in GSCs when injected into NOD/SCID mice.

W-1555

SILENCING OF THE NOVEL LEUKEMIA TARGET NR2F6 USING DIFFRONC SIRNA TECHNOLOGY INDUCES TERMINAL DIFFERENTIATION OF MURINE HEMATOPOIETIC STEM AND PROGENITOR CELLS

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To identify therapeutic targets, we previously adopted a novel single-cell approach that used microarray analysis of clonal siblings to identify the molecular signature of leukemia stem cells. Our work led to the discovery of the orphan nuclear receptor NR2F6 (EAR-2) as a potential leukemia stem cell therapeutic target. Previously we showed that NR2F6 is expressed greater in individual leukemia cells with clonogenic capacity than in leukemia cells that do not divide. In vitro, overexpression of NR2F6 inhibits differentiation of cell lines and primary bone marrow cells. In vivo, overexpression of NR2F6 leads to a rapidly fatal leukemia that is preceded by expansion of the stem cell compartment, c-kit+, sca-1+, lineage- hematopoietic cells (KSL cells) in mice. The discovery of the role of NR2F6 in leukemogenesis and the induction of differentiation of leukemia cell lines following silencing of NR2F6 expression suggests that this is a logical therapeutic target for differentiation therapy using gene silencing technology. Hence, we wished to show proof-of-concept that silencing of NR2F6 using the DiffronC siRNA gene silencing technology in primary mouse hematopoietic stem and progenitor cells promoted terminal differentiation. Herein, we show that while silencing of NR2F6 did not significantly reduce the number of colony forming units, it did significantly increase colony size. We then showed

that silencing of NR2F6 reduced the clonal longevity of bone marrow cells: In replating experiments we observed a significant decrease in secondary colonies in cells treated with NR2F6 shRNA. Furthermore, silencing of NR2F6 resulted in a drastic reduction in KSL cells after six days of ex vivo culture, and caused a dramatic decline in lineage negative cells, and a concurrent increase in cells expressing the myeloid markers CD11b and Gr-1, suggesting that they had differentiated into cells of the neutrophil lineage. This was confirmed by examination of the cytomorphology of the bone marrow cultures. Taken together, these results establish that NR2F6 is a negative regulator of terminal differentiation of the hematopoietic lineage and lays the foundation for use of gene silencing technology against NR2F6 as differentiation therapy for diseases with blocked differentiation such as leukemia and myelodysplastic syndromes.

W-1556

OLIGODENDROCYTE PROGENITORS AS POTENTIAL CELL-OF-ORIGIN OF HYPERACTIVATED RAF-INDUCED MALIGNANT ASTROCYTOMAS

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The expression of an activating mutation of BRAF (BRAFV600E) occurs in 39% of pediatric malignant astrocytoma (pMA) and in 7.7% of adult glioblastomas, and frequently coincides with homozygous deletion of cyclin-dependent kinase inhibitor 2A (CDKN2A). Our studies in genetically engineered mouse models have shown that the hyper-activation of MAPK pathway by BRAFV600E expression, and the deletion of CDKN2A, cooperatively transforms embryonic GFAP+ cells. BRAFV600E positive pMA xenografts respond only partially to the anti-proliferative effects of pharmacologic inhibitors of the mutant kinase. Since BRAFV600E inhibitors have entered clinical trials with glioma patients, it is important to understand and if necessary overcome therapy evasion. Our objective is to delineate the molecular mechanism of how BRAFV600E expression transforms CDKN2A-deleted neural progenitors into high-grade MA cells and generates cellular heterogeneity. Our investigations into the cellular composition of MA harboring these two mutations revealed that they consist of non-overlapping neural stem and oligodendrocyte progenitor (OP)-like cell subpopulations. They are different in their cell division mode, proliferation rate and in their responses to the BRAFV600E inhibitor vemurafenib. Here we test if BRAFV600E transforms CDKN2A-deleted neural progenitor cells, by disrupting their asymmetric cell division (ACD), thereby initiating a cascade of events that alters cell fate, enhances proliferation and leads to neoplastic transformation. The compound transgenic mice carrying Cre-inducible alleles of BRAFV600E and deletion of CDKN2A show a severe decrease in survival and develop high-grade astrocytoma when mutations were introduced in the germinal areas. Our data suggest that the tumorigenic cells can arise in part from non-stem cell regions. Lastly, we use an OPC-specific Cre driver to determine if OPC acquire plasticity and can give rise to astrocytic cells with NSC-like features as a consequence of these mutations. Due to the fact that stem and progenitor cells undergo ACD, we will discuss the combination of ACD agonists with BRAFV600E inhibitors as novel therapeutic approach for the treatment of pMA.

W-1557

NEURAL DIFFERENTIATION OF RAT C6 GLIOMA CELLS INDUCED BY TAXOL

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Glioblastoma is a common and aggressive type of primary brain tumor. Its effects on cell growth and morphological changes were observed when the cells from glioblastoma multiforme were treated with anti-cancer drugs. To clarify the differentiation patterns of rat C6 glioma cells treated with taxol, survived glioma cells were characterized by immunocytochemistry, real-time PCR and western blotting. After taxol treatment, part of the C6 cells died and morphological changes were observed in survived cells. Several neural differentiation markers, including betaIII-tubulin (for neurons), GFAP (for astrocytes) and CNP (for oligodendrocytes) could be detected in the C6 population after taxol and dbcAMP treatments. Quantitative analysis showed a significant increase of neural differentiated cells after treatments. To summarize, our results demonstrated that neuronal differentiation could be induced by taxol in glioma cells. The glial differentiation of C6 cells were enhanced by dbcAMP and taxol. Increased expression of neural differentiation markers in C6 cells by taxol treatment suggests that some anti-cancer drugs could be applied to eliminate the malignant cancer cells as well as induce the differentiation of putative cancer stem cells.

W-1558

BONE MARROW-DERIVED STROMAL CELLS ARE ASSOCIA-TED WITH GASTRIC CANCER PROGRESSION

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To clarify the role of bone marrow-derived stromal cells (BM-SCs) expressing CD271 in the development of gastric cancer. The effect of human BM-SCs on the proliferation and motility of six gastric cancer cell lines, OCUM-2M, OCUM-2MD3, OCUM-12, KATO-III, NUGC-3, and MKN-74, was examined. CD271 expression levels in BM-SCs were analyzed by flow cytometry. We also generated a gastric tumor model by orthotopic inoculation of OCUM-2MD3 cells in mice that had received transplantation of bone marrow from CAG-EGFP mice. The correlation between the clinicopathological features of 279 primary gastric carcinomas and CD271 expression in tumor stroma was examined by immunohistochemistry. Numerous BM-SCs infiltrated the gastric tumor microenvironment; CD271 expression was found approximately 25% of BM-SCs. Conditioned medium from BM-SCs significantly increased the proliferation of gastric cancer cell lines. Furthermore, conditioned medium from gastric cancer cells significantly increased the number of BM-SCs, while migration of OCUM-12 and NUGC-3 cells was significantly increased by conditioned medium from BM-SCs. CD271 expression

in stromal cells was significantly associated with macroscopic type-4 cancers, diffuse-type tumors, and tumor invasion depth. The overall survival of patients (n=279) with CD271-positive stromal cells was significantly worse than that of patients with CD271-negative stromal cells. This is the first report of the significance of BM-SCs in gastric cancer progression. BM-SCs might play an important role in gastric cancer progression, and CD271-positive BM-SCs might be a useful prognostic factor for gastric cancer patients.

W-1559

DNMT1-ASSOCIATED LINCRNAS REGULATE GLOBAL GENE EXPRESSION AND DNA METHYLATION IN COLON CANCER

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Genetically identical cells within a single multicellular organism display distinct phenotypes and functions, which are determined during development via changes in epigenetic modifications and consequently gene expression. Similarly during tumorgenesis, cells also undergo massive alterations of their epigenomes resulting in a change in both cell function and identity. However, the molecular mechanisms leading to these observed epigenomic changes are yet to be fully elucidated. Building on our previously discovery of human long intergenic non-coding (linc)RNAs and their functional interactions with several histone-modifying complexes, we postulated that deregulation of lincRNAs could lead to massive changes in the epigenome of cancer cells. To test our hypothesis, we utilized state of the art RIP-seq to identify a subset of lincRNAs that interact with the DNA methyltransferase DNMT1 in a colon cancer cell line HCT116. One such lincRNA, which we have designated as CURE1 (Colon UpREgulated DNMT1-Associated 1), shows notable tissue-specific and high expression in the normal colon. Furthermore, CURE1 is significantly down-regulated in a cohort of colon tumors and in numerous patient-derived colon cancer cell lines relative to matched normal tissue. Induction of CURE1 in patient-derived colon cancer cell lines demonstrated a tumor-suppressor function by significantly affecting global gene expression and DNA methylation patterns at multiple loci distributed throughout the human genome. CURE1 is also sufficient to up-regulate the expression of tight junction protein 1 (TJP1), a hallmark of epithelial cells. These results suggest that deregulation of DNMT1-associated lincRNAs contribute to aberrant DNA methylation and gene expression in cancer cells, and potentially to colon cancer etiology. Based on our findings, we propose that DNMT1-associated lincRNAs can reprogram the epigenome and consequently the identity of colon cancer cells and potentially other cell types.

W-1560

HEDGEHOG STEMNESS PATHWAY INDUCES ACQUIRED RESISTANCE TO EPIDERMAL GROWTH FACTOR RECEPTOR TYROSIN KINASE INHIBITOR (EGFR-TKI) IN LUNG ADENOCARCINOMA

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The development of EGFR-TKI has significantly provided survival benefit in lung adenocarcinoma (LAC) patients with activating EGFR mutations. However, the patients who respond to EGFR-TKI invariably acquire resistance, among which EGFR T790M secondary mutation accounts for >50% cases, and there are still ~30% cases without mechanisms identified for the resistance. Here in the current report, we found that Hedgehog (HH), a stemness pathway that regulates segments and organs formation during development, is frequently re-activated and induces EGFR-TKI resistance in LAC. In primary LAC tumor cells derived from patients who received EGFR-TKI treatment and relapsed without EGFR-T790M, HH pathway was frequently activated in a ligand-dependent manner, and the HH inhibitor significantly suppressed cell growth. Further investigations showed that Hedgehog-interacting protein (HHIP), a cell-surface protein that negatively regulates HH pathway, was epigenetically silenced in most LAC cells, which potentiated the activation of HH pathway and drug resistance. HHIP overexpression blocked HH signaling and inhibited the growth of EGFR-TKI resistant LAC cells. Finally, we showed that HH pathway induced HGF expression and the phosphorylations of MET and downstream AKT, improving cell survival in the condition of serum-starvation or drug treatment. In summary, our data indentified that HH stemness pathway is a novel mechanism of EGFR-TKI resistance in LAC, and HHIP is a critical regulator for the HH activation. HHIP or small molecule inhibitors of HH pathway may be applied in the treatment of EGFR-TKI resistant LAC in clinic.

W-1561

MIR367 PROMOTES PROLIFERATION AND STEM LIKE TRAITS IN MEDULLOBLASTOMA CELLS

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In pediatric medulloblastoma, abnormal expression of pluripotency factors such as LIN28 and OCT4 have been correlated with poor patient survival. The miR302-367 cluster has also been shown to control self-renewal and pluripotency in human embryonic stem cells and induced pluripotent stem cells, but there is limited, mostly correlational, information about these pluripotency-related miRNAs in cancer. We evaluated whether aberrant expression of such miRNAs could contribute to aggressiveness of medulloblastoma cells. Expression of primary and mature forms of miR-367 were assessed in four human medulloblastoma cell lines by real-time PCR. A transient overexpression was induced by transfecting cells with miR-367 mimic and the ensuing effects on tumor cell behavior, stem-like traits, and expression of putative miR-367 cancer-related targets were evaluated. All medulloblastoma cell lines displayed a basal miR-367 expression, which was found to be up-regulated upon enforced expression of OCT4A. Transient overexpression of miR-367 significantly 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. A concurrent down-regulation

of the miR367 targets, RYR3, ITGAV and RAB23, was also detected in miR-367 overexpressing cells. Overall, these findings support a pro-oncogenic activity for miR-367 in medulloblastoma and reveal a possible mechanism contributing to tumor aggressiveness, which could be further explored to improve patient stratification and treatment of this important type of pediatric brain cancer.

W-1562

IDH1R132H-INDUCED TRANSFORMATION OF GLIAL PROGENITORS INTO PRONEURAL GLIOMA

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Malignant glioma is the most common primary brain tumor. Most grade II-III gliomas and a subset of glioblastoma (GBM) display R132H mutation in the isocitrate dehydrogenase (IDH1) gene, resulting in improved overall patient survival. Genes associated with glial progenitors characterize the proneural transcriptomal profile of IDH1R132H mutant gliomas. By employing the RCAS/TVA system, we demonstrate that RCAS-IDH1R132H infection of postnatal day 4 (P4) OLIG2+ glial progenitors expressing the TVA receptor produce transcriptomal changes of genes involved in lipid metabolism and cell cycling. In addition, we found that IDH1R132H mutation robustly increases the levels of H3K27me3 and H3K9me3 in transformed tumor cells. IDH1R132H-induced in vivo transformation of OLIG2+ glial progenitors produce proneural gliomas in mice. Using the well-characterized RCAS-PDGFB model, we demonstrate that IDH1R132H mutation increases tumor latency in the presence of increased growth factor signaling. We will present data describing IDH1R132H-induced transformation in a growth factor-dependent context. The mesenchymal glioma phenotype is associated with invasiveness and treatment-resistance. Recurrent tumors in patients with IDH1R132H mutation seem to maintain their proneural phenotype, in contrast to patients with IDH1 wild-type tumors. We and others have recently demonstrated that ionizing radiation induces a proneural-to-mesenchymal transition in IDH1 wild-type proneural gliomas. We will present data demonstrating the effects of IDH1R132H mutation on mesenchymal transition following radiation. In summary, our work demonstrates that IDH1R132H mutation regulates glioma growth in a growth factor context-dependent manner and prevents radiation-induced mesenchymal transition.

W-1563

OVER-EXPRESSION OF THE CYSTINE-GLUTAMATE TRANSPORTER SYSTEM XC- PROMOTES A CANCER STEM CELL PHENOTYPE IN GLIOMA

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Glioblastoma multiforme (GBM), a grade IV astrocytoma, is the most common and aggressive primary brain tumor in adults with patient survival averaging only 14 months after diagnosis. GBM is characterized by a heterogeneous population of cells that are genetically unstable and resistant to chemotherapy. Additionally, GBMs display high activity of system xc- which is a sodium-independent transporter containing a catalytic subunit: xCT (SLC7A11). System xc- is capable of contributing to the antioxidant capacity by importing cystine into a cell, where it is then reduced to cysteine and used to synthesize glutathione (GSH). We performed in silico analysis that revealed glioma patients with high expression of SLC7A11 had a worse prognosis compared to patients with lower expression. Therefore, we generated stable SLC7A11-over-expressing and SLC7A11-knock-down U251 glioma cell lines to determine whether increased system xc- expression protects glioma from both oxidative- and genotoxic-stress. Over-expression of SLC7A11 resulted in increased GSH generation, resistance to oxidative stress and decreased chemosensitivity to the chemotherapeutic agent temozolomide (TMZ). The increased chemoresistance was also correlated with an increased cancer stem cell (CSC) phenotype. The SLC7A11-over-expressing U251 glioma cells had a significant increase in tumorsphere formation and a limiting dilution assay revealed an increased frequency of cancer stem/progenitor cells in the SLC7A11-over-expressing cells. Additionally, there was an elevated expression of the CSC-associated markers Nanog, Musashi-1, Sox-2, and Nestin. It has been established that CSCs possess enhanced mechanisms of protection from ROS that render them resistant to chemo- and radiotherapy. After enrichment for CSCs, the SLC7A11-over-expressing cells still maintained a higher viability compared to control cells treated with TMZ. Therefore, we demonstrate that system xc- in GBM not only promotes survival under oxidative- and genotoxic stress by inducing high intracellular GSH levels but may also modulate sensitivity to chemotherapy treatment by promoting a CSC phenotype. These findings indicate that therapeutic manipulation of system xc- either alone, or in combination with other treatments, may improve clinical outcome in GBM patients.

W-1564

GENE SIGNATURE OF INVADING HUMAN MELANOMA CELLS

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Due to its extremely high metastatic capacity, cutaneous malignant melanoma represents the most fatal skin tumor in industrialized countries. Up to date the only possible therapy to cure melanoma patients is surgical excision of localized, non-metastatic primary tumors. Unfortunately, many patients already present micrometastatic disease at the time of diagnosis resulting in a poor 5-year survival probability. Hope for a future therapy might therefore lie in the early identification of metastasizing melanoma cells and the elucidation of the mechanisms governing their dissemination. Melanoma arises in the epidermis from transformation of cells of the melanocytic lineage. Melanocytes originate from neural crest cells (NCCs). During development NCCs delaminate from the neural tube by undergoing an epithelial-to-mesenchymal transition (EMT). Upon EMT NCCs adopt a remarkable migratory capacity, which allows them to disseminate throughout the embryo and to colonize distant sites where they differentiate into specialized cell types. Intriguingly this process is highly reminiscent of metastasis formation during which tumor cells disseminate from the primary neoplasm to establish secondary tumors in distant organs. This raises the question of

whether the strong propensity of melanoma to metastasize reflects an intrinsic property of melanoma cells to disseminate by exploiting signaling cues normally active in migratory NCCs. To address this point we performed a microarray analysis of invading melanoma cells using a humanized in vivo model able to recapitulate the first steps of melanoma metastasis. With this approach we compared two populations, one positive for a known NCCs marker expressed during neural crest delamination, CD271, and the other negative for this marker. The analysis revealed that genes mostly involved in EMT were differentially modulated in the two populations. Currently we are investigating the mechanisms linking CD271 to melanoma invasion.

W-1565

EVALUATION OF A NEW AUTOMATED CELL TRACKING SOFTWARE FOR EASY CELL MIGRATION ANALYSIS OF LIVE CELL IMAGES - A COMPARISON TO MANUAL TRACKING

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Understanding the cell machinery in chemotaxis is vital for research in inflammation, stem cell homing and tumor biology. Migration experiments with time-lapse imaging are time-consuming to analyze manually. Available automated tracking softwares are complex to use, sensitive to difficult tracking conditions and lack user control. We lack fast and easy methods for analyzing cell migration of live cell images with accuracy comparable to manual cell tracking. The new automated cell tracking software, TrackingTool™ PRO, was evaluated by analyzing image sequences containing non-labeled cells like human neutrophils, prostate and breast cancer cells. The automated analysis was compared to data generated with manual tracking. The results show that TrackingTool™ PRO generates accurate data comparable to manual tracking. In addition, it was robust by being able to generate accurate migration data even during difficult tracking conditions such as when image quality is poor, frame rates are low and for cells with morphological changes.

W-1566

G PROTEIN COUPLED RECEPTOR X3 POSSESS HIGH CAPACITY FOR TUMORIGENICITY

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Cancer stem cells are considered to represent a small population of cancer cells. It is resistant to chemotherapy and radiotherapy through various mechanisms, and might be cause of tumor relapse and metastasis. Thus, cancer stem cell is an excellent therapeutic target for the complete elimination of cancer cells. In the present study we aimed to establish cancer stem cell marker. In this study, by using Digital Differential Display (DDD) database tool, we found X3 gene that is highly expressed in both Embryonic stem (ES) cell and cancer cell. Embryonic stem (ES) cells are pluripotent cells derived from early mammalian embryos. Interestingly, embryonic stem cell and cancer cell have similar characteristics. We investigated role of X3 in cell proliferation and molecular mechanism involved to regulate cell proliferation. We found that X3 gene regulates cell proliferation by the activation of the M3-muscarinic acetylcholine receptor. M3-muscarinic acetylcholine receptor agonist increased X3 gene expression in cultured F3 and HEK293 cell lines whereas the antagonist of M3-muscarinic acetylcholine receptor reduced X3 gene expression. Interestingly, in xenograft tumor growth, we observed significant tumor growth by using X3 overexpressed HEK293 cell whereas X3 overexpressed F3 cell showed no tumor growth. Taken together, X3 may involve in stemness regulation in normal stem cells, while in transformed cells it induce tumor formation.

W-1567

ESSENTIAL ROLES OF FUT1 AND FUT2 IN HUMAN BREAST CANCER STEM CELLS

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FUT1 and FUT2 encode alpha 1,2-fucosyltransferases which catalyze the addition of α1,2-linked fucose to glycans. Glycan products of FUT1 and FUT2, such as Globo H and Lewis Y, are overexpressed on malignant tissues including breast cancer. To further delineate the role of FUT1 and FUT2 in breast cancer, we used lentiviral vector to deliver shRNA to silence FUT1 or FUT2 in two human breast cancer cell lines: T47D which expressed significantly more FUT2 than FUT1, and MB157 which displayed the opposite characteristics with high FUT1 and negligible FUT2. The tumor growth rate in vitro and in vivo was significantly reduced in the FUT2- and FUT1-silenced T47D as well as FUT1-silenced MB157 in comparison with empty vector controls. Notably, marked reduction of the CSC subpopulation (CD44+/CD24-) from 52.4% to33.9% and 13.5% was observed upon FUT1 and FUT2 silencing, respectively, in T47D cells. In cultured cells or cells harvested from xenografted tumors of T47D, mammosphere forming capacity diminished significantly in shFut2 group compared to shLacZ controls. Similarly, FUT1 knockdown in MB157 cells significantly reduced CD44+/CD24- subpopulation and dampened mammospheres forming capacity of the resulting xenografted tumor cells. In addition, knockdown of FUT1 and FUT2 hindered epithelial-mesenchymal transition (EMT) as reflected by decreased wound healing rate and EMT markers such as twist, fibronectin and vimentin. Conversely, overexpression of FUT1 and FUT2 significantly increased cell migration, invasion in vitro and enhanced tumor growth in vivo. Silencing of FUT2, but not FUT1 impaired cell adhesion to polystyrene and extracellular matrix. During wound healing, Globo H was found to localize at the leading edge of migration. Addition of exogenous Globo H-ceramide ameliorated the inhibitory effects of FUT1 silencing on cell migration, but did not alleviate diminished cell adhesion by FUT2 knockdown. This suggests that at least part but not all phenotypic alterations induced by FUT1/2 silencing was mediated by Globo H. Our results implied that FUT1 and FUT2 play an essential role in regulating the growth, adhesion, migration ability and cancer stem cell properties of breast cancers, and may thus serve as therapeutic targets directed at breast cancer stem cells.

W-1568

A SMAD3 - RIF1 AXIS ON EMBRYONIC STEM CELL STABILITY

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Smad3 mediated TGF-β/Activin/Nodal signaling plays important roles

in many biological processes. Despite numerous studies regarding Smad3 function, the role of Smad3 in mouse embryonic stem (ES) cells is not well studied. To understand the function of Smad3 in mouse ES cells, we derived Smad3−/− ES cells and wild type ES cells. Smad3−/− ES cells display no defect on self-renewal. However, subcutaneous injection of Smad3−/− ES cells into nude mice leads to formation of malignant immature teratomas, whilst wild type ES cells tend to form mature teratomas, suggesting Smad3 depletion leads to ES cell adopt cancer cell like properties. By performing microarray analysis to compare the transcriptome of wild-type and Smad3 − /− ES cells, we found that Rif1 (RAP1-associated protein 1), a factor important for genomic stability, is significantly upregulated in Smad3 −/− ES cells. The expression level of Rif1 needs to be tightly controlled in ES cells, as a low level of Rif1 is associated with ES cell differentiation, but a high level of Rif1 is linked to ES cell transformation. In ES cells, Oct4 activates Rif1, whereas Smad3 represses its expression. Oct4 recruits Smad3 to bind to Rif1 promoter, but Smad3 joining facilitates the loading of a polycomb complex that generates a repressive epigenetic modification on Rif1 promoter, and thus maintains the expression of Rif1 at a proper level in ES cells. Interestingly, Rif1 short hairpin RNA (shRNA)-transduced Smad3 −/− ES cells showed less malignant properties than the control shRNA-transduced Smad3 − /− ES cells, suggesting a critical role of Smad3-Rif1 axis in maintaining the stability of ES cells during proliferation.

W-1569

RNA EDITING OF CDKN1A INDUCES ACCELERATED CELL CYCLE IN NORMAL HEMATOPOIESIS

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We have previously shown that inflammation-responsive RNA editase ADAR1 (ademosine deaminases acting on dsRNA) contributes to CML (chronic myeloid leukemia) disease progression. Whole transcriptome sequencing (RNA-Seq) data on primary CML chronic phase (CP) and CML blast crisis (BC) patient samples demonstrated over-representation of inflammatory IFN-pathways involved in hematological development. The resulting ADAR1 up-regulation plays important roles in both stem cell differentiation and self-renewal as indicated by in vitro colony-formation assay and in vivo CML xenotransplantation mouse model. Though we have established ADAR1-mediated RNA editing as a novel therapeutic target for treating CML, we do not yet understand the underlying mechanism of RNA editase's involvement in normal hematopoietic stem cells self-renewal and differentiation. In our new study, we describe ADAR1's role in cell cycle regulation of normal hematopoietic stem cell and its molecular editing targets. Whole transcriptome sequencing (RNA-Seq) data on cord blood CD34+ cells transduced with lentiviral ADAR1 revealed changes in cell cycle genes in hematological development. We further confirmed the finding using qRT-PCR microarray. Flow cytometry demonstrated that ADAR1 accelerated G0 to G1 transition in normal cord blood CD34+ cells, which resulted in an expansion of progenitor cells both in vitro and in vivo as demonstrated by engraftment in immuno-compromised mice. Interestingly, ADAR1 is able to binds to CDKN1a mRNA and inhibit its expression by A-to-I editing activity. Moreover, shRNA knockdown of ADAR1 in CML BC sample shows a reduction of engraftment in bone marrow and spleen, and an enrichment of G0 population in the remaining cells. A decrease of self-renewal capacity as demonstrated by serial engraftment suggests the residual LSC failed to propagate. Our finding suggests carefully regulated A-to-I editing by ADAR1 is essential for the maintenance of proper cell grow and proliferation in HSC. It is plausible that the elevated expression level of ADAR1 observed in CML BC LSC contributes to false regulation of cell cycle that leads to the expansion of malignant leukemia stem cells.

W-1570

A NOVEL MOUSE GLIOMA MODEL FOR ROBUST IN VIVO TARGET VALIDATION

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One major challenge for translational research is that faithful mouse models are required for preclinical target validation. In the field of brain tumor research, so far most of the target validation experiments were based on xenograft models. Under the requirement of more faithfully mimicking the genetic and biological features of human brain tumor, we established a primary glioma mouse model by overexpressing AKT and PDGFB in Nestin expressing cells of SVZ based on RCAS/TVA system. Histological and genetic analysis showed this tumor model had most of typical features of human glioma. For target validation, a Cre recombinase-inducible construct which allows conditional gene knockdown was delivered into Nestin expressing cells at the start of tumor induction. Tlx was shown to be a key regulator in neural stem cells and brain tumor stem cells. Using our system, we found that Tlx knockdown in brain tumor stem cells significantly slowed down tumor growth. In the cellular level, cells with Tlx knockdown were less proliferative than their counterparts without knockdown. Our system also showed its potential in single cell lineage tracing of brain tumor stem cells.

THURSDAY, 25 JUNE, 2015

Poster Presentations

18:00-19:00 ODD numbered posters presented
19:00-20:00 EVEN numbered posters presented

PRE-CLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL CELLS

T-1001

EQUINE ALLOGENEIC BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS ELICIT ANTIBODY RESPONSES IN VIVO

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This study tested the hypothesis that Major Histocompatibility Complex (MHC) incompatible equine mesenchymal stromal cells (MSCs) would induce cytotoxic antibodies to donor MHC antigens in recipient horses after intradermal injection. No studies to date have explored recipient antibody responses to allogeneic donor MSC transplantation in the horse. This information is critical because the horse is a valuable species for assessing the safety and efficacy of MSC treatment prior to human clinical application. Six MHC heterozygote horses were identified as non-equine leukocyte antigen (ELA)-A2 haplotype by microsatellite typing and used as allogeneic MHC-mismatched MSC recipients. Thoroughbred MHC homozygote horses of known ELA-A2 haplotype were used as MSC and peripheral blood leukocyte (PBL) donors. One ELA-A2 homozygote horse was the recipient of ELA-A2 donor MSCs as an MHC-matched control. Donor MSCs, which were previously isolated and immunophenotyped, were thawed and culture expanded to achieve between 30 x 10^6 and 50 x 10^6 cells for intradermal injection into the recipient's neck. Recipient serum was tested for the presence of anti-donor antibodies prior to MSC injection and every 7 days after injection for the duration of the 8-week study using the standard lymphocyte microcytotoxicity dye-exclusion test. In addition to anti-ELA-A2 antibodies, recipient serum was examined for the presence of cross-reactive antibodies. All MHC-mismatched recipient horses produced anti-ELA-A2 antibodies following intradermal injection of ELA-A2 MSCs and developed a wheal at the injection site that persisted for the duration of the experiment. Anti-ELA-A2 antibody responses were varied both in terms of strength and timing. Four recipient horses had high-titered anti-ELA-A2 antibody responses resulting in greater than 80% donor PBL death in the microcytotoxicity assays and one of these horses also developed antibodies that cross-reacted with PBLs from an ELA-A3 horse. In conclusion, allogeneic MSCs are capable of eliciting antibody responses in vivo that can be strong and also cross-reactive with MHC types other than that of the donor. Such responses could limit the effectiveness of repeated allogeneic MSC use in a recipient and could also result in untoward inflammatory responses in recipients.

T-1002

TALEN MEDIATED INDUCIBLE HEPATOCYTE GROWTH FACTOR ENHANCED ANGIOGENESIS ABLITY IN MESENCHYMAL STEM CELLS

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Therapeutic angiogenesis is a recent trend to treat serious vascular diseases, such as coronary arteriosclerosis, hind-limb ischemia and heart attack. Hepatocyte growth factor (HGF) is the one of key factors to accelerate angiogenesis. However, in vivo effects of recombinant HGF are limited because of a very short half-life (< 3~5min) at the site of injury. Continuous treatment of HGF is necessary to get valuable effects. To solve this problem, we made genetic engineered-mesenchymal stem cells (MSCs) capable of inducing conditional secretion of HGF. The expression of HGF was controlled depending on doxycycline (Doxy) concentration by Tet-on system. We also integrated the inducible HGF expression cassettes into the safe harbor site of MSC chromosome by transcription activator-like effector nuclease (TALEN) system, which HGF can permanently express in these MSCs, and then the conditional secretion of HGF in MSCs was confirmed by western blotting and enzyme-linked immunosorbent assay (ELISA). In the assessment of functional effects of HGF secreted from MSC, we showed improved migration effects in MSCs treated with Doxy, even though proliferation effects were insignificant. Moreover, long-term exposure of HGF secreted from MSCs induced anti-apoptotic effects against oxidative stress and showed the enhanced ability of tube formation compare with those of in non-Doxy treated condition. Taken together, the inducible HGF secreting MSCs are an effective tool to express HGF consistently and control the secretion of HGF effectively.

T-1003

INVESTIGATE THE PROTECT EFFECT AND MECHANISM OF RAT MESENCHYMAL STEM CELLS (MSCS) ON ISCHEMIC-REPERFUSIONAL INJURY AND MASSIVE RESECTION MODEL OF THE RAT SMALL INTESTINE

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The intestine is the most important organ for nutrition. 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. Stem cells have the ability of self-renewal and differentiating into many different cell types. 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 find out the possible mechanisms whereby MSCs protect ischemic and reperfusion injury of the intestine. Male Wistar rats will be used as subjects. We would also like to ascertain the mechanisms whereby MSCs reduce tissue damage in the ischemia-reperfusion model and massive small bowel resection model.

T-1004

GENERATION OF TALEN MEDIATED INDUCIBLE VEGF SECRETING STEM CELL FOR THE PROTECTION AND REGENERATION OF MYOCARDIAL INFARCTION

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Human umbilical cord blood-derived mesenchymal stem cell (HUCB-MSC) and Vascular endothelial growth factor (VEFG) has been widely used as a potent materials for the regeneration of injured hearts. However high levels of VEGF can arouse growth of abnormal blood vessels and hemangiomas. To overcome this problem, we made an inducible VEGF-secreting stem cell (VEGF/HUCB-MSCs) regulated by doxycycline (Doxy) and integrated AAVS1 locus (safe harvor site) by transcription activator-like effector nucleases (TALENs). VEGF/HUCB-MSCs were confirmed VEGF secretion at 48hrs post-Doxy and the integration of VEGF expression cassette into AAVS1 locus site by junction PCR. MSCs also were observed that they had an endothelial cell (EC)-like character by secreted VEGF. Next, we investigated this cell could improve cardiac function and protect from left ventricle remodeling in a rat model of myocardial infarction (MI). The increased viability, vascularization and functionality were observed in the rat transplanted with VEGF/HUCB-MSCs sheet. In echocardiogram and histological analysis, VEGF/HUCB-MSCs showed increased LV ejection fraction, fraction shortening and decreased MI size and fibrosis. From these result VEGF/HUCB-MSCs could be an effective cell treat for the treatment of MI.

T-1005

USING THE 'ACTIVATED PLATELET SUPERNATANT' AS A PRIMING AGENT ENHANCES THE EFFICACY OF MOBILIZED PERIPHERAL BLOOD STEM CELLS IN STEM CELL THERAPY

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Various methods are used to augment the efficacy of stem cell therapy in myocardial infarction (MI). Activated platelets excrete various cytokines/growth factors that play important roles in the healing process. Also monocytes polarized to M2 monocytes are known to play a key role in tissue regeneration by inhibiting tissue damage and reducing the inflammatory responses. In this study, we used the 'Activated Platelet Supernatant (APS)' of rats to prime autologous 'Granulocyte colony-stimulating factor mobilized peripheral blood stem cells (mobPBSCs)' and investigated the efficacy of these cells for stem cell therapy in MI. mobPBSCs were isolated from rats and were primed with APS for 6 hours. Then the APS-primed mobPBSCs were analyzed for their potential in tissue regeneration. To check the paracrine effect, we used the 36 hour culture supernatant of primed cells. For an in vivo model, APS-primed mobPBSCs were injected in a rat MI model. The rat APS contained high levels of various cytokines such as IL-1β, IL-10 and TGFβ. By APS-priming, mobPBSCs showed M2 polarization which was induced by the pSTAT3-SOCS1 pathway. Also expression of angiographic molecules (i.e. TEK, IL-10, CXCL1 and CX3CR1) was upregulated by APS-priming. APS-primed mobPBSCs had enhanced ability of migration and invasion, which likely resulted from the upregulated expression of integrins on the cell surface. To check the paracrine effect, rat endothelial cells were cultured in the 36 hour culture supernatant of Vehicle and APS-primed mobPBSCs culture supernatant. Rat endothelial cells showed augmented proliferation and capillary network formation. In vivo transplantation of APS-primed mobPBSCs into rat MI models showed reduction in fibrosis area and wall thinning, which lead to improvement in cardiac function measured by echocardiography. Our data reveals that APS-priming can enhance the wound healing potential of mobPBSCs. APS-primed mobPBSCs showed increased M2 polarization, angiogenic factor expression and superior paracrine effect that could augment the efficacy of stem cell therapy, which was proven in a rat MI model. Our results propose APS-priming as a promising method that can be used in future cell-based therapy of MI.

T-1006

ORAL PROGENITOR CELLS MODULATE T CELL BEHAVIOUR VIA THE RELEASE OF SOLUBLE FACTORS

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Our work has demonstrated that a progenitor cell (PC) population within the oral mucosal lamina propria (OMLP) has the capacity to modulate the immune response in vitro. The objective of this study is to elucidate the mechanism of OMLP-PC immunomodulation and their potential for use in the treatment of immune- related disorders. OMLP-PCs (n=3) were utilised in experiments (±100U/ml Interferon (IFN)γ). Cell surface human leukocyte antigens (HLA) I and II and co-stimulatory molecules were determined by flow cytometry and intracellular HLAII by Western Blotting. T-cells were activated (anti-CD2/CD3/CD28 beads) and co-cultured with OMLP-PCs. T-cell viability and apoptosis was determined by labelling with Live/Dead® cell stain and annexin V. T-cell anergy (in the presence of IL-2/IL-15) and proliferation (3H-thymidine uptake) was assessed. Flow cytometry was used to determine T-cell activation.

QPCR analysis of OMLP-PCs co-cultured with T-cells was performed for immunomodulatory signalling pathways. HLAI was constitutively expressed on OMLP-PCs. Intracellular HLAII was inducible after 24 hours of treatment with IFNγ however cell surface expression was not detectable until day 7. No expression of co-stimulatory molecules on the OMLP-PCs was detected. Co-cultures confirmed that OMLP-PCs suppress T-cell activation and proliferation. T-cell proliferation was restored on removal of the PCs, their secretome and re-stimulation. Reduction of T-cell proliferation was not due to an increase in cell death; annexin V analysis confirmed that OMLP-PC presence decreased the level of T-cell apoptosis. This study demonstrates the potent immunosuppressive capabilities of OMLP-PCs on T-cell proliferation in vitro. We have determined that OMLP- PCs utilise soluble factors to modulate pan T-cell proliferation without adversely affecting their viability. Investigations are now focussed on determining the mechanisms involved in mediating these effects.

T-1007

HUMAN SKIN-DERIVED STEM CELLS AS CELLULAR THERAPY FOR END-STAGE LIVER DISEASE

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Currently, the only effective treatment for patients suffering from severe liver failure is orthotopic liver transplantation (OLT), which is severely hampered by the overall shortage of healthy donor livers. Indeed, the number of patients on the waiting lists is 2- to 3- fold higher than the number of OLTs and it is estimated that between 5 to 10% of them will die before a donor is found. As such, there is an urgent need to develop novel treatments to address this problem. A plausible solution could be offered by stem cell technology as these cells exhibit the ability of self-renewal and multilineage differentiation. Stem cell transplantation could be particularly interesting for patients suffering from severe liver dysfunction with no immediate chance of OLT. With that goal, we investigated the potential of a multipotent human stem cell population with a high self-renewal capacity and cell plasticity that persists within the skin throughout adulthood for the treatment of end-stage liver disease. These cells, termed skin-derived precursor cells (SKP), are immunologically-privileged due to their absence of MHC class II antigens HLA-DR and co-stimulatory molecules, providing a solid basis for their future clinical applications. Indeed, SKPs successfully engraft, survive and repopulate hepatic tissue in a transgenic murine model of liver deficiency (uPA+/+/SCID) and contribute to the increase in liver mass. Most importantly, after oral administration of dianabol, these in vivo generated SKP-derived hepatocytes produce human-specific metabolites that can be detected in the urine of the chimeric mice demonstrating their in vivo biotransformation capacity. As such, we believe that SKPs have a major potential for the treatment of end-stage liver disease, especially when hepatocyte replacement is needed.

T-1008

INTRAVENOUSLY INJECTED NON-TUMORIGENIC PLURIPOTENT STEM CELLS, MUSE CELLS, HOME INTO THE DAMAGED LIVER AND GENERATE NEW HEPATOCYTE TO REPAIR THE TISSUE

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Multilineage-differentiating stress enduring (Muse) cells reside in the bone marrow as fraction and ~1% of mesenchymal stem cell (MSC) fraction. They are stress-tolerant, express pluripotency markers despite low telomerase activity, and are able to self-renew and generate cells of all three germ layers from a single cell. These cells can be identified as cells positive for both SSEA-3. Human Muse cells showed higher capacity for differentiating into hepatoblast/hepatocyte-lineage cells and higher migration toward the serum and liver of carbon tetrachloride-treated mice than did non-Muse cells (cells other than Muse cells in MSCs) in vitro. In vivo dynamics of cells showed that intravenously-injected human Muse cells efficiently homed into damaged SCID mouse liver, while non-Muse cells were not detected at day 14. Human Muse and non-Muse cells were separately injected intravenously into liver cirrhosis immunodeficient mouse models. Muse cells integrated into liver and expressed HepPar-1, human albumin and human anti-trypsin, and delivered recovery in total bilirubin and albumin as well as attenuation of fibrosis. Expression of the mature functional markers human CYP1A2 and Glc-6-Pase in Muse group liver suggested possible differentiation of Muse cells into functional hepatocytes. However these effects were not recognized in the non-Muse group. A unique and highly useful feature of Muse cells is their specific ability to detect damage signals, which allows them to migrate toward and home into damaged tissues when infused into the peripheral blood stream where they can spontaneously differentiate into cells compatible with the homed-into tissue. Thus, full utilization of Muse cells may improve the efficiency of currently performed MSC transplantation for liver diseases.

T-1009

SCALE-UP AND MANUFACTURING OF A MESENCHYMAL STEM CELL (MSC) THERAPY FOR THE TREATMENT OF GRAFT-VERSUS-HOST DISEASE (GVHD)

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Mesenchymal Stem Cells (MSCs) are an important tool in regenerative medicine and tissue engineering and are the focus of intense research towards clinical applications. For instance, acute steroid-refractory Graft-versus-Host Disease (GvHD) has been shown to be responsive to treatment with mesenchymal stem cells (MSCs) by several clinical studies. However, clinical expansion of these cells in culture environments with human or animal origin reagents such as media and/or extracellular matrix protein (ECM) coated surfaces requires expensive and time consuming testing for lot to lot variability and adventitious agents. Animal-free (defined as xeno-free and human origin components-free) culture environments

significantly reduce this risk. In this study human bone marrow derived MSCs, cultured on a synthetic fibronectin ECM mimetic surface in xeno-free medium, were expanded for treatment targeting GvHD. Production of cells for in vitro studies and pre-clinical development was accomplished through process scale up beginning in T-flask formats and transitioning to large closed system stacked cell culture vessels. Cells maintained their characteristic morphology and immunophenotyping (positive for CD13, CD29, CD44, CD73, CD90, CD105 and negative for CD45, CD80 and HLA-DR) and achieved comparable growth characteristics such as population doubling time and yield. Additionally, two other cell types, human glioblastoma (A172) cells and hamster kidney (BHK-21) fibroblasts, were cultured on the fibronectin peptide coated synthetic surface in both flask and stack vessels. Cell attachment, growth and morphology on ECM mimetic flask surfaces was demonstrated to be comparable to cells grown on the larger scale stack coated surfaces. These fibronectin peptide coated closed system vessels provide a xeno-free cell expansion system enabling scale up options for clinical research.

T-1010

XENO-FREE MANUFACTURING OF HUMAN ADIPOSE STEM CELLS (ASC) FOR THERAPEUTIC APPLICATIONS: PERFORMANCE OF ASC IN CORNING(R) STEMGRO(R) HMSC MEDIUM AND CORNING CELLBIND(R) SURFACES

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Adipose mesenchymal stromal/stem cells (ASC) are an appealing cell source for therapeutic applications due to their clinical potential. Manufacturing of cell-based products for therapeutic settings require current Good Manufacturing Practices (cGMP) to ensure reproducible results for release parameters such as cell yield, cell viability, and immunophenotype. Elimination of animal-derived products in manufacturing of these products is one of the crucial steps to minimize risks upon transplantation in humans. Therefore, the use of a chemically defined media for cell culture is essential. This study describes the isolation and expansion of ASC derived from xeno-free cGMP cryopreserved human stromal vascular fraction (SVF) by culturing in Corning stemgro hMSC medium using Corning CellBIND surfaces. SVF from 3 donors were plated on CellBIND surfaces using stemgro hMSC medium. Adherent cells, further referred as ASC were detached at approximately 80% of confluence, and sub-cultured at a density of 5000 cells/cm2 for two additional passages. Expanded cells were further characterized by flow cytometry at P1 and P3, and for their differentiation potential at P3. Elongated cells were visible at day 3, presenting spindle-like morphology. Cells proliferated with a similar pattern for ASC with standard culture media as described in the literature. Viability of ASC varied from 93-99%, and cultures had an average doubling time (DT) of 32-54h. Phenotypic characterization of ASC demonstrated the presence of CD73, CD90 and CD105 (MSC markers), and absence of CD45, CD34 and CD31. Concomitant expression of MSC markers ranged between 90.0-97.6%. Expression of CD45, CD34 and CD31 was below 2.5%. Trilineage (osteogenesis, adipogenesis and chondrogenesis) potential of expanded ASC was maintained after expansion for 3 passages in stemgro hMSC medium. In summary, stemgro hMSC medium enables the attachment and maintenance of ASC which have been derived from xeno-free cGMP cryopreserved SVF using CellBIND surfaces. Moreover, ASC expanded in stemgro hMSC medium for 3 passages retained typical elongated spindle-like morphology, surface markers profile, and multipotency.

T-1011

FACTORS PRODUCED BY HEPATOCELLULAR CARCINOMA MICROENVIRONMENT INDUCE CHANGES IN HUMAN MESENCHYMAL STROMAL CELLS

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Hepatocellular carcinoma (HCC) is the 2nd cause of cancer-related death and the majority of patients are diagnosed at advanced stages. New therapies are needed and those focused on the delivery therapeutic genes by mesenchymal stromal cells (MSC) are gaining interest. HCC produces factors that recruit MSC to the tumor. However, after MSC homing, these factors could modify MSC biology. The aim of this work was to study changes occurred in human MSC exposed to conditioned media (CM) derived from HCC tumors. Cytokines in CM derived from HCC tumors were examined by an antibody array. CM were obtained from fresh human HCC samples (PT-7, PT-12 and PT-19) or tumors subcutaneously generated by a primary culture from a patient with HCC (HC-PT-5) or HuH7 cell line in nude mice. All CM demonstrated similar expression of cytokines including GRO, MCP-1 and IL-8 being the latter with the highest concentration. We evaluated their role in MSC chemotaxis to HCC by a Boyden chamber using neutralizing antibodies anti-CXCR1, CXCR2 or MCP-1. Anti-MCP-1 reduced MSC migration towards CM from HCC around 20%. Similarly, anti-CXCR1 or CXCR2 reduced MSC in vitro migration to HCC approximately 20%. When both receptors were neutralized together MSC migration was reduced about 50%. To evaluate changes on MSC behavior by HCC released factors, MSC were pre-stimulated for 24 h with CM from HuH7 or HC-PT-5. Chemotaxis assay showed a 2-fold increase of stimulated MSC (sMSC) migration towards the CM in comparison with unstimulated MSC (usMSC). Gene expression profile of sMSC with CM from PT-7, PT-12, HC-PT-5 or HuH7 tumors showed that MSC differentially expressed 445, 511, 521 and 511 genes respectively in comparison with usMSC. Among these genes 46 are related with cell migration and invasion. The most important chemokines produced by sMSC were MCP-1, Nap2, IP-10, Mip1β and Eotaxin-2. Factors produced by sMSC were able to increase fibroblasts and endothelial cells chemotaxis in comparison to factors produced by usMSC. Finally, sMSC with HuH7 CM and then inoculated in HCC tumor bearing-mice did not modify tumor growth. We can conclude that factors produced by HCC are responsible from MSC chemotaxis and

induce changes in MSC gene profile, in the released factors and in their chemotactic capacity. However, these changes did not affect the aggressiveness of HCC in vivo.

T-1012

COMPARISON BETWEEN THERAPEUTIC POTENTIAL OF HUMAN ADIPOSE DERIVED PERICYTES AND STROMAL CELLS IN A MURINE MUSCULAR DYSTROPHY MODEL

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Progressive muscular dystrophies (PMD) are genetic diseases characterized by progressive muscle loss and weakness. Duchenne muscular dystrophy (DMD) is the most common and severe form, with incidence of 1 in every 3500-5000 boys. DMD patients usually loose ambulation around 9-12 years of age and without special care death occurs in the second decade due to respiratory or heart dysfunction. Cell therapy studies with mesenchymal stromal cells (MSC) at first aimed to repopulate the patient's muscle with normal cells but now it is known that therapeutic benefits may occur by the immunomodulation properties of these cells. MSCs comprise heterogeneous cell types and routinely cell sorting is performed to select a particular subpopulation. One of the cell populations of particular interest for therapeutic research are the pericytes, which are well defined regarding their in vivo function and location and have shown to increase significantly survival of double knockout mice (dko), negative for dystrophin and utrophin. Here we compared the effect of pericytes and MSCs derived from the same human adipose sample when injected in the dko mouse model without immunosuppression (n=20 per group). Mice were injected with 1 million of cells weekly during 8 weeks. Clinical evaluations included treadmill, weight, spinal curvature ratio by x-ray analysis and survival. Both pericytes and MSCs tend to improve the survival of treated mice when compared to vehicle injected mice, with a better effect of pericytes than MSCs. However, the trend was statistically significant only during the treatment period, suggesting that these cells have a short-term effect. The other clinical evaluations didn't differ among mice injected with pericytes, MSC or vehicle. Pericytes and MSC were evaluated for angiogenic and myogenic in vivo differentiation, but they didn't have potential to those differentiations. Immunomodulatory ability was showed by lymphocyte proliferation assay, in which both pericytes and MSC were equally able to suppress lymphocyte proliferation. In an attempt to ameliorate the therapeutic effect of these procedures other questions to be addressed are: the number and interval of injections, number of cells per injection and the duration of the treatment.

T-1013

EFFECT OF SERUM ON REPLICATIVE SENESCENCE OF MESENCHYMAL STEM CELLS

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A long-term growth curve of UC-MSCs cultured in FBS or CS established by calculating the cumulative population doubling (CPD). During early-phase, cells grown in FBS had a similar growth rate to cells grown in CS. However, a difference between the groups appeared during the middle-phase and slowly increased over time. Furthermore, Brdu test confirmed that cells cultured with FBS had a higher proliferative potential than those grown in CS. Moreover, the morphology and differentiate potential of UC-MSCs as well displayed different after a long time culture in FBS and in CS. The cells cultured in FBS showed more differentiate potential to osteoblasts and adipocytes compared with the cells in CS. The different growth features of UC-MSCs cultured in FBS- and CS-containing media were also proved with the cell apoptosis and cell cycle. Compared to cells grown in FBS, the UC-MSCs grown in CS expressed higher lever of Bax and more apoptotic cells were detected. Furthermore, a larger percentage of UC-MSCs grown in FBS was in proliferative phases of the cell cycle. The increase of β-galactosidase activity and ROS levels in the late cell passage showed by SA-β-gal and DCFH-DA staining respectively demonstrated that the cells experienced senescence-associated characteristics. Furthermore, a higher proportion of senescent UC-MSCs observed when grown with CS compared to FBS. The result of telomere length likewise confirmed more ageing features in the late passage cells, and the telomere length of cells cultured with FBS was longer than SC. The mRNA levels of p53, p21 and p16 increased gradually with cell passage, but were lower in FBS cultured cells compared to CS. The gene expression alterations were further confirmed at the protein level by immunoblotting. Furthermore, the production of IL-6 and IL-8 was dramatically increased by 10-fold in high-PD MSCs compared with low-PD, indicating that these cytokines are characteristic of senescent MSCs. When serum conditions are compared, CS induced higher expression of above cell cycle and inflammation associated proteins. This study demonstrated that FBS-supplemented media provided a better microenvironment for the expansion of UC-MSCs in vitro than CS-supplemented media. This work may provide some insights into MSC generation practices for use in bench research and clinical therapies.

T-1014

EFFECT OF FETAL MEMBRANE-DERIVED MESENCHYMAL STEM CELL TRANSPLANTATION IN RATS WITH ACUTE AND CHRONIC PANCREATITIS

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Mesenchymal stem cells (MSCs) have attracted attention as a valuable cell source in regenerative medicine. Several studies have demonstrated that MSCs are abundant in and can be easily isolated from fetal membrane (FM), especially from amnion. In this study, we investigated the therapeutic effect of rat FM-derived MSCs (FM-MSCs) and human amnion-derived MSCs (AMSCs) in rats with acute and chronic pancreatitis. The effect of rat FM-MSCs and human AMSCs on the inflammatory reaction of macrophages, pancreatic acinar cells and pancreatic stellate cells were investigated in vitro. Acute pancreatitis was induced by intraductal injection of 4%

taurocholate, and rat FM-MSCs (1.0 × 106 cells) were transplanted intravenously. Rats were sacrificed at day 5, and histological analyses and quantitative RT-PCR were performed. Chronic pancreatitis was induced by intravenous injection of 5 mg/kg dibutyltin dichloride and human AMSCs (1.0 × 106 cells) were transplanted intravenously at Day 5. Rats were sacrificed at day 14, and histological analyses and quantitative RT-PCR were performed. Inflammatory reaction of macrophages induced by lipopolysaccharide and trypsin was significantly suppressed by rat FM-MSC co-culture. Pancreatic acinar cell injury induced by cerulein was significantly ameliorated by human AMSC-conditioned medium. Pancreatic stellate cell activation induced by tumor necrosis factor-α was significantly decreased by human AMSC-conditioned medium. Transplantation of rat FM-MSCs significantly reduced the histological score and the infiltration of CD68-positive macrophages in the rat pancreas. Human AMSC transplantation significantly decreased the expression of MCP-1 and attenuated the downregulation of amylase expression in the pancreas. Pancreatic fibrosis tended to be reduced by human AMSC transplantation. Transplantation of FM-MSCs and AMSCs significantly improved the rat model of both acute and chronic pancreatitis, possibly through the inhibition of macrophage and pancreatic stellate cell activation, and by the suppression of acinar cell injury.

T-1015

MURINE MESENCHYMAL STROMAL CELLS PROTECT AGAINST BLEOMYCIN INDUCED LUNG FIBROSIS IN A HGF DEPENDENT MANNER IN VIVO

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Mesenchymal stromal cells (MSC) are adult multipotent cells, that have been proposed as a cellular therapy for a range of inflammatory diseases. MSC possess potent immunomodulatory and reparative capabilities and more recently studies on the therapeutic efficacy of MSC has centred around the paracrine mediated effector functions of MSC . This study has examined the ability of MSC to attenuate lung fibrosis in vivo. Pulmonary fibrosis is characterised by extensive scar tissue formation as a result of an abnormal wound healing response. This is due to excessive collagen deposition, causing architectural distortion and loss of function of the tissue. To examine the protective ability of MSC, bleomycin was delivered intranasally to mice to induce lung fibrosis. MSC were administered at two different time points to examine their ability to attenuate this fibrosis and determine the importance of timing of MSC delivery. Early MSC administration ameliorated the severity of lung fibrosis with a significant reduction in fibrotic lung pathology, collagen deposition and inflammatory and fibrotic cytokines. MSC administration resulted in an anti-apoptotic effect in the lung tissue exposed to bleomycin. In vitro studies identified important roles for PGE-2 and HGF in MSC promotion of wound healing and inhibition of apoptosis. HGF knock down MSC were utilised to examine the role of HGF in MSC protective and anti-apoptotic effects in lung fibrosis in vivo. The protective and anti-apoptotic ability of HGF knock down MSC was significantly impaired compared to the wild type MSC, highlighting the importance of HGF in MSC protection against fibrosis in this model. For the first time, this study identified a key role for HGF in MSC protective and reparative effects in a pre-clinical model of lung fibrosis in vivo. In addition, the data herein significantly adds to our understanding of the mechanisms involved in MSC promotion of wound healing.

T-1016

MSX1 GENE RELATED TO BIPHOSPHONATE-RELATED OSTEONECROSIS OF JAW ON MESENCHYMAL STEM CELLS

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Bisphosphonates are well-known as a drug of choice for the treatment of bone diseases such as osteoporosis and prevention of bone metastasis of some malignant disease such as breast cancer, multiple myeloma etc. One of bisphosphonates is a zoledronate that is widely used for the prevention of bone loss by inhibiting of bone resorption. However, there were a number of cases of dental disease reported as bisphosphonate-related osteonecrosis of jaw. In our experiments, we hypothesized that zoledronate treatment may influence the fate of mesenchymal stem cells such as periodontal ligament stem cells (PDLSCs) and bone-marrow stem cells (BMSCs) obtained from human teeth and supporting structures. We treated zoledronate with various concentrations (10-4, 10-6, 10-8, and 10-10) and time periods (24 hrs, 48 hrs, and 72 hrs) to PDLSCs and BMSCs. Following the zoledronate treatment, the cell proliferation, apoptosis, and ALP activity were measured. Also, we perform RT-PCR for osteoblast differentiation using osteoblastic gene markers (osteopontin, osteocalcin, ALP, Collagen I type, and EF-1a). As a result, PDLSCs and BMSCs are effectively increased the proliferation and cell counting at specific concentration of 10-8. Also, while the zoledronate are treated to both cell lines, the gene expression of osteocalcin and collagen type I are decreased at high concentration of zoledronate. In conclusion, at the concentration of 10-8 zoledronate, the proliferation of cells and bone-related osteoblastic activity are increased by inhibiting the apoptosis of cells. However, the high concentrations of zoledronate decrease the osteogenesis on both PDLSCs and BMSCs. Especially, MSX1 gene is suppressed with treatment of zoledronate on PDLSCs and BMSCs.

T-1017

COMBINED TREATMENT WITH HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AND METHYLPREDNISOLONE AMELIORATE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Experimental autoimmune encephalomyelitis (EAE) is a demyelinating disease of the CNS that shares clinical and pathological features with MS and is used as an animal model for the human disease. Several studies have demonstrated the therapeutic potential of administration of human bone marrow-derived mesenchymal stem cells (hBM-MSCs) in EAE. Methylprednisolone (MP) as a synthetic glucocorticoid drug, exhibited anti-inflammatory and used as a treatment for MS. In this study, we evaluated whether the combination of hBM-MSCs and MP could produce enhanced therapeutic effects in EAE. The animal models were induced in C57BL/6 mice by using immunization with myelin oligodendrocyte glycoprotein 35-55 (MOG 35-55). Pro-inflammatory cytokines (IFN-γ, TNF-α, IL-12) and anti-inflammatory cytokines (IL-4, IL-10), hallmark cytokines that direct Th1 and Th2

development, were detected with enzyme-linked immunosorbent assay (ELISA). The effects of combination therapy on T cell proliferation and apoptosis were determined by flow cytometry (FACS). MP did not affect the viability, phenotype and differentiation ability of hBM-MSCs. The combination treatment significantly reduced clinical symptom compared with either single treatment, along with attenuation of inflammation and demyelination in EAE spinal cord. Furthermore, combined treatment promotes a shift from Th1 to Th2 cytokine balance in EAE mice. FACS analysis with cell surfaces markers suggested that combined treatment reduced proliferation and induced apoptosis in MOG-activated T cells. Thus, the combination of hBM-MSCs and MP provides a novel treatment protocol to enhance the therapeutic effects in MS.

T-1018

CHARACTERIZATION AND FUNCTION ANALYSIS OF MICROVESICLES DERIVED FROM FETAL CARTILAGE PROGENITOR CELLS

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Microvesicles (MVs) are thought as a key mediator of the paracrine effect of stem cells. MVs have an important function in communication between stem cells and with other cell types and modulating by stem cells of various events such as immune response. We have previously shown that fetal cartilage-derived progenitor cells (FCPCs) have stem cell properties and therapeutic effects on inflammatory reaction. In this study we isolated MVs from FCPCs and investigated their basic characteristics and therapeutic effect comparing to Mesenchymal stem cell (MSC) on the OA chondrocytes and synoviocytes. Human FCPCs were isolated from human fetal cartilage tissue with informed consent. After FCPCs cultured for 7 days, washed and incubated for 24 h in serum free medium. MVs were collected from the culture medium. Medium was concentrated and ultracentrifugation in a sucrose gradient. MVs collected via purification using Optiprep density gradient. Finally, the purified MVs were analyzed FACS and Nanosight against commonly found MVs markers or size distribution. To trace MVs, collected MVs were then labeled with PKH26 dye. OA chondrocytes and synoviocytes were isolated from the cartilage and synovium of OA patients during the total knee arthroplasty. Both cells were treated with FCPCs-MVs or MSCs-MVs at 10 μg/ 1 x 105 cells, then mRNA was extracted after 24 h and real time PCR was performed. The size of FCPCs-MVs has a mean value of 156 nm. They contained common MV's markers such as CD63, CD9. In the functional analysis, FCPCs-MVs were incorporated well in OA chondrocytes and synoviocytes. Real time PCR analysis revealed that the expression of MMP1 and MMP3 in OA chondrocytes was lower than in the control. In OA synoviocytes, MVs reduced the expression of inflammatory factors such as IL-1β, IL-6, IL-8 and TNF-α. Overall, their activity was comparable to that of MSCs-MVs in regulating OA phenotypes. This study showed that FCPCs-MVs exerted anti-inflammatory effects on OA synoviocytes and reduced the protease expression in OA chondrocytes as efficiently as MSCs-MVs. Future studies will address the therapeutic potential of FCPCs-MV on OA development and progression.

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T-1019

HUMAN BONE MARROW DERIVED MULTIPOTENT STROMAL CELLS AUGMENT ISLET REGENERATIVE CAPACITY THROUGH SECRETION OF WNT SIGNALING PROTEINS

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Multipotent stromal cells (MSCs) are an attractive transplantable cell source for regenerative medicine. MSCs can be easily harvested from many sources which include: umbilical cord tissue, umbilical cord blood, bone marrow, adipose tissue as well as amniotic fluid, making them ethically and readily available for therapeutic use. With the prevalence of diabetes on the rise, new strategies to combat this metabolic disease are needed. Islet transplantation has shown the ability to reverse the insulin dependence observed in patient with severe type 1 diabetes. However this approach has many drawbacks. Most recently, MSCs have surfaced as an attractive strategy to combat diabetes. MSCs have been shown to recruit to the injured pancreas and induce endogenous β-cell regeneration to improve islet function. It is believed that these MSCs secrete previously undetermined cytokines, matrix modulating factors, as well as other supportive proteins that modify or establish a regenerative niche. The current study employs the use of highly sensitive mass spectrometry to elucidate these previously unknown secreted factors. The regenerative capacity of the MSCs is determined by transplanting culture expanded MSCs into NOD/SCID mice and measuring the glucose response after STZ treatment. MSC lines are further sub-classified into two groups: regenerative MSCs and non-regenerative MSCs. Both types are then cultured in defined SILAC media that either contained heavy or intermediate arginine and lysine for 9 days to allow complete incorporation of isotopically-labeled amino acids. A total of 4000 proteins have be identified by mass spectrometry in all cell lines. The proteomic screen has revealed that the regenerative MSCs secrete more proteins, a large number of which correspond to Wnt signaling pathway. Two secreted proteins that were only detected in the secretome of regenerative MSCs, WISP1 and WISP2, are members of the CCN family of proteins that are responsible for a number of cellular processes. To further probe the WNT pathway, quantitative real-time PCR was performed on 84 genes. Key findings suggest that WISP1 is 3 fold more prevalent in regenerative MSCs, and Wnt inhibitors such as WIF1 and DKK1 are 5 and 2.5 fold higher in non-regenerative MSCs, suggesting that the Wnt signaling pathway is inactive.

T-1020

OSTEOGENIC CAPACITY OF CANINE ADIPOSE DERIVED MESENCHYMAL STEM CELLS ON ROLL SHEET TYPE POLY EPSILON-CAPROLACTONE/BETA-TRICALCIUM PHOSPHATE SCAFFOLD

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Successful repair of bone defects injuries are a major issue in reconstructive surgery. Bone tissue engineering using a suitable scaffold has been developed as an alternative strategy of bone graft. In addition, multipotent cells have capable of differentiation into osteoblast. It can enhance the bone repair. The aim of this study was to investigate osteogenic capacity of canine adipose tissue-derived mesenchymal stem cells (AD-MSCs) with poly ε-caprolactone (PCL)/β-tricalcium phosphate (β-TCP) composite scaffolds. Sheet form scaffolds of PCL/β-TCP were fabricated. AD-MSCs were seeded into the scaffolds and were exposed to proliferation or osteogenic differentiation condition. After 14 days of in vitro culture, osteogenic differentiation capacity of AD-MSCs were evaluated by using the alkaline phosphatase (ALP) activity assay and reverse transcriptase polymerase chain reaction (RT-PCR) technique. For in vivo study, sheet form PCL/β-TCP scaffolds alone, undifferentiated AD-MSCs with scaffolds and osteogenic differentiated AD-MSCs with scaffolds implanted into segmental bone defects in dogs. The sample harvested at 12 weeks after implantation, measured newly formed bone mass by micro-computed tomography. The ALP activities in osteogenic differentiated AD-MSCs group was significantly higher than undifferentiated AD-MSCs group. The ALP, runt related transcription factor 2 and osteocalcin mRNA levels of the osteogenic differentiated AD-MSCs group up-regulated compared to the undifferentiated group. In addition, the amounts of new bone of the osteogenic differentiated group was significantly higher than scaffold alone group and undifferentiated group. The AD-MSCs can proliferate and osteogenic differentiate on the PCL/β-TCP scaffolds. Also osteogenic differentiated AD-MSCs show osteogenic capacities and enhance new bone formation. These results indicated that the osteogenic differentiated AD-MSCs with PCL/β-TCP scaffold can potentially be used for clinical bone tissue regeneration.

T-1021

THE COMPARISON OF FRESHLY ISOLATED RAT MONONUCLEAR CELLS AND MESENCHYMAL STROMAL CELLS FROM THE BONE MARROW FOR THE USE IN TRACHEAL TISSUE ENGINEERING

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Tracheal tissue engineering commonly involves adult bone marrow derived stem cells seeded to a biocompatible scaffold to replace damaged airways. The choice of cell source for preclinical studies play a pivotal role to provide insights into potential mechanisms involved in tissue remodeling and regeneration. Mononuclear cells (MNCs) contain both hematopoietic and non-hematopoietic stem cells such as mesenchymal stromal cells. They can be easily isolated from the bone marrow and rapidly seeded on the day of harvesting. In contrast, MSCs require ex vivo expansion, can be costly and associated with a risk of microbial contamination. Here, we report the comparison of freshly isolated MNCs to MSCs in a rat model. MNCs were isolated by ficoll gradient separation, while MSCs were isolated by their plastic adherent capacity. Although we noticed that MNCs and MSCs were morphologically similar with a fibroblastic-like appearance, and could robustly be directly differentiated into mesenchymal tri-lineages, by real time RT-PCR, MNCs had higher expression for CD45 but lower expression for CD90, CD34, CD105 and CD73. Further characterization including colony forming unit assay, flow cytometry and immunocytochemical detection were used to determine their multipotency. Both cell populations readily adhered and survived on clinically applied synthetic electrospun tracheal nanomaterial. Mathematical modeling was further used to estimate the MSC population size presented in MNCs. Whether MNCs and MSCs can be interchangeably utilized without limitation; this must further be elucidated through functional assays and in vivo studies.

T-1022

AFFINITY SELECTION OF HEPARAN SULFATE GLYCOSAMINOGLYCANS: A PROMISING SCALE UP STRATEGY FOR STEM CELL THERAPY

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Human mesenchymal stem cells (hMSCs) have become a valuable resource for cell-based therapy and been vigorously assessed in various clinical trials. A main challenge for their more widespread application is the low abundance of stem cells in native tissues, demanding strategies to maximize ex vivo expansion of hMSCs without compromising their stemness. Fibroblast growth factor (FGF) 2 increases hMSC proliferation and is widely used as a stem cell culture adjuvant. Another valid strategy is to manipulate extracellular matrix (ECM) components, such as heparin and more specifically heparan sulfate (HS). As a class of glycosaminoglycans abundant in ECM, they are known to regulate the activity of many growth factors, most notably FGF2. Heparin has been used extensively to support human stem cell expansion as an analogue of the more physiologically appropriate HS; however, our recent work suggests that heparin adversely affects hMSCs, resulting in senescence, which is not surprising given heparin's promiscuous interactions with many proteins. To obviate the need for heparin, we have instead isolated HS variants more specifically targeted to FGF2. An affinity chromatographic approach was utilized to extract such HS, dubbed HS8, using peptide sequences derived from the heparin-binding domains of FGF2 as the ligand bait. ELISA assays demonstrated that HS8 bound to FGF2 with much higher affinity than to other FGFs or other heparin-binding factors such as PDGF or VEGF. Furthermore, the melting temperature of FGF2 was markedly increased by HS8, indicating HS8 acts to stabilize FGF2, so prolonging its activity. Both FGF2-stimulated ERK signaling and proliferation was amplified by HS8 in hMSCs. Importantly, hMSC cultures expanded with HS8 supplementation yielded a subpopulation cells that were enriched for the stemness marker STRO-1+ as well as greater CFU-F capacity. When applied into critical-size calvarial defects in rats, HS8 significantly accelerated bone healing as confirmed by micro-CT scanning. Our work demonstrates that affinity-selection is able to enrich for HS variants that can trigger faster hMSC ex vivo expansion without adversely changing their biological properties.

Better preparation of HS is a promising avenue for stem cell scale-up technology required to meet the rapidly expanding clinical need.

T-1023

LONG TERM EFFECTS OF MESENCHYMAL STROMAL CELLS ON RETINAL GANGLION CELLS SURVIVAL AND REGENERATION AFTER INTRAVITREAL TRANSPLANTATION AFTER OPTIC NERVE INJURY IN ADULT RATS

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We have investigated the therapeutic potential of mesenchymal stromal cells (MSC) transplanted after optic nerve crush (ONC), which causes retinal ganglion cell (RGC) death and optic nerve degeneration. Lister Hooded rats underwent unilateral ONC followed by an intravitreal injection of 5 x 105 MSC (treated) or the vehicle (untreated). MSC were labeled with iron-oxide particles for cell tracking. Optomotor response was evaluated from 1 to 59 days after ONC. On the day before euthanasia, rats were kept for 24h in the dark and then exposed to light for 2h. The brains were analyzed for the expression of the immediate early gene NGFI-A in the superior colliculus (SC). This gene is upregulated in neurons of the SC after activation of the NMDA receptor by RGC axons and it is downregulated in the absence of visual stimuli or connections from the retina to the SC. The retinas were immunostained for Tuj1 to estimate RGC number. Axons were anterogradely labeled with cholera toxin B (CTB). Tuj1-positive cell numbers in the central retina were increased from 88,88 ± 8,337 cells/mm2 in the untreated group (n=9) to 260,4 ± 16,60 cells/mm2 in the treated group (n=8, mean ± SEM, P<0.001), 28 days after injury. In addition, the number of CTB-positive axons 0.5 mm beyond the injury site was 2.7-fold increased in the treated group. MSC remained in the vitreous body for at least 18 weeks. Optomotor response was completely lost in the ipsilateral eye of treated and untreated groups, and it was not restored after 59 days. NGFI-A-positive cells in the contralateral SC were significantly increased from 0,1965 ± 0,02703 in the untreated group to 0,4059 ± 0,02377 in the treated group (% to ipsilateral SC, n=3, P<0,01). Cell counts 60 days after injury showed 67,98 ± 27,55 Tuj1-positive cells/mm2 in the untreated group (n=3) and 104,1 ± 15,49 cells/mm2 in the treated group (n=4). These preliminary results suggest that RGC degenerate overtime in both groups, but the number of cells is higher in the treated animals for at least 60 days after injury. Although this is not associated to the recovery of the optomotor response, NGFI-A expression was increased in the SC of treated animals, suggesting that axons regenerated and made synapses to their target cells in the brain. Further analysis of the visual response will be performed at 90 and 120 days after treatment.

T-1024

THERAPEUTIC POTENTIAL OF HUMAN ADIPOSE TISSUE DERIVED MULTI LINEAGE PROGENITOR CELLS IN NON ALCOHOLIC FATTY LIVER DISEASE

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Nonalcoholic fatty liver disease (NAFLD) is a common and increasing cause of chronic liver disease and broadly defined by the presence of steatosis with inflammation and progressive fibrosis. Recently, we have reported the therapeutic potential of adipose tissue-derived multi-lineage progenitor cells (ADMPCs) in liver fibrosis using CCl4-induce chronic mice model. These findings lead us to plan next study, whose aim was to assess the effectiveness of ADMPCs in improving NAFLD. ADMPCs were isolated from inguinal adipose tissues of C57 BL/6 mice and expanded. NAFLD model was induced by a single subcutaneous injection of 200 μg STZ 2 day-after birth followed by feeding a high fat diet beginning at 4 weeks of age. After randomization of animals, the NAFLD mice received ADMPCs (n=4) or placebo control (n=5) via tail vein injection at an age of 6 weeks and were applied for histological and blood examination at an age of 9 weeks. NAFLD model mice with ADMPCs injection exhibited a significant reduction in liver fibrosis and inflammation areas, as evidenced by Sirius red staining and F-4/80 immunohistochemical-positive inflammatory cells infiltration area %, respectively, compared with those without ADMPC injection. NAFLD activity score was also reduced from 4.75 (placebo controls) to 4 (ADMPC-injected). Moreover, blood examination showed that plasma adiponectin (APN) levels in ADMPCs-treated NAFLD model mice were higher than those in placebo controls. in vitro-Production of anti-inflammatory cytokines (PGE2 and Glutathione peroxidase), fibrinolytic enzymes (matrix metalloproteinase-3 and -9) and hepato-protective cytokines (HGF and APN) examined by ELISA were higher than those of and BM-MSCs, suggesting the mode of action of ADMPCs. These results showed the mode of action and proof of concept of systemic injection of ADMPCs in NAFLD, which is a promising therapeutic intervention for the treatment of patients with NAFLD.

T-1025

EVALUATION OF CELL THERAPY IN EXPERIMENTAL MODEL OF CHAGAS' DISEASE USING MAGNETIC RESONANCE IMAGING AND SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY

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Chagas' disease is a leading cause of cardiomyopathy with no effective treatment. In previous work we observed that the injection of adipose tissue-derived mesenchymal cells (ASC) prevented cardiomyopathy progression. For further inquiries about cardiac function and perfusion, it is essential to use in vivo analysis. The objective of this work was to demonstrate whether Magnetic

Resonance Imaging (RMI) and Single Photon Emission computed Tomography (SPECT) were able to evaluate mouse's heart infected with T. cruzi treated with ASC. ASC were isolated from subcutaneous adipose tissue of transgenic mice expressing the green fluorescent protein gene under the control of the β-actin promoter through enzymatic digestion and maintained until third passage. Three days post-infection (3dpi) 106 cells were injected ip. Parasitemia was evaluated between 5 and 34dpi by parasite count in the peripheral blood. RMI studies measured ejection fraction (EF), end diastolic volume (EDV), end systolic volume (ESV) and ventricular area (Va) in both ventricles; and the cardiac perfusion was assessed by SPECT. Two-way ANOVA and Student's t test was used for statistical analysis. Treatment significantly decreased the amount of parasites in Infec + ASC group compared to Infec + PBS (3.94±3.6 vs 10.37±7.06 x 105 Trypomastigotes/mL of blood). MRI analysis showed that Infec+mASC group showed reduction Va, EDV and ESV right ventricle in comparison to the Infec+PBS group values (11.93±0.84 vs 16.04±0.84mm2; 43.06±3.97 vs 59.41±3.52μL; 24.62±3.66 vs 39.77±2.83μL, respectively). In scintigraphy a discreet reperfusion was found. However, when we look for these fluorescent cells in the cardiac tissue, we found no sign of them. In conclusion, MRI and SPECT were able to evaluate cardiac function and perfusion respectively. The images acquired showed that ASC treatment was able to prevent right ventricular dilation caused by the infection as well as increase myocardial perfusion.

T-1026

IMMUNE ACTIVATION OF ENDOGENOUS PAIN MODULATION AS A MECHANISM OF BONE MARROW STROMAL CELL-PRODUCED PAIN RELIEF IN RATS

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We have shown that intravenous infusion of rat bone marrow stromal cells (BMSC) suppressed pain hypersensitivity in rat persistent pain models and the effect was reversed by systemic naloxone, an opioid receptor antagonist. Studies suggest that BMSC produce their therapeutic effects through secretion of chemical mediators that interact with the body's immune system. In the present study, we tested the hypothesis that BMSC produce the pain-relieving effect, or antihyperalgesia, through interactions with immune cells and involve activation of the endogenous opioid system. A rat model of myogenic orofacial pain was produced by ligation of the tendon (TL) of the masseter muscle. Primary culture of rat BMSC were obtained from donor rats and human primary BMSC cell line was purchased (STEMCELL Tech.). BMSC were injected (1M cells/rat, i.v.) at 7 d after TL. We show that the mu opioid receptors (MOR) expression in the rostral ventromedial medulla (RVM), a key structure in endogenous pain modulation, was upregulated following infusion of both rat and human BMSC. BMSC's antihyperalgesic effect and upregulation of MOR was attenuated by depletion of the monocyte/macrophage population of immune cells. Direct injection of peripheral blood monocytes (PBMC, 10K cells) from BMSC-treated rats into the RVM attenuated hyperalgesia in TL rats. Interestingly, injection of serum from BMSC-treated rats into the RVM produced similar pain attenuation, suggesting that chemical mediators from PBMC mediated BMSC's effect. Antibody array of serum and chemokine array of PBMC derived from BMSC-treated rats consistently detected an increase in CXCL1 chemokine levels in BMSC-treated rats, compared to control rats. Immunostaining showed localization of CXCR2, the receptor for CXCL1, in RVM neurons, but not glial cells. CXCR2 expression was also shown in RVM neurons immunoreactive for MOR. Injection of the CXCR2 antagonist SB 225002 (100 pmol) and transfection of Cxcr2 shRNA into the RVM both diminished BMSC-produced antihyperalgesia. The BMSC-induced upregulation of MOR in RVM was also attenuated after RNAi of Cxcr2. These results suggest that immune activation of endogenous pain modulation plays an important role in BMSC-produced antihyperalgesia and monocyte-derived CXCL1 is one critical mediator of BMSC-produced effects.

T-1027

THERAPEUTIC EFFECTS OF HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELLS WITH METHYLPREDNISOLONE TREATMENT IN RAT SPINAL CORD INJURY

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Methylprednisolone (MP), a glucocorticoid steroid, has an anti-inflammatory action and seems to inhibit the formation of oxygen free radicals produced during lipid peroxidation in a spinal cord injury (SCI). Currently MP is the standard therapy after acute SCI on reported neurological improvements. The combination therapeutic effect of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) for transplantation time (1d, 7d, and 30d) after MP treatment on the axonal regeneration and on the behavioral improvement in SCI were studied in the rat. The spinal cord was injured by contusion using a weight-drop at the level of T9 and MP (30 mg/kg, i.m., 10 min and 4 h) was acute administered after injury. hUCB-MSCs were labeled GFP and our study was performed the efficacy for transplantation time (1d, 7d, and 30d) of hUCB-MSCs into the boundary zone of injured site. Efficacy was determined by histology, anterograde and retrograde tracing, and behavioral test. We found that hUCB-MSCs with MP treatment exerted a significant beneficial effect by neuroprotection and reducing cavity volume. Also the transplantation of hUCB-MSCs with MP treatment was significantly improved functional recovery. Combined transplantation at 7d after SCI provided significantly greater efficiency than combined transplantation at 1d and 30d. These results suggest that transplantation time window of the hUCB-MSCs with MP treatment give rise to an earlier neuron protection strategy and effect of cell grafting in SCI. Thus our study may be considered as a therapeutic modality for SCI.

T-1028

THERAPEUTIC EFFICACY OF ISCHEMIC SERUM PRECONDITIONED MESENCHYMAL STEM CELLS USING DIFFUSION TENSOR IMAGING IN EXPERIMENTAL STROKE MODEL

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Magnetic resonance diffusion tensor imaging (DTI) is widely used to investigate brain white matter microstructure noninvasively using anisotropic motion of water molecule. DTI indices such as fractional anisotropy (FA) and mean diffusivity (MD), and neural fiber tractography have been used to study various neurological disorders including dementia, multiple sclerosis, traumatic brain injury, and ischemic stroke in both human and animal models. In this study, we aimed to observe the regeneration effect of bone marrow derived mesenchymal stem cells (MSCs) which were cultured in the ischemic stroke patient's serum. We also observed the recovery radiologically using DTI technique. The in vitro vertical migration system was used to evaluate the migration capacity of MSCs toward the ischemic location. Transient middle cerebral artery occlusion rat model was used in this study. Immunohistochemical assay was performed with anti-ß2-MG which is one of the human stem cell marker to confirm the migrated MSCs in the ischemic lesion. To evaluate the functional recovery, behavior test (modified neurological severity score) was performed 1 day to 5 weeks after experimental stroke. DTI image was acquired from the experimental stroke model using 7T small animal MR scanner. Diffusion indices such as FA and MD, and the neural fiber tractography were computed for assessing regenerated neural tracts. We observed the migration capacity of ischemic serum preconditioned MSCs toward the ischemic environment compared to the MSCs which were cultured in FBS by applying the in vitro vertical migration system. In in vivo, we observed more ß2-MG positive MSCs in case of ischemic serum preconditioning than FBS and also, functional recovery was improved in that case. In DTI indices results, FA shows higher value in the ischemic serum preconditioned MSCs injected group than FBS and PBS group. Also, tractography results show high number of fiber tracts in the ischemic serum preconditioned group at 2 weeks later after ischemic stroke. Ischemic serum preconditioned MSCs can improve a migration capacity toward the ischemic lesion and regeneration after stroke. Using DTI method, we can assess the recovery of the brain microstructure in various neuronal disorders.

T-1029

THERAPEUTIC EFFECT OF INTRAVENOUSLY ADMINISTERED CLINICAL-GRADE HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS (HUC-MSCS) IN A RODENT MODEL OF ISCHEMIC STROKE

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In this study, we aimed to provide preclinical evidence showing the therapeutic effects of intravenously (IV) administered, GMP-generated human umbilical cord-derived mesenchymal stem cells (hUC-MSCs)using a rodent model of middle cerebral artery occlusion (MCAo). To do this, we transplanted hUC-MSCs into 90-min MCAo rats with various cell doses and at different time points. We examined the behavioral, histological and molecular changes at the acute and late phases of ischemic stroke. We also investigated the in vitro effect of hUC-MSC-derived conditioned medium (CM) on immunomodulation. Among various treatment conditions, we found that IV administration of hUC-MSCs with dose of 1 x 10^6 cells at 1 day and 7 days after MCAo was effective in functional improvement and infarct size reduction. The acute treatment (1 day) of hUC-MSCs attenuated astroglial activation with anti-inflammatory macrophage polarization on ischemic brain. Additionally, hUC-MSCs altered the expression of immune response-related genes with prominent upregulation of interleukin-1 receptor antagonist (IL-1ra) in astroglial cells of ischemic brain. Treatment with CM of hUC-MSCs increased IL-1ra in macrophages via activation of Akt-CREB signaling. Additional IV administration of hUC-MSCs during sub-acute phase (7 days) after MCAo showed further functional improvement via mechanisms of promoting endogenous repair, such as enhanced neurogenesis and angiogenesis on damaged brain. According to our study, single or repeated IV administration of hUC- MSCs at acute (1 day) and sub-acute (7 days) phases can lead to the histological, biochemical and functional improvement in ischemic stroke. The therapeutic effect of hUC-MSCs was time-dependent, with attenuation of inflammation in early phase and enhanced endogenous repair in late phase of cerebral ischemia. Enhanced astroglial IL-1ra up-regulation is one of the therapeutic mechanisms of hUC-MSCs in ischemic stroke. *This study was supported by a grant of the Korea Healthcare technology R&D project, Ministry for Health, Welfare & Family Affairs (A121964).*

T-1030

MESENCHYMAL STEM CELL-DERIVED MICROVESICLE THERAPY FOR STROKE: NEUROGENIC/ANGIOGENIC EFFECTS AND BIODISTRIBUTION IN A RAT STROKE MODEL

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We hypothesized that mesenchymal stem/stromal cells (MSCs) exert their action via microvesicles in the ischemic brain and that MSC-derived microvesicles could minimize cell trapping within organs that filter the bloodstream of systemically introduced stem cells. Thus, we evaluated the neurogenic and angiogenic potential of microvesicles and their biodistribution in a rat stroke model. Microvesicles were obtained from supernatants of MSC cultures after treatment with ischemic brain extracts. MSC-derived microvesicles were injected stereotaxically or intravenously in a rat stroke model. When neural stem cells and endothelial cells were treated with 100 μg/mL microvesicles, neurogenesis and angiogenesis

increased in a dose-dependent manner ($p<0.01$). MSC-derived microvesicles stimulated neurogenesis and angiogenesis. To test microvesicle biodistribution, microvesicles and MSCs were labeled with either fluorescent dyes (CFSE) or GFP transfection and intravenously injected. On western blots, higher GFP levels were observed in the infarcted brain of microvesicle-treated rats than MSC-treated rats, whereas GFP levels in the lung and liver were higher in MSC-treated rats than microvesicle-treated rats. Mortality rates were higher in MSC-treated rats than microvesicletreated rats (50% vs. 5%, $p<0.0001$). Microvesicle-treated rats exhibited greater behavioral improvements than control rats ($p<0.05$). Cargo protein analysis showed that microvesicles included VEGF/VEGFR-2, HGF/c-Met, SDF-1/CXCR-4, synaptophysin, and TGF-beta. Additionally, microvesicles contained various miRNAs associated with neurogenesis and angiogenesis. MSC-derived microvesicles promote neurogenesis and angiogenesis in the injured brain. Stem cell-derived microvesicle therapy could be a novel, feasible, and safe strategy that avoids cell-associated problems.

T-1031

COMPARISONS OF DIFFERENTIATION POTENTIAL IN HUMAN MESENCHYMAL STEM CELLS FROM WHARTON'S JELLY, BONE MARROW AND PANCREATIC TISSUES

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Type 1 diabetes mellitus results from autoimmune destruction of pancreatic islet β-cells. Insulin-producing cells (IPCs) differentiated from mesenchymal stem cells (MSCs) in human tissues decrease blood glucose levels and improve survival in diabetic rats. We compared the differential ability and the curative effect of IPCs from three types of human tissue to determine the ideal source of cell therapy for diabetes. We induced MSCs from Wharton's jelly (WJ), bone marrow (BM) and surgically resected pancreatic tissue to differentiate into IPCs. The in vitro differential function of these IPCs were compared by insulin-to-DNA ratios and C-peptide levels after glucose challenge. In vivo curative effects of differentiated cells transplanted into diabetic rats were monitored by weekly blood glucose measurement. WJ-MSCs showed better proliferation and differentiation potential than pancreatic MSCs and BM-MSCs. In vivo differentiated WJ-MSCs significantly reduced blood glucose levels at first week after transplantation and maintained <200 mg/dl for > 6 weeks, while differentiated BM- MSCs and pancreatic MSCs reduced blood glucose levels at first week and maintained 250 mg/dl for 5 weeks and 300 mg/dl for 4 weeks, respectively. WJ-MSCs are the most promising stem cell source for β-cell regeneration in diabetes treatment.

T-1032

EFFECT OF VEGF TREATMENT ON HUMAN FETAL MESENCHYMAL STEM CELL ADHESION, MOTILITY AND MIGRATION IN FACILITATING TISSUE REPAIR

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Mesenchymal stem cells (MSC) have great potential as a form of stem cell therapy in regenerative medicine. However, trials done have shown poor engraftment rates leading. We thus hypothesize that stimulation by VEGF can modulate various properties of hfMSC to support tissue repair and augment wound healing. We first investigated the static adhesive properties of several sample lines onto fibronectin (FN) and VCAM-1. Following 4 hours of VEGF treatment, 2 (S118 and S125) out of 4 samples tested demonstrated an increase in adhesion to FN (1.47X and 1.89X, $p<0.05$) and VCAM-1 (1.33X and 1.92X) compared to the untreated group (UTX). Tracking the motility of the cells on FN over a 40-hour imaging, we saw an increase in the instantaneous cell velocity from 0.0024 ± 0.0001 to 0.0028 ± 0.0001 µm/min ($p<0.05$) following VEGF treatment compared to UTX. Using a transwell system, there was also an increase in hfMSC migration towards SDF-1 compared to spontaneous basal migration (1.77X, $p<0.05$, n=8). In comparison, UTX only showed a 1.36X increase in migration in response to SDF-1. The treatment of AMD3100 (antagonist to CXCR4), in some lines, resulted in a subsequent decrease ($p<0.05$) in the number of cells migrating towards SDF-1 following VEGF treatment. Following treatment, some of the lines also showed an increase in transmigration capability across a HUVEC layer as compared to UTX. Through FACS, we analysed that VEGF-treated hfMSC did not show an increase in percentage of cells expressing cell surface CXCR4 or MFI. Instead, through western blot, we found that in comparison to UTX, there was an upregulation in the phosphorylation of serine residues on CXCR4 in the VEGF group. This suggests that VEGF influences hfMSC response to SDF-1 by modulating the activity of CXCR4. There was also an improvement in cell survival (0.76X drop in annexinV+ cells, $p<0.01$) and VEGF secretion (>2X increase, $p<0.001$) following VEGF treatment compared to UTX, suggesting possible augmentation in cell survival and retention following engraftment, as well as promoting angiogenesis/vasculogenesis during wound healing. Our data supports our hypothesis that recruitment of hfMSC can be modulated by VEGF and that it may be used as a pre-treatment option to enhance the migration, engraftment and support tissue reparation properties of selected hfMSC lines.

T-1033

REGENERATIVE EFFECT OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS IN FOOT FAT PAD

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The foot fat pad (FFP) ultimately bears body weight at standing and absorbs striking shocks during walking and running. The thinning

or atrophy of FFP during aging is a source of foot pain, affects the function of other joints in the lower extremity and makes the foot more susceptible to pressure ulcer, especially for diabetic patients. In this study, we implanted autologous adipose tissue derived mesenchymal stem cells (AT-MSC) in rats for FFP regeneration. A total of 30 male Sprague-Dowley rats were operated to harvest fat tissue from both sides of inguinal areas for isolation of AT-MSCs. AT-MSCs were induced for adipogenic differentiation for one week. On the day of injection, the cells were labeled with fluorescent dye Vybrant®Dil. The Dil-labeled adipogenically differentiated AT-MSCs (5x104 suspended in 50ul saline) were injected into the second infradigital pad in right hind foot of the rat of origin. Saline only (50ul) was injected into the corresponding fat pad in the left hind paw of each rat. A group of rats (n = 10) was euthanized at 1, 2, and 3 weeks and the second infradigital fat pads were dissected from both sides for histology and MT-MSC tracking. Cell tracking showed that the injected AT-MSCs were largely alive through the 3-week experimental period. Fat pad unit (FPU) is a histological hallmark of FFP and consists of a cluster of adipocytes surrounded with dense elastin fibers. On histology, the number of FPUs in the fat pads that received AT-MSC injections was greater than that in the control fat pads. The difference, however, was only significant at week 2. The areas of FPUs in fat pads that injected with AT-MSCs, increased as compared with the controls. Moreover, elastin fiber density in the fat pad received injections of AT-MSCs was increased at weeks 2 and 3. This is the first study to apply AT-MSCs for treating age-related FFP degeneration. Injection of AT-MSCs increased the number and area of FPUs and the elastin fiber density in the fat pads. Given that aging-related FFP degeneration is primarily a result of reduced adipocytes per FPU, AT-MSC stimulation on individual FPUs may be more significant than regeneration of new FPUs for potential treatment of aging-related pathology in FFP.

MESENCHYMAL STEM CELL DIFFEREN-TIATION

T-1034

VARIABILITY IN OSTEOGENIC CAPACITY OF BONE MAR-ROW MESENCHYMAL STROMAL CELLS IN MIDDLE-AGED WOMEN CORRELATE WITH HIP IMPLANT HEALING

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Bone healing is regulated by a variety of cells and signaling molecules, including local and systemic mesenchymal stromal cells (MSCs). Age-related dysfunction of MSCs is suggested as main cause of altered repair capacity with aging. Decreased MSC capacity with age is well documented in vitro, but it is unclear how well this reflects in vivo bone healing capacity of the donors. We recently showed that aging, low BMD and geometric changes of the proximal femur are risk factors for early migration and delayed osseointegration of cementless hip implants in postmenopausal women. Individual variations in MSC capacity may influence the implant healing process. Therefore, we investigated how in vitro assayed osteogenic capacities of patient's MSCs reflect in implant healing. We hypothesized that patients with altered osteogenic capacity of their MSCs would show increased implant migration and delayed osseointegration. This study included postmenopausal women (mean age 64 years, range 41-78) with primary hip osteoarthritis and well-defined preoperative bone quality, undergoing cementless total hip arthroplasty (THA). A subgroup of 19 women underwent successful preoperative analysis of BM-MSCs and two-year radiostereometric analysis (RSA) of implant migration as a measure of implant healing. Implant healing was analyzed as 1) change in stem position, 2) total distance of migration 3 to 24 months, and 3) time point for osseointegration (i.e. implant stability as sign of complete healing). Defined MSCswere evaluated for growth kinetics and osteogenic capacity (ALP expression and mineralization). Osteogenic capacity of THA patients' MSCs correlated with preoperative systemic BMD (r=0.59, p=0.002), serum levels of 25(OH)D (r=-0.48, p=0.009), and serum bone turnover markers ALP (r=0.40, p=0.041) and TRACP-5b (r=0.44, p=0.023). In patients with MSCs of low osteogenic capacity, the total distance of implant migration was greater (p=0.038), and time point for implant healing was delayed (p=0.030). In accordance with current concepts, MSCs from female THA patients displayed individual differences in osteogenic and proliferative capacity in vitro, which reflected in the process of in vivo bone healing represented by cementless implant fixation.

T-1035

REGULATION OF STEM TO PROGENITOR TRANSITION IN THE MESENCHYME STEM CELL NICHE

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Adult mesenchymal stem cells (MSCs) provide a source of cells for tissue growth and repair. Unlike human teeth, mouse incisors grow continuously, which is a process achieved by stem cells residing at their apical end and can therefore provide an ideal model to study MSCs and their niche in vivo. We show here that expression of Celsr1, Thy1 and Ring1b in the MSC niche correspond to quiescent cells, slow cycling stem cells and fast cycling transit amplifying cells respectively in the mouse incisor. Members of the PRC1 complex are specifically expressed in the fast cycling transit amplifying cells of the incisor. Ring1a/b double knock-out (DKO) mice show dramatically reduced proliferation. Interestingly, slow cycling stem cells can be observed outside their niche following Ring1 deletion. We identify specific CBX proteins rather than RYBP protein as components of the PRC1 complex together with Ring1a/b in the mouse dental pulp cells. Co-immunoprecipitaion and flow cytometry identify the co-localization of Ring1b with CBX7 and H3K27me3. Gene microarray profiling identifies the CDK inhibitor Cdkn2a as significantly up-regulated in Ring1a$^{-/-}$; Ring1b$^{fl/fl}$ *cre+* cells compare to the Ring1a$^{-/-}$; Ring1b$^{fl/fl}$ *cre-* cells. Based on the repressive role of PRC1 complex, our data suggest that Ring1b interacts with CBX7 and binds to trimethylated H3K27 to repress Cdkn2a expression in progenitor cells and thus regulate the transition from slow (stem) to fast (progenitor) cycling cells in the MSC niche.

T-1036

XENO-FREE DIFFERENTIATION OF HUMAN ADIPOSE DERIVED AND BONE MARROW DERIVED MESENCHYMAL STEM CELLS TOWARDS MYOCYTES FOR APPLICATIONS IN TISSUE ENGINEERING

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Tissue engineering is a growing field which is dealing inter alia with bio-functionalization of synthetic materials using stem cells. Examples for applications are the coating of bone replacement materials to improve ingrowth in critical size defects or the coating of stents in cardiovascular tissue engineering. Most tissue engineered constructs fail on their way to the market because of several limitations. A common known limitation is that a huge amount of cells is needed to produce an autologous tissue engineered construct. Human mesenchymal stem cells (MSC) derived from bone marrow are widely used for tissue engineering settings, but there is only a limited amount of cells available based on the characteristic of the tissue source. Another limitation of tissue engineered constructs on their way to the market is the usage of fetal calve serum (FCS) in cell culture. A promising tissue source for MSC and FCS-free culture media need to be investigated. Lipoaspirate is such a promising tissue source for MSC. In this study, human adipose-derived stem cells (ADSC) are isolated from tissue lipoaspirates and compared to bone marrow derived MSC (BM-MSC). The ADSC are characterized by flow cytometry using the typical MSC marker panel (CD73, CD90, CD104, CD44, CD34, CD45) as well a series of other markers known to be present in adipose derived MSC (AD-MSC) (CD49d, CD29, CD106, CD166). Cells from both sources are differentiated under xeno-free conditions towards myocytes following a cytokine-induction protocol. Functional analysis of myocytes is performed by using lifetime imaging with a carbachol contraction assay. AD-MSC and BM-MSC are further compared under xeno-free and FCS-based medium expansion conditions for their proliferation potential. In the present study we pave the way for a myogenic differentiation of AD-MSC and BM-MSC under xeno-free conditions for future tissue engineering applications.

T-1037

EFFECTS OF A DEFINED XENO-FREE MEDIUM ON THE GROWTH AND NEUROTROPHIC PROPERTIES OF HUMAN ADULT STEM CELLS

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Mesenchymal stem cells (MSC) are promising tools for a broad range of clinical applications including nerve repair. Our previous studies have shown that human MSC stimulated with a mixture of growth factors (bFGF, PDGF-AA, neuregulin and forskolin) release high levels of neurotrophic molecules and enhance in vitro neurite outgrowth and axon regeneration in vivo. For clinical applications, our standard cell culture techniques which use animal-derived reagents need to be adapted. The aim of this study was therefore to investigate the growth properties and neurotrophic effects of human bone marrow derived stem cells (BMSC) and human adipose tissue derived stem cells (ASC) cultured in a defined xeno-free medium (MesenCult™-XF, STEMCELL Technologies Inc). Both cell types showed better proliferation in MesenCult™-XF medium compared with standard MEM-Alpha medium containing 10% (v/v) foetal calf serum (FCS). At early passage, the population doubling time for BMSC in FCS medium was 87,14 ± 13,47 h versus 46,96 ± 3,21 h ($P<0.001$) in MesenCult™-XF medium. Similar values were obtained for ASC. RT-PCR showed that both types of stem cells expressed a range of neurotrophic and angiogenic molecules including NGF, BDNF, GDNF, VEGF-A and angiopoietin-1. Growth factor stimulation of the cells up-regulated BDNF and VEGF-A gene expression under both FCS and Mesencult™-XF conditions. Changes in gene expression correlated with enhanced protein secretion. ELISA analysis showed that stimulation under MesenCult™-XF conditions produced the highest levels of secreted proteins. To test the biological activity of the stem cell conditioned media it was applied to the human SH-SY5Y neuronal cell line. Neurite outgrowth was increased in the presence of medium from stimulated BMSC and ASC. The greatest effect was produced by the ASC stimulated under MesenCult™-XF conditions. These results indicate that the neurotrophic effects of adult stem cells can be reproduced under defined xeno-free conditions and provides further evidence for their potential clinical application for the treatment of nerve injuries.

T-1038

ENHANCEMENT OF DIFFERENTIATION AND SURVIVAL ON HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS BY PULSED ELECTROMAGNETIC FIELD DURING NEURONAL INDUCTION

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Mesenchymal stem cells (MSCs) have multipotency for differentiation into other cell types, which makes them applicable to medical therapies. Three distinct advantages of using MSCs in stem cell therapy are their reportedly low tumorigenic potential, wide availability, and origin from an autologous source. Especially, one of these cell types, bone marrow-derived MSCs (BM-MSCs), has the potential to overcome their lineage restriction and differentiate into nerve-type cells. Mechanical signals have great potential to regulate biochemical signal pathways induced by soluble factors for the control of stem cell differentiation. In this study, we investigated that the differentiation and survival effect against mechanical signal like Pulsed Electromagnetic Field (PEMF) in chemical-based neuronal cell induction media. We used 50Hz, 10mT PEMF, and we thought the energies of PEMF are transferred to cells invasively because of low frequency. To induce neuronal differentiation, cells were cultured in DMEM/F12 supplemented with Insulin, Forskolin, Hydrocortisone, KCl, Valproic acid and Butylhydroxyanisole. To control neuronal differentiation, we changed the concentration of each chemical component. When neuronal differentiation properties of hBM-MSCs were enhanced by PEMF, and also the cell viability was increased.

At that time the cell morphology was changed. At 6 hours the cell morphology was close to neuron, and after 24 hours 41 ± 6 % in no exposed groups and 61 ± 14 % cells in PEMF exposed groups were alive. Within induction media PEMFs helped neuronal differentiation of hBM-MSCs, and during this process CREB was activated. Cell viability was enhanced by PEMF after 24 hrs. And p90RSK and ERK were activated at 3 hours. But each level was decreased at 24 hours. We confirmed that mechanical signals can affect cell differentiation and viability invasively through activation of various signaling molecules.

T-1039

NEW GERM CELL FORMATION DURING THREE DIFFERENT PERIODS OF TIME AFTER AUTOLOGOUS BONE MARROW MESENCHYMAL STEM CELLS TRANSPLANTATION INTO THE TESTES OF INFERTILE MALE RATS

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Recent studies propose that mesenchymal stem cells (MSCs) are a potential choice for use as treatment for male infertility. Although male germ cells (GCs) have been derived from MSCs in the laboratory, the effects of MSCs on reconstruction of germinal epithelium and restoration of spermatogenesis remain controversial. In this research we have evaluated and compared the fate of autologous bone marrow (BM)-MSCs during three different periods of time- 4, 6 and 8 weeks after transplantation into the testes of busulfan-induced infertile male rats. BM samples were collected from rats' tibias under anesthesia. The samples were directly cultured in complete culture medium. BM-MSCs were characterized based on their behavioral characteristics, flow cytometric analysis of CD-markers and the trilineage (bone, fat and cartilage) differentiation test. 10-14 days after BM sampling, the rats received 40 mg/kg BW busulfan for induction of infertility. After about 28 days, passage-3 to 5 BM-MSCs of each male rat were labeled with PKH26 and autologous transplantation into the testes was performed. Immunohistochemical analysis showed that transplanted cells survived in all three groups, although the percentages of positive tubules were different ($p<0.05$) in different times (in 4, 6 and 8 week testes were about 12.2%, 4.8% and 1.2% respectively). Some of the cells homed at the germinal epithelium and expressed spermatogonia markers (Dazl and Stella). The numbers of homed spermatogonia-like cells in 4-week (0.14%) testes were more than the 6-week (0.072%) testes. The 8-week testes (0.0068%) had the least numbers of homed cells ($p<0.05$). We observed only one GC colony that originated from the transplanted cells in a testis from the 4-week group. There was no further differentiation from the spermatogonia stage observed in any of the three groups. Totally, the number of donor cells were decreased in the testis with time progressed. Immunohistochemistry for vimentin showed that transplanted BM-MSCs did not differentiate into the sertoli cells. Our results indicated that BM-MSCs could survive in the testis and differentiate into new spermatogonia. More detailed investigations should be done to achieve the knowledge of GCs production in the laboratory and find new methods to establish an effective GC or gamete producing system from MSCs.

T-1040

TRANSPLANTATION OF HUMAN UMBILICAL CORD STEM CELLS WITH HIGH EXPRESSION OF TGF-BETA RECEPTORS IN DEGENERATED INTERVERTEBRAL DISC OF RABBIT

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Mesenchymal stem cells (MSCs) have been considered to hold promise for treating intervertebral disc (IVD) degeneration and cell carrier has been regarded as important tool for avoiding the formation of osteophyte, which has been observed as a result of cell leakage in animal models of IVD degeneration. However, difference in MSC efficacy has been a major problem and the optimum derivation of MSCs for use in IVD degeneration remains unclear. Additionally, no data are available on the efficacy of Wharton's Jelly-derived MSCs (WJ-MSCs) transplantation in an animal model of IVD degeneration. Therefore, the main objective of this study was to evaluate the effectiveness of human WJ-MSCs loaded in a cross-linked hyaluronic acid (XHA) scaffold for IVD regeneration according to the levels of transforming growth factor-β (TGF-β) receptor I/activin-like kinase receptor 5 (TβRI/ALK5) and II (TβRII) in a rabbit model. We compared the degree of IVD regeneration between high TβRI/ALK5 and TβRII expressing WJ-MSCs (MSC-highTR) and low TβRI/ALK5 and TβRII-expressing WJ-MSCs (MSC-lowTR) using magnetic resonance imaging (MRI) and histological study. T2 MRI performed 12 weeks after transplantation showed significant restoration of disc water content when treated with MSC-highTR loaded XHA as compared to the scaffold only and MSC-lowTR loaded XHA. In addition, morphological and histological analyses revealed higher IVD regeneration in MSC-highTR-XHA-treated groups than in scaffold only or MSC-lowTR-XHA-treated groups. Taken together, our results suggest that MSC-highTR-loaded XHA provides more effective IVD regeneration compared with MSC-lowTR-loaded XHA. This study supports the potential use of MSC-highTR -loaded XHA for clinical application to halt the IVD degeneration or to enhance the IVD regeneration.

T-1041

A STEM CELL BASED SCREENING PLATFORM FOR DISCOVERY OF CHONDROGENIC DRUGS AND PUTATIVE DRUG TARGETS

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Drugs that promote chondrogenesis could be used to induce self-repair and regeneration of articular cartilage after traumatic injury or osteoarthritis (OA). OA is the most common degenerative disorder of the joints, effecting nearly half the elderly population worldwide for which there are no cures. We present here a workflow for designing a stem cell based drug screening activity and innovative target prioritisation approach for OA drug development. We believe that the resident chondroprogenitor cell populations that exist within or adjacent to the articular cartilage are a suitable target cell

type for chondrogenic drug screening. Using Plasticell's bead-based combinatorial cell culture system CombiCult, we have developed a number of highly efficient, serum-free chondrogenic media capable of generating chondroprogenitor cells from human MSC monolayer cultures. Chondroprogenitor cells derived in this way were further characterized by phenotype, stability and suitability for compound screening. We then carried out phenotypic drug screening on annotated chemical libraries consisting of ~7000 diverse set of compounds selected over a broad range of stem cell signaling and regulatory pathways. Through this approach, we discovered a number of hits that are capable of inducing chondrogenesis in vitro. Potency and reproducibility of the hits were assessed using an 11-point dose response curve. Target analysis revealed several promising reservoir of putative drug targets where one fifth represented by ~50% of all the hits. The top target was hit by 7 compounds represented by multiple chemotypes. We ranked our drug targets according to a selection criterion based on hit rate, EX50, maximum assay signal and biological relevance, then carried out RNAi knock down studies to validate targets. Using this approach we were able to identify and prioritise drug targets and potential drug leads for an OA program. Unlike the conventional single-target paradigm, this novel "multi-target" phenotypic screen is expected to reduce attrition rates and help to speed-up the drug development process in general. We anticipate intra-articular administration of drugs developed from these hits will target the resident chondrogenic progenitor cell populations in the joint to promote repair and regeneration of damaged cartilage.

T-1042

OSTEOBLAST DIFFERENTIATION AND BONE FORMATION OF HUMAN BONE MARROW MESENCHYMAL STROMAL CELLS IS ENHANCED IN CO-CULTURE WITH PERIPHERAL BLOOD MONONUCLEAR CELLS AND EXOGENOUS VEGF

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Despite of recent advances in bone tissue engineering, efficient bone formation and vascularization still remains a challenge for clinical applications. Hypothesis: The aim of this study was to investigate if the osteoblastic differentiation of human mesenchymal stromal cells (MSCs) can be enhanced by co-culturing them with peripheral blood (PB) mononuclear cells (MNCs), with and without vascular endothelial growth factor (VEGF), a coupling factor of bone formation and angiogenesis. Human bone marrow (BM) derived MSCs from eight donors were co-cultured with PB-MNCs in osteogenic medium with or without VEGF. Osteoblastic differentiation and bone formation were studied by staining for alkaline phosphatase (ALP) and mineral deposition (von Kossa), respectively, and by measurements for ALP activity and calcium concentration (Ca). Cell proliferation was assayed with Alamar blue. The mechanism(s) were further studied by Transwell® cell culture experiments. MSC-MNC co-cultures supplemented with exogenous VEGF resulted in increased ALP activity ($p<0.01$) and strongest ALP staining confirmed by quantifying the positively-stained surface areas ($p=0.04$-0.001) as well as mineralization (Ca) ($p=0.017$ - $p<0.001$) compared to plain MSC cultures. VEGF alone had no effect on osteoblastic differentiation of MSCs. The mechanism for enhanced bone formation was shown to require cell-cell contact between MSCs and MNCs and the factors contributing to further differentiation appear to be soluble. No differences were observed in cell proliferation. Our study demonstrates that the in vitro ALP activity and mineralization of human BM-MSCs is more efficient in the presence of PB-MNCs, and exogenously added VEGF further enhances the stimulatory effect. This indicates that PB-MNCs could be a potential cell source in development of co-culture systems for novel tissue engineering applications for enhanced bone healing.

T-1043

LAMININ MATRIX PROMOTES HEPATOGENIC TERMINAL DIFFERENTIATION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS

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Objective(s): In vitro production of human hepatocytes from stem cells is of increasing importance in tissue engineering and biotherapy of liver diseases. Considering the lack of the optimal in vitro model; this study was designed to examine the effect of laminin matrix on the improvement of in vitro differentiation of human BM-MSC into the more functional hepatocyte-like cells. Characterization of the human BM-MSCs was performed by immunophenotyping and their differentiation into the Mesenchymal-derived lineage. Then, cells were seeded on the laminin-coated or the tissue culture polystyrene. The differentiation was carried out during two steps. Afterward, the expression of hepatocyte markers such as AFP, ALB, CK-18, and CK-19 as well as the expression of C-MET, the secretion of urea and the activity of CYP3A4 enzyme were determined. Moreover, the cytoplasmic glycogen storage was examined by PAS staining. The results demonstrated that the culture of hBM-MSC on laminin considerably improved hepatogenic differentiation compared to the control group. A significant elevated level of urea biosynthesis and CYP3A4 enzyme activity was detected in the media of the laminin-coated differentiated cells ($p<0.05$). Furthermore higher expressions of both AFP and ALB were observed in the cells differentiated on laminin matrix. The intracellular glycogen accumulation on day 21 of hepatogenic differentiation was observed on laminin matrix, while no accumulation was detected in the control MSCs. Despite using different protocols and cytokine cocktails to improve the in vitro culture systems, still they do not recreate all needed signals present in vivo. So far reported differentiated hepatocyte-like cells display several hepatic functions, however levels of albumin secretion, urea production, glycogen storage, CYP450 and GST activity are still approximately 5 to 10 folds lower than in mature hepatocytes. Our findings indicate that laminin matrix can improve terminal differentiation of hepatocyte-like cells from human BM-MSCs comparing to that of previous reports. Thus, it suggests that laminin as one of the important liver tissue specific ECM components can be considered as a suitable coating matrix in hepatic differentiation and tissue engineering designs.

T-1044

SCREENING OF CELL DIFFERENTIATION PEPTIDES FOR ENHANCING BONE REGENERATION

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The long-term efficacy of bone implants critically depends on the regeneration efficacy of the disturbed tissue surrounding the implant. Late regeneration can burden patient with cost and time, and can decline QOL of the patient. Peptides are one of most promised bio-compatible materials that could be chosen for surface modification, since it could eliminate both risks of animal-derived infections and interspecific immune response found in other large proteins and antibodies. There had been many researches that reports cell adhesion peptides, such as RGD peptides, found from extracellular matrixes (ECM) to be introduced for medical device surface function. To solve the regeneration of bone tissue, we focused on cytokines that have a role of cell adhesion, proliferation, differentiation and maturation. Thus we hypothesized that peptides included in cytokines have ability that enhance bone regeneration. And we searched the sequence of cytokines that are related to bone regeneration comparing with several species. Previous study, we have screened several cell-adhesion peptides, using peptide array method utilizing SPOT-synthesis technique. In this study, we applied this method for proliferation and differentiation of mesenchymal stem cells (MSCs) and osteoblast cells (OBCs).

T-1045

SERUM-FREE CULTURE MEDIUM STK2 SUPPRESSES THE EXPRESSION OF MANY LYSOSOME-RELATED GENES AND THE DECREASE IN THE GROWTH RATE ASSOCIATED WITH AGING OF HUMAN MSC

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For safe and efficient stem cell-based therapy, serum-free media are essential. We developed a chemically defined serum-free medium STK2, which increases the proliferation of mesenchymal stem cells (MSCs) and their differentiation potential compared with DMEM with 10% FBS (DS Pharma Biochemical, Osaka, Japan). In this study, we examined a relationship between aging and lysosome-related gene expression in MSCs in the presence of either STK2 or DMEM with 10% FBS, because STK2 seems to suppress the decrease in the proliferation capability of MSCs in late passage cultures. Human synovium-derived MSCs, human marrow-derived MSCs and human fibroblasts were maintained through sequential passage using STK2 or DMEM with 10% FBS. Gene expression levels in cultures grown with STK2 and DMEM with 10% FBS were compared by DNA microarray and quantitative PCR (qPCR) analyses. In DNA microarray and qPCR analyses, STK2 suppressed the expression of many lysosome-related genes, including various cathepsin genes, glycolytic enzyme genes, and lysosome-associated membrane protein genes, in MSCs. In DMEM with 10% FBS, the lysosome-related genes expression levels markedly increased as the passage number increased. This increase was associated with the decrease in the growth rate and changes in cell size and cell shape. However, in STK2, the lysosome-related gene expression was maintained at low levels, the growth rate was maintained in high levels, and the cell size and shape did not undergo the senescence-related changes even in late passage cultures. In conclusion, the serum-free culture medium STK2 can suppress the decline of the growth rate, the increase in the cell size, cell flattening, and the increase in the lysosomal related gene expression, which are associated with the aging of cultured MSCs. We are now investigating which growth factors are involved in the suppression of the aging of MSCs.

T-1046

5-AZA AND TSA INCREASE MULTILINEAGE DIFFERENTIATION CAPACITY OF MESENCHYMAL STROMAL CELLS

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This research designs to confirm enhancement of MSC multi lineage differentiation capacity when 5-aza, methylation inhibitor and TSA, HDAC inhibitor treat alone or together. Furthermore, this study addresses the expression of key regulator gene and change of epigenetic status which are involved in each lineage differentiation by epigenetic regulator. Neurogenic, osteogenic, and chondrogenic differentiation was induced following 7-21 day culture of cells in lineage specific differentiation medium and differentiation was assessed by immuno-histo/cytochemistry and immuno blotting. The changes of lineage specific transcription regulator gene expression and its relations to methylation status was investigated by real-time PCR and pyro-sequencing. In conclusion, our study demonstated that epigenetic regulator enhances lineage specific differentiation from BMMSCs. And also, suggested that gene activation and silencing controlled by epigenetic modulation, were crucial to MSC lineage differentiation.

T-1047

ASSOCIATION OF LINE-1S EXPRESSION WITH DIFFERENTIATION POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS

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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. Recently, it was reported that reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) activates LINE-1s expression, suggesting that LINE-1 expression is negatively and positively related to the cell differentiation and the differentiation potential of iPSCs, respectively. Although LINE-1s mRNA is also known to be expressed in embryonic stem cells, it has been unclear whether LINE-1s is expressed in multipotent somatic stem cells. In the present study, we examined the LINE-1s expression in human bone marrow-derived

mesenchymal stem cells (hMSCs) and its association with the differentiation potential of hMSCs. Quantitative RT-PCRs indicated that LINE-1s mRNA was significantly expressed in all the hMSCs we tested, whose levels were higher, compared with those in human iPSCs. However, the expression of LINE-1s in hMSCs was down-regulated as their passage number increased. In addition, treatment with a culture medium for adipogenic differentiation attenuated the mRNA expression of LINE-1s in hMSCs. These findings suggest that the LINE-1s expression is associated with the differentiation potential not only of pluripotent stem cells, but also of multipotent somatic stem cells.

T-1048

COMPARATIVE ANALYSIS OF CELL DIFFERENTIATION AND GENOTOXIC RESPONSE IN HUMAN MESENCHYMAL STEM CELLS FROM BONE MARROW, ADIPOSE TISSUE AND ENDOMETRIUM

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Mesenchymal stem cells (MSCs) are shown to be effective in cell therapy treatments of many diseases and injuries. MSCs can be isolated from a variety of adult tissues. Different types of MSCs vary in their differentiation properties. We performed a comparative study of the neurogenic potential of three types of human MSCs derived from bone marrow (BMSCs), subcutaneous adipose tissue (AMSCs) and endometrium (isolated from the menstrual blood) (eMSCs). It was shown that all three types of MSCs cultures are multipotent and predisposed to neurogenesis, based on the expression of pluripotency marker SSEA-4 and neuronal precursor markers nestin and beta-III-tubulin. RT-PCR analysis revealed basal level of expression of neurotrophin-3 in undifferentiated AMSCs. Additionally, a significant basal level of brain-derived neurotrophic factor (BDNF) in eMSCs culture was observed by ELISA analysis. An induction of neuronal differentiation in the studied MSCs induced changes in the cells morphology, the increase of β-III-tubulin expression, and the appearance of neuronal markers GFAP, NF-H, NeuN and MAP2. During the differentiation the BDNF secretion was significantly enhanced in the BMSCs and decreased in the eMSCs cultures. We had also compared the response on these three types of human MSCs to the genotoxic stress. The cytotoxic effect of the anticancer drug doxorubicin (DR) has been examined. DR induced arrest of cell proliferation and formation of gamma-H2AX foci. It was found that BMSCs are more sensitive to DR than AMSCs and eMSCs. Thus, we demonstrated that MSCs isolated from different tissues are distinguished in their differentiation plasticity and resistance to genotoxic stress.

T-1049

CARDIAC DIFFERENTIATION OF MESENCHYMAL STEM CELLS BY SUPPRESSING MICRORNA TARGETING MULTIPLE KEY CARDIAC TRANSCRIPTION FACTORS

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Increasing number of stem cell-based therapies utilizes induced pluripotent stem cells (iPSCs) that can circumvent the ethical issue of embryonic stem cells. However, the reprogramming rate of somatic cells into iPSCs is still low and the process is somewhat time-consuming becuse the actual differentiation of cells into the desired type of cells can be initiated only after the generation of iPSCs. Controlled differentation of stem cells is especially important for the substitution of specialized cells such as cardiomyocytes. Since cardiomycytes need to be synchronized with surrounding cells to relay the electric signals in heart, transplantation of cells with cardiac characteristics would be preferable. Thus, if transdifferentiation of stem cells, such as mesenchymal stem cells (MSCs), into cardiomyocte without iPSCs formation step can be achieved, it may revolutionize the way of stem cell-based thearpy for cardiac disease, such as myocardial infarction (MI), is conducted. In the present study, we identified microRNA that suppresses expression of key transription factors enriched in cardiomyocytes, namely T-box 5 (TBX5), myocyte enhancer factor 2C (MEF2C), and GATA binding protein 4 (GATA4). Based on miRNA database search, miR-222-3p was identified as the miRNA targeting all three of these transcription factors. Intersting enough, the expression of miR-223-3p was significantly lower in cardiomyocytes compared to undifferentiated MSCs. Thus, it was hypothesized that if neutralize the miR-222-3p in undifferentiated MSCs using anti-miRNAs specific to miR-222-3p, then it may promote expressions and transcriptional activity of those transcription factors leading to initiation of cardiac differentiation program of MSCs. Our data indicate that quenching functional miR-222-3p of MSCs using anti-miR-222-3p significantly increased the expression of above mentioned transcription factors, and as a consequence, the expressions of cardic marker genes that are under transcriptional regulation of those transcription factors were also increased. Our study demonstrate that miRNAs play a important roles in differentiation of cells, and furthermore, neutralizing miRNAs suppresing expressions of cell type specific molecules can be an effective way of inducing transdifferentiation program of MSCs.

T-1050

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA NEGATIVELY TRGULATES THE DIFFERENTIATION OF BONE MARROW MESENCHYMAL STEM CELLS TOWARD MYOFIBROBLASTS IN LIVER FIBROGENESIS

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We recently reported that bone marrow mesenchymal stem cells (BMSCs) are the main origin of hepatic myofibroblasts (MFs), which contribute to fibrogenesis in chronic liver diseases. When BMSCs differentiate toward MFs, α-smooth muscle actin (α-SMA) appears and collagen α1(I) (Col α1(I)) and collagen α1(III) (Col α1(III)) are markedly increased. Peroxisome proliferator-activated receptor gamma (PPARγ), a ligand-activated transcription factor belonging to the nuclear receptor superfamily, has gained a great deal of recent

attention as it is involved in the fibrosis and cell differentiation. However, whether it regulates the differentiation of BMSCs toward MFs remains to be defined. The aim of this study was to investigate the function of PPARγ in the process of primary mouse BMSCs differentiation toward MFs in liver fibrogenesis. Here, carbon tetrachloride or bile duct ligation was used to induce liver fibrosis in mice. Expressions of PPARγ, α-SMA, Col α1(I) and Col α1(III) were detected by qRT-PCR and Western blot or immunofluorescence assay. High content analysis was utilized to measure nuclear/cytoplasmic ratios for PPARγ. Our results demonstrated that PPARγ expression were decreased in mouse fibrotic liver tissue. PPARγ mRNA level had inverse correlations with Col α1(I), Col α1(III), α-SMA or transforming growth factor β1 (TGF-β1) mRNA level in liver tissue. In addition, PPARγ was declined during the differentiation of BMSCs toward MFs induced by TGF-β1 in vitro. There were also negative correlations between mRNA level of PPARγ and α-SMA, Col α1(I) or Colα1(III) in the differentiation process. Activation of PPARγ stimulated by natural (15d-PGJ_2) or synthetic (troglitazone or ciglitazone) ligands suppressed differentiation of BMSCs as the expression of α-SMA, Col α1(I) and Col α1(III) were reduced, which was blocked by PPARγ antagonist (GW9662). Additionally, knock down of PPARγ by siRNA contributed to BMSCs differentiation. Moreover, nuclear/cytoplasmic ratios for PPARγ were elevated by application of specific PPARγ agonists. The data suggest PPARγ is an essential modulator in BMSCs differentiation; it negatively regulates the differentiation of BMSCs toward MFs in liver fibrogenesis via nuclear translocation, which highlights a further mechanism implicated in the BMSCs differentiation.

T-1051

ADIPOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS REVERSES THEIR IMMUNOSUPPRESSIVE EFFECTS

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Mesenchymal stem cells (MSC) communicate with endothelial cells (EC), modulate their ability to respond to pro-inflammatory cytokines, and suppress leukocyte recruitment. Here we examined whether differentiation of bone marrow (BM)MSC into adipocytes altered their immunosuppressive capabilities. MSC may lose their immunosuppressive effects by differentiation which could contribute to the inflammatory response. EC were co-cultured with BMMSC or adipocytes derived from them on opposite sides of a porous filter for 24h. Alternatively, EC were treated with conditioned medium from these co-cultures for 24h. EC or co-cultures were stimulated with tumour necrosis factor α (TNFα) for 4h and incorporated into a flow adhesion assay. Purified neutrophils or peripheral blood lymphocytes were perfused and their adhesion to the EC was assessed. BMMSC significantly suppressed neutrophil and lymphocyte recruitment to EC stimulated with 100U/ml TNFα. These effects were recapitulated by culturing EC in conditioned media from EC:BMMSC co-cultures and were reversed by neutralising IL-6 generated in co-culture. In contrast, differentiated adipocytes lost the ability to suppress neutrophil, and to a lesser extent lymphocyte recruitment, when compared to BMMSC. Indeed, adipocytes enhanced neutrophil recruitment to EC treated with low dose TNFα (0.1 or 1U/ml). Conditioned media from adipocyte mono- or co-cultures were also unable to suppress neutrophil recruitment, despite containing higher levels of IL-6. MSC are endogenous tissue-resident regulators of inflammation that modify the EC response to cytokines and limit the recruitment of leukocytes, partially through IL-6. Upon differentiation MSC lose this regulatory effect, potentially adopting a pro-inflammatory, stimulatory phenotype. This could contribute to pathogenic inflammatory responses through the release of bioactive, soluble factors that may alter the way in which IL-6 exerts its effects.

T-1052

CPNE7 REGULATES ODONTOBLASTIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS

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Tooth development involves sequential interactions between dental epithelial and mesenchymal cells. Our previous studies demonstrated that preameloblast-conditioned medium (PA-CM) induces the odontogenic differentiation of human dental pulp cells (hDPCs) and promotes dentin formation. The protein Cpne7 was identified in PA-CM by liquid chromatography-tandem mass spectrometry and suggested as a candidate signaling molecule regulating odontoblast differentiation. In the present study, we investigated biological function and mechanisms of Cpne7 in regulation of odontoblast differentiation. Cpne7 was expressed in preameloblasts and secreted to the extracellular matrix during ameloblast differentiation. After secretion, Cpne7 protein was translocated to differentiating odontoblasts. In differentiating odontoblasts, Cpne7 overexpression or treatment with recombinant Cpne7 significantly promoted the expression of Dspp in vitro. In contrast, siRNA-mediated inactivation of Cpne7 suppressed Dspp expression. In in vivo transplantation experiments, Cpne7 induced odontoblast differentiation and promoted formation of dentin/pulp-like tissue in dental mesenchymal cells, such as hDPCs. Moreover, Cpne7 induced differentiation into odontoblasts of undifferentiated mesenchymal C3H10T1/2 cells in vitro and of non-dental mesenchymal cells, such as human bone marrow mesenchymal stem cells, with formation of dentin-like mineralized tissues including the structure of dentinal tubules in vivo. Mechanistically, Cpne7 interacted with Nucleolin and modulated odontoblast differentiation via the control of Dspp expression. These results suggest Cpne7 is a diffusible signaling molecule that is secreted by preameloblasts and regulates the differentiation of mesenchymal cells of dental or non-dental origin into odontoblasts. Therefore, regulation of Cpne7 expression in mesenchymal stem cells could be a novel therapeutic approach for the treatment of diseased dentin-pulp complex.

T-1053

INTRACELLULAR CALCIUM SIGNALING IN TLR 3 AND 4 PRIMED HUMAN MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) are of special attention as therapeutic agents in the settings of both chronic inflammatory and autoimmune diseases due to their repair function. Changes in intracellular Ca2+ concentration ([Ca2+]i) as many physiological

roles including proliferation, differentiation, apoptosis, gene expression and release of neurotransmitters. However intracellular Ca2+ signaling in MSCs remains poorly understood and it is unclear under the activation of TLRs-related immune responses such as to poly(I:C) and LPS. In this study, we directly measured [Ca2+]i changes using fura-2/AM system and compared gene expression level related intracellular Ca2+ signaling using real-time PCR in TLR 3- and 4-primed MSCs by exposing them to poly(I:C) and LPS, respectively. The extracellular application of cholinergic agonist carbachol, producing InsP3 to activate InsP3Rs, and CPA, an inhibitor of the SERCA (sarcoplasmic/ER Ca2+-ATPase) induced a large intracellular Ca2+ transient, suggesting the involvement of Ca2+ release from ER in these cells. Compare the carbachol and CPA, Bay K-8644 which specific agonist of voltage dependent L-type Ca2+ channel, showed weak response on [Ca2+]i. The patterns of intracellular Ca2+ changes and gene expression were displayed different between two conditioned-MSCs which will be served to understanding critical role for Ca2+ mobilization in MSCs.

T-1054

PHORBOL MYRISTATE ACETATE INDUCED CARDIAC DIFFERENTIATION OF MESENCHYMAL STEM CELLS IS MEDIATED BY TRANSCRIPTIONAL ACTIVATION OF NFATC AND MYOD

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Current stem cell-based therapies generally involves in vitro expansion of stem cells to acquire sufficient number of cells and re-introducing the cells to damaged tissues for regeneration. However, certain tissues requires more than just high number of therapeutic cells and myocardium is one of such tissues. Since cardiomycytes are required to work with neighbouring cells for conducting electrical signaling, therapeutic cells that are more differentiated toward cardiomyocytes would be better in terms of their functionality after transplantation compared to simple in vitro expanded undifferentiated stem cells. The concept of changing the fate of stem cells using small molecules have been introduced about a decade ago and our group also empirically proved that it is possible to direct cell fate using different small molecules. For example, we previously reported that phorbol 12-myristate 13-acetate (PMA) treatment induced cardiac differentiation of mesenchymal stem cells (MSCs) with increased expressions of cardiac marker genes but decreased MSC-specific marker CD90. However, in that particular study we were unable to identify key molecular mechanisms of how PMA triggers cardiac marker gene expressions in details. Thus, in the present study, we investigated how PMA induces cardiac differentiation of mesenchymal stem cells focusing on its effect on transcription factors responsible for the increased cardiac marker genes such as cardiac troponin T (cTnT), myosin light chain (MLC), as well as myosin heavy chain (MHC). Our data indicate that PMA induces transcriptional activity of nuclear factor of activated T cells (NFATc) and myogenic differentiation 1 (MyoD) that have been associated with increased expressions of cTnT and MHC, respectively. Experiments using key kinase inhibitors indicated that protein kinase C (PKC) was mediating the effect of PMA on both transcription factors examined. Furthermore, PMA treatment increased cell-cycle regulator p27 expression, and this was also mediated by NFAT transcription factor. Our study indicated that PMA triggers activation of cardiac differentiation program in MSCs by regulating key cardiac transcription factors, and it also simultaneously regulate cell-cycle progression to priming cellular environment for differentiation rather than proliferation.

T-1055

EXPRESSION OF NEURAL CELL ADHESION MOLECULE AND POLYSIALIC ACID IN HUMAN BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS

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Mesenchymal stromal cells (MSCs) are attractive candidates for cellular therapy and regenerative medicine. Detailed molecular characterization of human MSCs is important in the context of cell therapy to guarantee the stability of transplanted cells, preservation of functional characteristics, and patient safety. Neural cell adhesion molecule (NCAM, CD56) is a transmembrane glycoprotein modulating cell-cell and cell-matrix interactions. An additional key modification of NCAM results from post-translational addition of α2,8-linked polysialic acid (polySia), which has an important role in maintaining developmental plasticity and cell migration. Expression of NCAM and polySia is widespread during organogenesis, particularly in relatively undifferentiated cells of mesenchymal character. NCAM is often looked on as a marker of neural lineage commitment and, thus, NCAM expression on multipotent cells has been considered undesirable. On the other hand, several studies have shown that lack of NCAM expression reduces the differentiation potential of MSCs in animal models. Human bone marrow-derived MSCs are considered to be devoid of NCAM expression, but more rigorous characterization of the presence of this molecule is needed. We have extensively utilized mRNA detection, flow cytometry, and immunofluorescence staining to determine cell surface features and observed that human bone marrow-derived MSCs may sometimes express NCAM. However, there is little - if any - polySia expressed on these cells. In addition, it is possible that NCAM expressing MSCs are capable of multilineage differentiation, and if so, NCAM might not be a direct sign of neural lineage commitment.

T-1056

METHYLOME, TRANSCRIPTOME, AND PPAR-GAMMA CISTROME ANALYSES REVEAL TWO EPIGENETIC TRANSITIONS IN FAT CELLS

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Although DNA modification is adaptive to extrinsic demands, little is known about epigenetic alterations associated with adipose differentiation and reprogramming. We systematically characterized

the global trends of our methylome and transcriptome data with reported PPARγ cistrome data. Our analysis revealed that DNA methylation was altered between induced pluripotent cells (iPSCs) and adipose derived stem cells (ADSCs), surprisingly there were no obvious changes when differentiation from ADSCs to mature fat cells (FatCs) occurred, indicating that epigenetic predetermination of adipogenic fate is almost established prior to substantial expression of the lineage. Furthermore, the majority of the PPARγ cistrome corresponded to the pre-set methylation profile between ADSCs and FatCs. In contrast to the pre-set model, we found that a subset of PPARγ-binding sites for late-expressing genes such as Adiponectin and Adiponectin receptor2 were differentially methylated independently of the early program. Thus, these analyses identify two types of epigenetic mechanisms that distinguish the pre-set cell fate and later stages of adipose differentiation.

T-1057

PROLIFERATIVE AND GENOTOXIC EFFECT OF PERIVITELLINE FLUID FROM HORSESHOE CRAB ON HUMAN DENTAL PULP STEM CELLS

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The aim of this study was to evaluate the effect of perivitelline fluid (PVF) on the proliferation, chromosome aberration and mutagenicity of dental pulp stem cells (DPSCs). Crude extracts were prepared from the PVF collected from horseshoe crabs. For cytotoxicity test (MTT assay), the DPSCs were subjected to different concentrations of PVF crude extract. Two inhibitory concentrations (IC50=26.887 mg/ml and IC25=14.093 mg/ml) and two other concentrations (0.278 mg/ml and 0.019 mg/ml) which produced higher cell viability were selected for downstream experiments. The proliferative effect was assessed using AlamarBlue® assay for a period of 10 days and the population doubling times (PDTs) were also calculated based on this assay. Chromosome aberration and Ames tests were employed for the evaluating the genotoxicity. Statistical analysis was carried out using independent t-test to assess the significant differences ($p<0.05$). AlamarBlue® assay showed that PVF groups produced comparable proliferation activity to negative (untreated) control. The PDTs between PVF groups and negative control were also insignificantly different ($p>0.05$). Both the chromosome aberration test and Ames test did not show any significant differences in the PVF treated groups. Hence, it can be concluded that PVF from horseshoe crabs produced insignificant proliferative activity and was non-genotoxic on DPSCs based on chromosome aberration and Ames tests.

T-1058

MAPPING BONE MARROW ADIPOGENESIS IN HOMEOSTASIS AND APLASIA

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Adipocytes are the most frequent cell type in the adult human bone marrow (BM). Their formation occurs at the expense of the differentiation of preadipocytes and mesenchymal stem progenitors cells (MSPCs) that have been shown key to hematopoietic stem cell (HSC) support. Different subsets of such MSPCs have been described, including human CD146+ cells, and mouse Nestin+, Sca1+ ALCAM-, LeptinR+ cells. The hematopoietic (red) and adipocytic (yellow) marrow are heterogeneously distributed within the BM depending on skeletal location and physiological conditions. A transition of red to yellow marrow occurs with aging, and in juvenile development when distal parts of the skeleton become fully adipocytic during growth as described by Neumann's law. BM aplasia, whether secondary to chemotherapy or inherited BM failure syndromes, is also systematically accompanied by adipocytic infiltration of the marrow. Intriguingly, BM adipocytes accumulate different lipids than other adipose compartments, and they have features of brown fat-like tissue, compatible with electron microscopy descriptions of multivacuolar adipocytes within the marrow. Given the inhibitory role of adipocytes in hematopoiesis, we are interested in characterizing the transition from red to yellow marrow in the context of ageing, gender and BM aplasia. To this end, we have developed a semi-automated imaging analysis tool for quantification of BM adipocytes and hematopoietic cells in histological sections. Morphometric analyses are compared with micro-computerized tomography and magnetic resonance imaging in mice as a prospective non-invasive technique to survey whole skeleton BM adipogenesis. HSCs, MSPCs, and their progenitors are characterized by flow cytometry and gene expression analysis in different skeletal locations, while mapping the MSPC progenitors within the white to brown fat axis. To test the efficiency of red and yellow marrow components in recruiting BM-forming units, different methods of ossicle formation and heterotopic BM adipogenesis are applied. Correlation of findings in mouse to human will be done by analysis of surgical debris from various skeletal locations and by retrospective analysis of human BM adiposity to time to engraftment in recipients.

T-1059

HUMAN MESENCHYMAL STEM CELL DIFFERENTIATION MONITORED BY SINGLE-CELL GENE EXPRESSION ANALYSIS

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Typical cellular and molecular biology approaches for measuring gene expression utilize bulk-scale measurements that yield averaged values for cell populations. However, these approaches fail to report the phenotypes of individual cells and, therefore, mask heterogeneity within cell populations. Development of stem cell-based therapies will require an understanding of the homogeneity of the obtained cells on an individual cell basis. In the case of mesenchymal stem cells, biochemical compounds (e. g., bone morphogenetic proteins) have been utilized to promote their osteogenic differentiation. Our research provided evidence that, in the absence of exogenous biochemical compounds, alternating electric current (a biophysical stimulus), induces exclusive osteodifferentiation of adult human mesenchymal stem cells (MSCs) at the population level. The present study investigated the effects of alternating electric current on MSC osteodiffereniation at the single-cell level. MSCs were cultured on flat, indium-tin-oxide-coated glass (an electrically conductive substrate pre-coated with fibronectin), and exposed to a sinusoidal,

10 μA, 10 Hz, alternating electric current for 6 hours daily, for 7 and 21 consecutive days; these experimental conditions had been determined optimal in inducing exclusive osteodifferentiation of MSCs. Control cells and MSCs following exposure to alternating electric current were collected at 7 and 21 days and used for single-cell qRT-PCR measurement of mRNAs for 45 genes indicative of MSC osteogenic differentiation using the Fluidigm C1 Single-Cell Auto Prep and BioMark HD Systems. Similar gene expression for bone morphogenetic protein 2, bone sailoprotein, and osteonectin were observed for MSCs under control and the alternating electric current conditions tested. In contrast, compared to the respective controls, there was greater homogeneity in genes (specifically TAZ and RUNX2) indicative of the early stages of osteodifferentiation in cells exposed to alternating electric current for both 7 and 21 days. These results provide the first glimpse of the degree of gene expression heterogeneity among differentiating MSCs, and suggest that cell exposure to alternating electric current promotes a more homogeneous response towards early osteoblastic differentiation.

T-1060

ODONTOGENIC DIFFERENTIATION OF DENTAL PULP DERIVED STEM CELLS ON TRICALCIUM PHOSPHATE VERSUS TREADED DENTIN MATRIX SCAFFOLDS

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The regeneration of dental-related tissue is a major problem in dentistry. Thus, it is valuable to develop dental constructs that are fabricated with dental pulp stem cells (DPSCs) and an appropriate scaffold. The present study investigates the level of odontogenic differentiation of dog DPSCs on commercially-available tricalcium phosphate (TCP) compared with treated dentin matrix (TDM) that was extracted from mongrel dog teeth according to the protocol described in literature. For this purpose, we isolated pulp stem cells from dog premolars and culture-expanded them through several successive subcultures. The cells from passage 3 were then loaded onto TCP and TDM scaffolds and treated with odontogenic supplements (OSs) that included vitamin D3 for a period of 21 days. DPSCs cultivated on scaffolds without OS, a monolayer culture treated with OS, and normal pulp tissue were the controls. We compared the groups in terms of odontogenic differentiation markers. According to our findings, the amount of culture mineralization (obtained by quantitative alizarin red staining and alkaline phosphatase activity assay), as well as the expression levels of dentin sialophosphoprotein (DSPP) and dentin matrix acidic phosphoprotein 1 (DMP1) genes were significantly higher in the three-dimensional (3D) cultures treated with OS compared to those 3D cultures without OS and the monolayer culture with OS ($P<0.05$). Furthermore, we found that the mineralization level of TCP cultures was considerably more than TDM cultures ($P < 0.05$). The level of genes expression in 3D culture on TCP and TDM without OS, increased relative to control group that showed induced odontogenic property of the TCP and TDM biomaterial. There was no significant difference among the studied scaffolds in terms of the expression level of odontoblast marker. Taken together it could be concluded that TCP possess more an odontogenic-inducing property than TDM in vitro.

MESENCHYMAL CELL LINEAGE ANALYSIS

T-1061

TOPOGRAPHICAL BRIDGING BETWEEN BONE AND NACRE

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While vertebrates commandeer calcium phosphate for skeletal fabrication, invertebrates routinely make use of calcium carbonate to create hard tissue structures such as shells. This juxtaposition of phosphate and carbonate is defined as the "Bone-Shell Divide". The discovery of fully integrated shell implants to replace lost teeth in Mayan skulls however, showed that there may be an exception to this divide. Further exploration in human jaw reconstructions and sheep femur implant, ascertained that invertebrate calcium carbonate $CaCO_3$ shells have good osteogenic and osteointegrative properties. Here we investigate whether different topographies presented by the main $CaCO_3$ polymorphs (aragonite nacre or calcite prisms) found in shells produce topography-specific responses in vertebrate stem cells. By reproducing high fidelity patterned replicas of aragonite nacre and calcite prisms of the bivalve mollusc Pinctada maxima on polycaprolactone (PCL), we show that the shell nano/microtopography alone is a potent determinant in stimulating stem cell fate. There is a clear discrimination of MSC response to the topography of nacre and prisms. Exposure to nacre topography results in significant increase in osteocalcin and osteopontin production indicating osteogenesis. The topography of calcite prisms promotes only growth as indicated by significant increases in CD63 and STRO-1 expression suggestive of growth with retained multipotency. These findings form important bases for development of novel bone therapies and the potential to generate ample quantities of autologous stem cells in vitro for generative therapy and tissue engineering.

T-1062

BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS RECRUITMENT IN A T-CELL LYMPHOBLASTIC LEUKEMIA (T-ALL) TUMOR MODEL

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Mesenchymal Stem Cells (MSCs) are components of the

tumor stroma with the ability to actively modulate the tumor microenvironment. This is due to a number of biological properties which include migration, differentiation, immunomodulation and angiogenesis enhancing. In order to study the contribution of human bone marrow derived-MSCs to tumor stroma formation we established a new in vivo model for T-cell lymphoblastic leukemia (T-ALL) in SCID mice using the human cell line CUTLL-1 (Columbia University T-cell Lymphoblastic Lymphoma 1). In contrast to previous models, CUTLL-1 displays biological responses to NOTCH inhibition thus representing the disease's etiology and serving as a relevant model for pre-clinical and drug screening assays. We studied the incorporation of MSCs into the tumor stroma by measuring the homing capacity of systemically delivered pre-labelled MSCs (DiR+/CMDil+). Biodistribution was analysed through in vivo imaging and fluorescence microscopy. Tumors developed after subcutaneous inoculation of a minimum dose of 2x10e7 tumor cells becoming visible 2.5 weeks post-inoculation. We intravenously administered pre-labeled MSCs (1x10e6 cells) into lymphoma-bearing mice. Infrared signal associated to MSCs homing reached the highest values at smallest tumor volume (4,03x10e5 ± 0,37 x 10e5 p/sec/cme2/sr and 1193 ± 249.1 mme3 tumor volume VS. 0,62x10e5 p/sec/cme2/sr and 2848 mme3 tumor volume) indicating a negative correlation between tumor size and MSCs homing ability and pointing at the high homing MSCs capacity into a niche of haematological neoplasic cells. Microscopic examination revealed MSCs incorporation as tumor stromal cellular elements, with a perivascular location. Microenvironmental tumor factors may directly affect cell migration and promote tumor progression. We demonstrated that MSCs displayed a high chemotactic response to soluble factors present in the lymphoblastic tumor cells conditioned medium. This coincides with the high MSCs in vivo tumor homing response. Our data suggests that tumor recruited MSCs may be involved in tumor development in this model. By discerning the mechanisms that govern MSCs/tumor cells cross-talk we could contribute to elucidate underlying mechanisms involved in tumor stroma transformation and acquired metastatic properties.

T-1063

HYPOXIA AND SERUM DEPRIVATION DO NOT IMPAIR CELLULAR VIABILITY, PROLIFERATION CAPACITY AND ANTIOXIDANT ENZYMES EXPRESSION IN MESENCHYMAL STEM CELL DERIVED FROM MENSTRUAL BLOOD

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Most of mesenchymal stem cells (MSC) injected in ischemic tissue die in a few days. Thus, it is essential to identify a MSC capable to survive in an inhospitable environment for cell therapy assays. Among the different sources of MSC, the ones derived from menstrual blood (mbMSC) might survive since their niche undergoes with intense necrosis and increased oxidative stress monthly. The aim of this work was to investigate the impact of reduced oxygen concentration and the absence of fetal bovine serum (FBS) in mbMSC adhesion, proliferation and resistance to oxidative stress. Menstrual blood was obtained from healthy women. Our local institutional review board approved all experiments below. The experimental conditions used in vitro were: normoxia (21% O2) and hypoxia (5% and 1% O2) in the presence and absence of FBS. Cells cultured in normoxia with serum were used as controls. Up to 48 hours, percentage of adhered cells was similar, in all conditions. Additionally, after 7 days in culture it was no difference in apoptotic cells number (<1.5%) comparing to control detected by annexin assay. Hypoxia (5% and 1%) in serum presence induced a significant increase in BrdU+ cells compared to control (79%, 75% and 53% respectively). However, only hypoxia 5% kept cellular proliferation (73%) in the absence of FBS compared to hypoxia 1% and control (39% and 10% respectively). Independently of serum presence, oxygen deprivation increased about 5-fold higher for VEGF, catalase, SOD1, 2 and 3 in 5% of hypoxia and in 1% of O2 RNA levels increased about ten-fold for VEGF, SOD2 and 3 in qPCR assay after 48 hours in culture. In conclusion, the mbMSC, exposed to hypoxic environment and serum deprivation, maintained adhesion, proliferation ability and increased the antioxidant enzymes expression. Also, oxygen deprivation, either in presence or absence of serum, leaded to an increased expression of VEGF. Taken together, these data suggest that mbMSC might be an excellent cell option since they can survive in conditions similar to ischemic area and may induce neovascularization.

T-1064

LONG- AND SHORT-LIVING CORD BLOOD MULTIPOTENT STROMAL CELLS AT THE CROSSROAD BETWEEN EPIGENETICS AND BIOENERGETICS

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Human cord blood multipotent stromal cells (CBMSCs) demonstrated peculiar biological properties compared to MSCs derived from other sources in in vitro and in vivo preclinical studies. Intriguingly, we recently described the existence of two different stromal populations in cord blood characterized by distinct morphology, clonogenicity, growth properties and differentiation potentials. One CBMSC subset is mainly short-living and less proliferative (SL-CBMSCs), while the other is long-living, with higher proliferation rate, and, very importantly, with significantly ($p \leq 0.01$) longer telomere (LL-CBMSCs). Further ongoing analysis are revealing major differences in population doubling time (SL-CBMSC=96 hours; LL-CBMSC=48 hours), measured by Microculture Tetrazolium (MTT) assay, in absence of DNA damage (single and double strand breaks), detected by single-cell gel electrophoresis (SCGE). In addition, preliminary results are showing how LL-CBMSCs seem to possess a different mitochondrial (mt)DNA copy number and mtDNA deletion ratio compared to SL-CBMSCs, both assessed by Real Time qPCR. Moreover, global genomic DNA methylation was esteemed measuring the methylation of some repetitive element subfamilies by bisulfite-treatment and subsequent highly quantitative PCR-pyrosequencing. The analysis showed that LL-CBMSCs possessed a significantly distinct methylation profile, compared to SL-CBMSCs, suggesting possible differences in the chromatin structure. These new data confirm and better define the distinct identity of these populations, shedding light on some molecular determinants which may be correlated to the different cell-culture behaviours of SL- and LL-CBMSCs.

T-1065

CLONAL DYNAMICS OF PROGENITORS DURING AXOLOTL APPENDAGE REGENERATION

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After amputation, regenerating organisms such as the axolotl produce a mass of undifferentiated cells beneath the wound epidermis, termed the blastema, that will go on to faithfully reproduce the missing segments. Far from being a static process, the formation of the regenerating blastema is a dynamic combination of cellular dedifferentiation, proliferation, migration, and cellular rearrangements that results in the morphogenesis of differentiated structures. While we know that there is lineage restriction of tissues during regeneration, the dynamics and behavior of cells on a clonal level, so far, has not been possible. Using a multi-color fluorescent reporter and embryonic transplantation methods, we can for the first time visualize single cell clones as they migrate into the blastema and contribute to limb regeneration. In particular, I chose to clonally trace the connective tissues of the axolotl limb. These diverse tissues such as dermis, cartilage, and interstitial tissue represent a large percentage of the early limb blastema and are known to impart patterning information on the re-forming limb. Despite the importance of connective tissue to regeneration, we know little about the contribution of individual cells and tissue types as they enter the blastema and dedifferentiate. By stochastically inducing multi-colored, single-cells clones I can determine if all cells are equally competent to participate in regeneration or if stem-like progenitors exist that preferentially supply the cells for regeneration. By examining the dynamics of cells in real time we can determine the niche tissue for regenerating cells and if this process is primarily stochastic (i.e. right place, right time), or proceeds via a stereotypical recruitment of cells from specific compartments. We can also resolve whether cells have a predetermined potency by observing the outcomes of the same clone through sequential rounds of regeneration.

T-1066

REGENERATION-COMPETENT CONNECTIVE TISSUE PROGENITORS IN MAMMALIAN DIGIT TIP REGENERATION AND WOUND HEALING

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Although mammals lack the ability to regenerate fully patterned appendages as in salamanders and teleost fish, mice retain a limited ability to re-form the digit tip throughout their lifespan. This regeneration process is restricted to the distal half of the terminal phalanx (P3), whereas amputation at more proximal levels results in wound healing (P2). Digit tip regeneration mirrors the regenerative process in salamanders, where a mass of undifferentiated cells accumulate under the wound epidermis (blastema) and are able to form the diverse tissues lost after amputation. In the Axolotl (Ambystoma mexicanum), a major proportion of blastema cells re-express transcriptions factor associated with early limb bud progenitors of the lateral plate mesoderm that give rise to the bone, cartilage, and soft connective tissues of the limb. In addition, connective tissue-derived blastema cells express positional identity markers such as HoxA9-13 genes that are critical for patterning the correct proximal to distal limb segments. One important question is the role that various connective tissue cell types play in determining if the mammalian digit will regenerate or not. To answer this question, we specifically looked at the connective tissue cell populations identified by markers that are also expressed in the embryonic limb bud and salamander blastema. We hypothesized that if cells still expressed these markers in adult tissue it might reflect a retained progenitor population, similar to limb bud and blastema cells. Using transgenic mouse lines that drive Cre-ERT from the promoters of several of these progenitor markers we are tracing cells at different stages of digit tip regeneration and wound healing. By assaying their contribution to digit regeneration as well testing their ability to form various tissues in vitro, we can identify sub populations of mesenchymal cells that have stem-like potential and are able to positively respond to injury instead of fibrosis.

T-1067

BIOACTIVE LIPID MEDIATORS IN THE FUNCTIONALITY OF MESENCHYMAL STROMAL CELLS

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Mesenchymal stromal cells (MSC) have shown great potential in cell therapy. In addition to their regenerative properties, MSCs are immunomodulative and anti-inflammatory. The immunomodulative response of MSCs resembles the resolution phase of inflammation. MSCs are easily obtained from various sources like bone marrow (BM) and umbilical cord blood. MSCs have been used in several clinical trials for the treatment of immunological disorders, e.g. graft-versus-host disease and Crohn's disease, with promising results. It has previously been shown in our research group that supplementation of arachidonic acid (AA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (EPA) modifies the lipid composition of the cell membrane of BM MSCs. These changes also influence immunomodulative capacity of MSCs. According to our hypothesis, the derivatives of these fatty acids are key players in the immunomodulative response of MCSs. To investigate whether BM MSCs produce resolution phase lipid mediators derived from AA, EPA and DHA under inflammatory conditions The media of BM MSCs were supplemented with either AA, EPA or DHA. In addition, the cells were given an inflammatory stimulus (tumor necrosis factor α 10 ng/ml, interleukin-1β 10 ng/ml and lipopolysaccharide 100 ng/ml). The cells were analysed by LC-MS/MS and the quantitative gene expression by QPCR. BM MSCs produced PGE2 and expressed the gene for cyclo-oxygenase (COX)-2, the enzyme that begins PGE2 synthesis, regardless of fatty acid supplementation. These results are in agreement with previous studies. PGE2 has been shown to be responsible for at least part of the immunomodulative effects of MSCs, e.g. by inducing polarization of macrophages towards an anti-inflammatory phenotype. The inflammatory stimulus increased the production of PGE2 and expression of COX-2. Moreover, BM MSCs produced the intermediates of resolution phase lipid mediators after supplementation of n-3-fatty acids EPA or DHA. These novel results show that MSCs produce bioactive lipid mediators which may play an important role in the immunomodulatory responses of MSCs.

T-1068

THE IMPAIRED IMMUNOSUPRESSIVE POTENTIAL OF MENSTRUAL BLOOD IN COMPARISON WITH BONE MARROW DERIVED STEM CELLS RAISES CONCERN REGARDING THEIR CLINICAL USE

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A non-invasive and highly proliferative stem cell population was recently identified from menstrual blood (MenSC). Although its therapeutical potential is currently being investigated in the clinic, little is known about its immunomodulatory properties. In this context, we studied the suppressive properties of MenSC and compared it with the well-characterized BM-MSC source. We found that MenSC exert less suppressive effect than BM-MSCs in the proliferative response of activated peripheral blood mononuclear cells In line with these results, a significantly higher number of proinflammatory CD4+IFN-γ+ and CD8+IFNγ+ cells was observed. Moreover, IFN-γ and IL-1β-activated MenSCs produced a reduced amount of immunosuppressive factors such as IDO, PDL-1, PGE2 and TGF-β1 with a substantial decrease in IFN-γ receptor subunits expression level when compared to BM-MSC In the collagen induced arthritis (CIA) model, only BM-MSCs exhibited a therapeutic effect alleviating most of the induced disease symptoms. This beneficial effect mediated by BM-MSC paralleled a significant decrease in proinflammatory T cell frequency. In addition, compared to BM-MSCs treated mice, mice injected with MenSCs displayed an increase of the pro-inflammatory mediators in the sera. Similarly, in the xeno-GVHD model, MenSC treated animals had increased spleen infiltration of human CD45+ cells, Surprisingly, despite their reduced immunosuppressive potential in vitro, MenSC significantly increased the survival of GVHD animals, effect that was associated with a higher migratory potential. This impaired immunosuppressive property entails a distinct clinical potential between the two cell sources and should be taken into consideration for the development of safe and effective cell therapies with limited undesired effects.

T-1069

HYPOXIA INDUCIBLE FACTORS SUPPRESSE P53 TO ENHANCE THE STEMNESS AND CELL SURVIVIAL IN HUMAN YOUNG CARTILAGE-DERIVED STEM CELLS

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Inhuman organisms, O_2 concentration varies significantly between the tissues: in well irrigated parenchymal organs it is comprised between 14% and4%. In other tissues, relatively less irrigated, O_2 concentration is even lower (brain, 0.5-7%; eye, 1-5%; bone marrow, 0-4%). Stabilization of HIF-1 transcripts is inversely proportional to O_2 concentration between anoxic condition and 5% O_2. For many cell populations these oxygen concentrations (1-5% O_2), called by "physiological hypoxia" are conditions of the physiological oxygenation, that is, the steady state oxygenation or "in situ normoxia". Most of adult stem cells reside in defined hypoxic microenvironments termed niches which regulate stem cell fate. It has been reported that hypoxia inducible factors are involved in regulating the crucial cellular processes such as stemness, proliferation, and differentiation potential. However, it is not clear whether hypoxic condition is critical for stem cell function. This study investigates the importance of hypoxia inducible factors (HIFs) in regulating the hypoxic responses of human young cartilage stem cells (hCSCs). The cultivation at 5% oxygen increased cell proliferation and resulted in a significantly increased expression of OCT4, SOX2, and NANOG mRNA as well as OCT4 protein compared with normoxic condition. Long-term cultivation of hCSCs in normoxic condition significantly induced the expression of p53, p21 and BAX, whereas hCSCs in hypoxic condition markedly enhanced the expression of BCL2. Silencing of HIF1A and HIF2A resulted in a significant decrease in OCT4, SOX2, NANOG, and BCL2 expression while tumor suppressor gene, p53, was increased. In addition, senescence significantly decreased in hypoxic cells, as evaluated by the expression of senescence-associated beta-galactosidase. These results strongly demonstrate that a low oxygen tension is preferential for maintaining the stemness. *This research was supported by Fundamental Technology Development Project through the National Research Foundation of Korea (NRF)funded by the Ministry of Science, ICT and Future Planning (2011-0019730).*

T-1070

DIRECT VISUALIZATION AND CHARACTERIZATION OF MESENCHYMAL STEM CELLS AND ENDOTHELIAL BONE MARROW NICHE CELLS USING A CD73-BAC-EGFP LIVE REPORTER MOUSE

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Mesenchymal stem cells (MSCs) are multipotent cells residing in the bone marrow and other tissues. In vitro MSCs are well characterized by their immunophenotype and differentiation potential to mesenchymal lineages. However, the current understanding of their localization and function in vivo remains elusive. We therefore generated a transgenic mouse model in which MSCs are labeled by a live reporter gene. CD73, also known as ecto-5'-nucleotidase, is a membrane-bound enzyme catalyzing the dephosphorylation of AMP to adenosine. It is a well-established marker for MSCs with only moderate distribution in other mesodermal cell types. We confirmed high expression of CD73 in various MSC populations by FACS, immunostainings and qPCR. Next, a reporter vector expressing EGFP under control of the CD73 promoter was created from bacterial artificial chromosomes (BACs), and transgenic mice were generated. As expected from the literature, kidney and lung epithelium showed a strong fluorescent signal, proving specificity of the construct. The expression in other organs like white fat,

uterus, and liver showed a typical perivascular distribution pattern as would be predicted for MSCs in vivo. In developing bones EGFP+ cells emerged at the sites of peri- and endochondral ossification, labeling mesenchymal precursor cells. Primary cultures from cortical bone, epiphysis, bone marrow (BM), and white fat gave rise to adherent growing EGFP positive cells that were able to differentiate into mesenchymal lineages, underlining their MSC character. Bone sections also revealed a distinct pattern of EGFP-labeled cells forming elongated structures in the BM. By FACS, RNAseq, and immunohistochemical analysis we identified these as sinusoidal endothelial cells. Interestingly, hematopoietic progenitor cells localized in close vicinity of these EGFP+ structures. In summary, our CD73-BAC-EGFP live-reporter mouse enables the direct visualization of MSCs in various organs. In addition, we also found that it labels endothelial sinusoidal cells in the bone marrow and these cells appear to support the hematopoietic stem cell (HSC) niche. Therefore this mouse model will enable the characterization of specific niche components and potentially also the identification of molecular cues playing a role in HSC development or fate.

T-1071

PERICYTES ACCUMULATING LIPIDS EXPRESS INFLAMMATORY CYTOKINES IN THE MURINE AGING THYMUS

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During aging, adipose tissue cells secrete inflammatory cytokines. TNFα, IL-6 and LIF were shown to promote thymic T cell death. In the aging thymus, T cell death is associated with increased perithymic adipose tissue. In addition, the number of lipid-laden cells inside the thymus increase with age. Because mesenchymal stem cells first differentiate into multilocular preadipocytes and then to unilocular mature adipocytes, intrathymic lipid-laden multilocular cells (LLMC) could be preadipocytes derived from the perithymic adipose tissue. However, we recently found α-smooth muscle actin+ (SMA) pericytes expressing perilipin (PLIN), a lipid-droplet marker, in the thymic parenchyma, suggesting that these cells could differentiate into preadipocytes in the aging thymus. Here, we aimed to identify in situ intrathymic lipid-laden pericytes and analyze whether these cells could express inflammatory cytokines. For this, we standardized in situ laser microdissection of intrathymic LLMC from mice with 22 months of age. We performed thymic tissue cryosectioning and lipid staining with Oil Red O in RNase-free conditions and verified the RNA quality of extracted cells using a bioanalyzer. Following RNA amplification, RT/PCR was performed using specific primers for epithelial cells (CK8), brown adipocytes (UCP1), preadipocytes (PREF1), macrophages (CD68) dendritic cells (CD11c) and pericytes (NG2 and PDGF receptor). We found that two cells expressed genes for NG2 and PREF1, suggesting preadipocyte differentiation from pericytes; and one expressed genes for NG2 and PDGFr, another marker for pericyte. We also verified that these three Oil Red O+ pericytes express genes related to inflammation, including TNFα and IL-6, as verified by RT real time PCR. In conclusion, our results indicate that pericytes might contribute to increase intrathymic adipocyte population as well as for the proinflammatory state in the aging thymus.

T-1072

BIOMATERIALS GRAFTED WITH EXTRACELLULAR MATRIX AND CYCLIC RGD HAVING DIFFERENT ELASTICITY FOR PLURIPOTENT MAINTENANCE OF AMNIOTIC FLUID-DERIVED STEM CELLS

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Human amniotic fluid-derived stem cells (AFSCs) are pluripotent fetal cells capable of differentiating into multiple lineages, including representatives of the three embryonic germ layers. Stem cells which derived from human amniotic fluid may become a more suitable source of stem cells in regenerative medicine and tissue engineering. However, stem cell characteristics, such as proper differentiation and maintenance of pluripotency, are regulated not only by the stem cells themselves but also by their microenvironment. Furthermore, physical characteristics of cell culture substrates may influence the fate of stem cell differentiation, such as substrate elasticity. We investigated AFSCs characteristics on the biomaterials grafted with the cyclic RGD oligopeptide to investigate whether the biomaterials could support the cell adhesion with high efficiency and keep proliferation. Here we report pluripotent maintenance and differentiation efficiency of AFSCs cultured on cell culture substrates immobilized extracellular matrix-derived oligopeptides containing different cyclic RGD combination ratio, which have different elasticity. We prepared dishes coated with polyvinylalcohol-co-itaconic acid (PVA-IA) films having different elasticity by controlling the crosslinking time in crosslinking solution containing glutaraldehyde. The PVA-IA dishes were grafted with several ECM-derived oligopeptides and cyclic RGD peptides though N-(3-dimethylaminopropyl)- N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) chemistry in an aqueous solution. Pluripotent gene expression, Nanog, Oct4 and Sox2 were evaluated by the qRT-PCR measurements. We found that there is an optimal elasticity of cell culture matrix to keep pluripotency of AFSCs for their culture. It is suggested that physical cues such as stiffness of culture materials as well as biological cues of extracellular matrix components can guide and decide pluripotency and proliferation of stem cells.

T-1073

EXOSOMES DERIVED FROM MESENCHYMAL STEM CELLS MINIMIZED THE LPS ACUTE KIDNEY INJURY BY RENAL PLURIPOTENT CELLS ACTIVATION

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We investigated the effects of mesenchymal stem cells (MSCs), your conditioned medium (CM) or exosomes (EXOs) in a LPS-induced nephrotoxicity and the role of renal stem progenitor cells (RPCs). Rats received i.v.: LPS (10 mg/B.W.) or PBS (CTL) with MSCs (1x106), CM (500 µl) or its EXOs (100 µg/ml) from MSCs incubated or not for 12 hours with cytochalasin B (CB; 1 µM) or actinomycin D (AD; 2.6 µM) given in 1 or 3 doses and sacrificed after 72 hours. Blood, urine samples were collected for creatinine (sCr), urea (sU) and FENa. Kidneys were analyzed for HE, KI67, caspase 3, RPCs as Wnt1, BrDU, PAX2, EXOs as CD9, CD63. Y chromosome, IL6,

TNF-α, INF-γ and IL10 were evaluated. It was observed increases in sCr, sU, FENa, caspase 3 marking, proinflammatory cytokines and reduction of KI67 with lesions in proximal tubules induced by LPS. However, these parameters were ameliorated with MSCs, CM or EXOs treatments. LPS and LPS+EXOs increased BrDU, Wnt1, PAX2 and CD63 expressions indicating activation of RPCs. CB and AD inhibited the protective effect of EXOs. The effect of 3 times administration of MSCs or CM or EXOs decreaed the mortality in LPS. Therefore, results support that the MSCs and its CM and EXOs protected from LPS-nephrotoxicity. It is reasonable to suggest that the mediation by EXOs is, at least in part, by stimulating RPCs in this cascade of events and those EXOs alone could be employed in order to ameliorate LPS cute kidney injury (AKI).

HEMATOPOIETIC CELLS

T-1075

HEMATOPOIETIC STEM CELL GENE THERAPY AND SELECTION STRATEGY AGAINST HIV INFECTION

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HIV/AIDS has devastated the world since its discovery, drastically decreasing life expectancy and quality of life for infected individuals. HIV uses the co-receptor CCR5 to enter and infect a T-Cell, leading to a body wide infection. We aim to transduce hematopoietic stem cells with a lentiviral vector containing a short hairpin RNA sequence to knock down the expression of CCR5 thus not allowing HIV to infect its progeny T-Cells. Similar gene therapy strategies in the past fall short in two aspects, low engraftment and the risk of oncogenesis. By knocking down a second gene, HPRT, we have developed a reliable selection strategy to select and deselect transduced cells in culture with the use of two drugs, 6TG and MTX. We have effectively developed a model to control the growth and decline of gene modified cells in culture. We have found that we are able to use a lentiviral vector containing an shRNA to suppress the expression of CCR5 and HPRT, and reliably select and deselect these cells in culture. We selected gene modified cells in culture from an initial 5% to over 90% of overall cells within culture in 14 days of 6TG treatment, and a knockdown from over 90% to below 40% within 14 days of MTX treatment. This shows that by knocking down HPRT, we are able to reliably select and deselect cells within culture with this selection system, which was developed to furthermore move this strategy to modify hematopoietic stem cells.

T-1076

EFFICIENT AND SPECIFIC CRISPR/CAS9-MEDIATED GENE ABLATION IN PRIMARY HUMAN HEMATOPOIETIC CELLS

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Cell-based gene therapy has the potential to change the way we treat disease. The hematopoietic system has been at the forefront of the field, as the cells can be readily obtained, manipulated and reintroduced into patients. Genome editing has dramatically improved over the past five years, holding great promise for the advancement of human gene therapy. CRISPR/Cas9-mediated genome editing has rapidly become the method of choice due to its ease of design and high on-target efficiency. However, the use of CRISPR/Cas9 in primary cells remains untested. Here, we targeted two clinically relevant genes, CCR5 (HIV co-receptor) and B2M (MHC class I accessory chain), in primary human CD34+ hematopoietic stem and progenitor cells (HSPCs) and CD4+ T cells. We observed that a single guide RNA strategy achieved efficacious gene targeting in HSCPCs, but not in CD4+ T cells. A dual guide RNA strategy, where two different guide RNAs targeting the same locus were introduced into cells, increased gene ablation efficiency, particularly in CD4+ T cells. In addition, this approach led to predictable deletions, opening the possibility of targeting regulatory regions controlling the expression of disease-related genes. Importantly, CRISPR/Cas9-edited HSPCs retained full multi-lineage potential in vitro and when transplanted into mice. Finally, targeted capture and extremely deep sequencing revealed a very high on-target mutation rate and a vanishingly small off-target mutation rate. These results provide the first proof of principle that CRISPR/Cas9 technology can be used to ablate clinically relevant genes in primary HSPCs with high efficiency and specificity, greatly enabling the manipulation of the hematopoietic system for several clinical settings, such as the treatment of HIV. Further detailed analysis of CRISPR/Cas9 targeting of primary human hematopoietic cells and their use in a preclinical/clinical setting is ongoing and will be presented.

T-1077

CDC42 MEDIATED EPIGENETIC REGULATION OF HEMATOPOIETIC STEM CELL AGING AND REJUVENATION

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Aging can be broadly defined as the time-dependent functional decline that affects most living organisms and it is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death. Some common denominators of aging can be identified among different organisms and somatic stem cell activity is one of these, critical for tissue regeneration. Recent publications indicate that distinct undesirable phenotypes associated with aged stem cells can be ameliorated by a targeted pharmacological approach, demonstrating that the functional decline of aged stem cells and tissues may be reversible. Previously we showed that hematopoietic stem cell (HSC) aging is characterized by a loss of polar distribution (epigenetic polarity) and a decrease in the level of acetylation on histone 4 on lysine 16 (AcH4K16). Treatment with CASIN, a Cdc42-activity inhibitor that can functionally rejuvenate aged HSCs, re-establishes epigenetic polarity and increases level of AcH4K16. Based on these observations, we hypothesize that alterations in the distribution and/or level of additional epigenetic marks together with AcH4K16 might be linked to Cdc42 activity level and polarity in HSCs and critically impact on HSC function upon aging and rejuvenation. Results demonstrate that aged HSCs show apolar distribution and

decreased level of AcH4K12, similarly to AcH4K16. Distribution of 2meH3K9 and 3meH3K27 are also altered upon aging of HSCs. In vitro treatment of aged HSCs with butyrate, a known class I and II histone deacetylase inhibitor, increases levels of overall acetylation on histone 4 but does not affect epigenetic polarity of AcH4K12 and of AcH4K16 and, most importantly, function of aged HSCs. In summary we gain insights into epigenetic effects of Cdc42 activity inhibition in aged HSCs via CASIN by showing that this treatment affects epigenetic features specific of HSCs and is impacting on nuclear distribution of AcH4K12 in addition to AcH4K16.

T-1078

TISSUE- SPECIFIC INTERACTORS OF ENDOSOMAL NETWORKS REGULATE STEM CELL POTENCY AND CONTROL HEMATOPOIESIS

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Endosomal trafficking regulates diverse biological processes including stem cell differentiation. We recently showed that in Drosophila haematopoiesis and mouse embryonic stem cells (ESCs), endosomal control by Asrij specifically regulates stem cell phenotype in a dynamic environment. Using transcriptome and proteome analysis we now identify novel components of the Asrij interactome, whose disruption leads to aberrant signal generation or attenuation in Drosophila, mouse and human hematopoiesis, resulting in perturbed homeostasis and cancer. Using the Notch and JAK/STAT signalling pathways as examples we show that this mechanism is conserved in evolution. Asrij null mice show splenomegaly, increase in bone marrow LT-HSCs and perturbed steady-state hematopoiesis. Biochemical analysis shows constitutive reduction of phospho-STAT3 and activation of Notch and the ubiquitin and signallosome machinery, possibly due to an endosomal sorting defect. The outcome is increased activation of AKT (S473) that predisposes Asrij null mice to a leukemic state. In human ESCs Asrij is expressed at low levels and forced expression drives hESC towards a naïve pluripotent state. Further, regulated overexpression of Asrij during mesodermal differentiation accelerates the epithelial to mesenchymal transition with increased early mesodermal progenitors. Preliminary analysis shows increased haematopoiesis as well as effects on MAPK signalling. Thus we show that the tissue-specific endosomal regulator Asrij plays a vital role in controlling ubiquitous cellular circuits such as Notch, JAK/STAT and PI3K/AKT pathways to maintain the balance between stemness and differentiation. We extend the current model of signalling in maintaining stemness and show that Asrij-associated endosomes are pivotal for spatial and kinetic regulation of networks. Our study also provides new avenues to generate and amplify lineage-specific precursors and will aid understanding of early human development.

T-1079

LINEAGE ANALYSIS AND FUNCTIONAL CHARACTERISATION OF APJ EXPRESSING CELLS IN THE HAEMATOPOIETIC SYSTEM

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Haematopoietic stem cells (HSCs) can reconstitute the entire haematopoietic system and are used to treat blood cell disorders including leukaemia. However they represent a very small fraction of adult bone marrow and it is has not been possible to produce, expand or maintain these cells in vitro. There is a great need to identify and characterise novel cell surface markers and their ligands that can be used to track the development of HSC, to aid in their isolation and expansion and to develop strategies for their production from pluripotent stem cells (PSCs) in vitro. Enforced expression of HOXB4 enhances the production of HSCs from PSCs and we demonstrated recently that this transcription factor can exert its effects in a paracrine manner. We subsequently identified Apelin and its receptor, APLNR/APJ as potential mediators of this effect. The aim of the present study is to determine the identity of APJ-expressing cells in vivo; specifically whether APJ marks the HSC and/or their precursors or cells which act as a supportive environment for their development. To this end we have performed flow cytometry analyses of adult bone marrow and embryonic haematopoietic tissue using a panel of antibodies that mark haematopoietic stem and progenitor cells and mesenchymal cells associated with their microenvironment. We have shown that APJ is expressed on a proportion of the lineage-, Sca+, Kit+ (LSK) HSC containing population. However cell sorting experiments indicate that the in vitro haematopoietic colony-forming (CFU-C) activity is associated with the APJ- population. This could suggest a supportive role for APJ+ cells or perhaps APJ is marking a pre HSC population. In keeping with the idea that APJ may be expressed in supportive cells we also found APJ expression to be enriched in a CD45-, CD73+, CD105+ CD90+ mesenchymal cell population of the bone marrow. In order to determine whether haematopoietic cells are derived from APJ expressing precursors we have designed a Crispr-Cas9 targeting strategy that will allow Cre-mediated lineage tracing of APJ-expressing cells. Analysis of mice expressing ERT2CreERT2 under the control of the APJ promoter crossed with reporter mice will indicate whether haematopoietic cells or niche /supportive cells arise from APJ-expressing precursor cells.

T-1080

ROLE OF HEME OXYGENASE-1 IN AGING OF HEMATOPOIETIC CELLS

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Heme oxygenase-1 (HMOX1) is a heme degrading enzyme with cytoprotective and antioxidative activities. Its deficiency disrupts the response of hematopoietic stem cells (HSC) to acute stress. We investigated whether HMOX1 may play a role in the steady-state function of HSC in young (~3 months) and old (~12 months) mice. To this aim we compared the HSC properties in wild type (WT) and HMOX1-/- (KO) animals. We demonstrated that LT-HSC pool was more expanded in young KO than in young WT mice. Young KO LT-HSC proliferated much faster than their WT counterparts, while downstream progenitors proliferated with the same rate. Furthermore, HSC in young KO mice lost their quiescent character, and much more cells entered the G1 cell cycle phase, in comparison to WT. Young KO individuals had also more myeloid cells in peripheral blood, and their single sorted HSC showed increased differentiation towards macrophages in vitro. Expansion of LT-HSC

and myeloid bias is typical for aged hematopoietic system and the observed differences between WT and KO mice disappeared in old individuals. We also found that expression of GCSF receptor on HSPC in young KO mice was very low, while in young WT was high until decreased with age. Lastly, we showed that LT-HSC in young KO had a higher fraction of phosphorylated gH2aX-high cells, that may indicate the enhanced DNA damage. The expression of HMOX1 in HSPC was relatively low. Much higher level was found in the HSC niche, especially in CAR reticular cells and CD31+Sca1+ endothelial cells, suggesting that HMOX1 may be more important in the niche than in HSC themselves. To verify this supposition we isolated WT LT-HSC and transplanted them to KO or control WT mice. After 32 weeks the HSC transplanted into KO animals gave worse reconstitution of peripheral blood. Then we performed a secondary transplant to the young WT animals. We observed stable reconstitution only if the first recipient was of WT genotype. In contrast, HSC which were initially transplanted into KO animals did not reconstitute any secondary recipient. To sum up, HSC in HMOX1 KO mice show features of premature aging. We suppose that lack of HMOX1 in the bone marrow niche precludes a proper function of LT-HSC.

T-1081

EFFECT OF RAF-1 KINASE INHIBITORS IN THE PATHWAY OF ERYTHROID DIFFERENTIATION

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Rubiolo C et al reported that Raf-1 regulates eryrthoird differentiation by controlling the level of FAS expression in cells lacking Raf-1. We analyzed the relationship between FAS and Raf-1 using the Raf-1 kinase inhibitors in the context of erythroid differentiation from the cord blood derived CD34+ cells into mature RBCs for 21 days in culture. The CB-derived CD34+ cells were expanded and differentiated in serum- free erythroid medium (StemPro) supplemented with human EPO, SCF, and IL-3 for 21 days. In cultured for differentiation, 1 μM Raf-1 kinase inhibitors (RKI) was added in media after 10 d in culture. The differences of apoptosis between control and PKI treated RBCs were tested by FACS analysis with Annexin V stain and western blotting with FAS and FAS ligand antibodies. The mRNA expression of GATA binding protein 2 quantitated by Real-time RT-PCR and protein expression by immunocytochemistry. When apoptosis was assessed by annexin V cell staining during RBC differentiation, Raf-1 kinase inhibitor decreased apoptosis in terminal erythroid differentiation. Also, Raf-1 kinase inhibitor increased FAS expression and decreased FAS Ligand expression. Raf-1 kinase inhibitor induced burst-forming erythroid (BFU-E) from CD34+ cells in colony formation assay. Consistently, Raf-1 kinase inhibitor maintained the expression of the gene essential for early phase of erythropoiesis of GATA binding protein 2 (GATA2) and delayed the change from GATA2 to GATA 1. Our data shows that Raf-1 kinase inhibitors indirectly accelerate into maturation of RBCs from CD34+ stem cells and cell survival rate with 21 day in culture.

T-1082

HEMATOPOIETIC STEM/PROGENITOR CELLS FROM HUMAN PLACENTAL TISSUE, UMBILICAL CORD BLOOD, AND FETAL LIVER

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We investigated the similarities and differences of hematopoietic stem/progenitor cells (HSPCs) population from full-term placental tissue (FTPT) compared to HSPCs from cord blood (CB) and fetal liver (FL). HSPCs of FTPT origin obtained by enzymatic method. Immunophenotyping performed on BD FACSAria (USA). MethoCult (Canada) was used for differentiation assays. FTPT was cryopreserved by special program in software based freezer. FTPT characterized by higher phenotypic heterogeneity compared to CB and contained cell subpopulations at different stages of differentiation as CD34+/lowCD45low/-, CD34++CD45low/-, CD34+++CD45low/-, CD34+/lowCD45hi, and CD34++CD45hi. The content of "true" HSPCs (CD34+CD45lowSSClow) among viable CD45+ cells from FTPT tissue was 0.56 % (0.39 - 0.76 %, n = 16). HSPCs from FTPT expressed the CD90 at a significantly higher level in compare to CB and the same level of CD133 and CD31. The CD133 was shown to be strictly specifically expressed on CD34++CD45low subset of both FTPT- and CB-derived cells. The CD34+++CD45low/- cells similar to CD34++CD45low/- cells carry the immunophenotype CD33-/lowCD14-/lowCD235-CD19-CD7 /lowCD45RA-, but were heterogeneous by morphology .The HSPCs compartment of FTPT contains significantly higher level of lineage committed cells in compare to CB (namely CD14+CD33+, CD14+, CD235+ cells) but significantly lower than FL (namely CD33+, CD7+, CD19+ cells). The expression increase of CD14, CD33, CD7 and CD19 was accompanied by the decreasing intensity of CD34 expression on CD45low/-cells of the FTPT origin. HSPCs of FTPT origin gave rise to various type of fetal origin (proved by FISH) colonies in vitro and their ratios were not significantly differ in compare to CB, in addition the ratios of myeloid and erythroid progenitors among the lineage committed HSPCs were the same for FTPT and CB. Differences and similarities of HSPCs from studied tissues evidence about placental hematopoiesis and suggest FTPT is a valued source of HSPCs for medicine. We obtained viable HSPCs from cryopreserved placental tissue fragments. Optimization of method of tissue cryopreservation gives the possibility of banking and testing it for clinical use.

T-1083

ESL-1 IS A HEMATOPOIETIC-BORNE REGULATOR OF THE BONE MARROW MICROENVIRONMENT

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Maintenance of hematopoietic stem and progenitor cells (HSPC)

is regulated through cell-autonomous mechanisms as well as extrinsic factors produced by the hematopoietic niche. However, the contribution of the HSCs pool to the properties of their own niche remains unclear. Here we report a new role for the selectin ligand ESL1-TGFβ axis in the hematopoietic stem cell pool. By analyzing ESL-1 deficient mice, we find evidence that hematopoietic precursors are able to regulate the proliferative status and size of the stem cell pool in a paracrine manner. Mice deficient in ESL-1 showed marked reductions in HSPC proliferation, expansion of the immature pool, and reductions in niche size. Functionally, this proliferative impairment of the stem cell compartment resulted in elevated resistance to exhaustion after a genotoxic insult. Surprisingly, this phenotype was both transplantable and dominant when mutant and wild-type precursors coexisted in the same environment, and was largely independent of E-selectin, the vascular receptor for ESL-1. Instead we find that the enforced quiescence generated by ESL-1 mutants is generated by the abnormal secretion of the cytokine TGFβ by hematopoietic cells, including HSPC. Mice reconstituted with mutant marrow showed increased levels of this cytokine and in vivo or in vitro blockade of TGFβ restored the homeostatic properties of the hematopoietic niche. Thus, these results show that homeostatic secretion of TGFβ by HSPC is autonomously restricted by ESL-1 but can contribute to essential features of the hematopoietic niche by creating an anti-proliferative environment.

T-1084

THE CHARACTERIZATION OF ENDONUCLEASE-INDUCED CHROMOSOMAL REARRANGEMENTS AT THE BETA- AND DELTA-GLOBIN GENE LOCI IN HEMATOPOIETIC CELLS

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The use of site-specific endonucleases such as transcription activator-like effector nucleases (TALENs) and clustered, regularly interspaced, short palindromic repeats (CRISPRs) for targeted gene correction of the sickle mutation in hematopoietic stem cells (HSCs) is a promising gene therapy strategy for the treatment of sickle cell disease (SCD). It has been shown that targeted gene editing via the introduction of a locus-specific endonuclease combined with a DNA donor template, containing flanking regions homologous to the endonuclease cut site, increases the success of targeted gene addition. However, due to a high sequence homology existing between the beta- and delta-globin genes, off-target endonuclease cleavage events have been observed at delta-globin when using endonucleases targeted to exon 1 of beta-globin. It has also been shown that the introduction of double stranded breaks by site-specific endonucleases has the potential to induce intrachromosomal rearrangement events or structural variations such as translocations, deletions, duplications, and inversions. Additionally, multiple groups are using integrase defective lentiviral vectors (IDLVs) as their currently-optimized DNA donor template delivery strategy (for SCD and X-linked severe combined immunodeficiency [X-SCID]), which have the propensity to be end-captured at the DSB site. This work focuses on the evaluation and characterization of these potential structural variations, chromosomal rearrangements, and end-captured DNA at the beta- and delta-globin gene loci as a result of endonuclease-induced DSBs. PCR analysis using primer sets designed to detect these chromosomal rearrangements show that both deletion and translocation events are present in K562 cells treated with TALENs specific to the sickle mutation site in the beta-globin locus. Of note, these rearrangements are not detected in cells treated with CRISPRs known to not induce cleavage at delta-globin. Additionally, PCR analysis of K562 cells treated with TALENs and a GFP-IDLV show evidence of end-capture of the GFP-IDLV in the off-target delta-globin locus. These results demonstrate the necessity for characterization of endonuclease-induced chromosomal rearrangements and end-capture events in CD34+ hematopoietic stem/progenitor cells.

T-1085

MITOCHONDRIAL DYNAMICS REGULATE LYMPHOID POTENTIAL OF HEMATOPOIETIC STEM CELLS

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Hematopoiesis is sustained over an entire lifetime through continuous maintenance and self-renewal of hematopoietic stem cells (HSCs). However, a coherent picture of how these processes are achieved by the HSC to regulate homeostatic responses of the hematopoietic system in vivo has not yet emerged. Moreover, the molecular mechanisms underlying the recently identified clonal heterogeneity among HSCs in lineage potential and self-renewal capacity are unknown. The transcription factor Prdm16 is required for HSC maintenance and self-renewal. We show that regulation of mitochondrial dynamics is a target of the Prdm16 transcriptional program. Our data show that Prdm16 maintains mitochondrial hyperfusion in HSC through transcriptional activation of Mitofusin 2 (Mfn2). HSCs retrovirally transduced with Mfn2 showed enhanced function in competitive and serial repopulation assays compared to control vector. Furthermore, our studies reveal that Mfn2 and mitochondrial hyperfusion facilitates increased steady-state intracellular [Ca2+] buffering in HSCs, leading to decreased baseline [Ca2+] and consequently, lower calcineurin phosphatase activity thereby inhibiting nuclear translocation of the transcription factor, NFAT. Pharmacological inhibition of NFAT increased the mRNA expression of lymphoid-biased HSC markers IL7Ra and Sox4 in vitro. Furthermore, NFAT inhibition increased the lymphoid reconstitution potential of HSCs. Despite the prevalent use of glycolysis in HSCs for energetic metabolism, we show mitochondria play a critical role in conferring lymphoid lineage potential and may underlie the conspicuous heterogeneity of HSC differentiation potential. Insights from these findings will broadly impact our understanding of HSC biology and advance the field towards improving targeted therapies to treat diseases of HSC origin.

T-1086

CHEMICAL SCREENING USING ZEBRAFISH EMBRYO CULTURES IDENTIFIES ADENOSINE AS A MODULATOR OF HEMATOPOIETIC STEM AND PROGENITOR CELL INDUCTION

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Hematopoietic stem cells (HSCs) have been used clinically to reconstitute the bone marrow, but half of all patients lack a matched donor marrow. iPSC-derived HSCs hold great promise for treating these patients, but our understanding of the pathways involved in HSC formation is incomplete. We previously reported a chemical genetic screen that identified inducers of myogenesis using an embryonic blastomere culture system in zebrafish. Here, we have adapted this culture screening system to find inducers of c-myb, a marker of hematopoietic stem and progenitor cells (HSPCs), to identify new pathways that expand HSPCs. In this study, *c-myb:GFP* zebrafish are grown to 50% epiboly and dissociated into single cells, which are then plated in 384 well plates. The cultured cells are treated with 3,840 independent compounds in duplicate, and after two days in culture, GFP fluorescence is read out. We identified 14 chemicals (z score >10; hit rate 0.36%) that induce *c-myb* expressing cell expansion and performed dose response curves. Among these hits was the adenosine analog 3-deazaadenosine, suggesting adenosine regulates HSPC formation. We treated whole embryos with different adenosine analogs, including the adenosine receptor agonist 5'-N-ethylcarboxamidoadenosine (NECA) and antagonist CGS15943, to examine effects on HSPC development. Confocal imaging of the aorta-gonad-mesonephros (AGM) region, the site where HSPCs are specified, showed a significantly increased number of HSPCs in *cmyb:GFP* embryos (52.8 ± 4.8) vs DMSO control (43.2 ± 2.4) due to NECA treatment, and significantly decreased (36.0 ± 2.4) due to CGS15943 treatment. Since HSPCs originate from hemogenic endothelium by undergoing endothelial-to-hematopoietic transition (EHT), we wanted to observe whether adenosine has an effect on endothelial cells by time-lapse imaging. Morpholino A_{2b} receptor injections to knockdown this adenosine receptor caused more endothelial cells, marked by *flk1:GFP*, to abort EHT. Taken together, we conclude that adenosine signaling is required for EHT and proper HSPC development. Factors revealed in our zebrafish culture system establish new pathways that can be useful targets to promote iPSC-derived HSPC expansion; our findings identify an important role of adenosine signaling in HSPC emergence.

T-1087

ENHANCED LONGTERM MULTI-LINEAGE REPOPULATION OF KIT-MUTANT IMMUNODEFICIENT NRG MICE BY TRANSPLANTS OF PRIMARY NORMAL AND LEUKEMIC HUMAN HEMATOPOIETIC CELLS

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Improvements in xenograft models to characterize the growth potential of primitive subsets of normal and leukemic human hematopoietic cells have transformed basic and translational research. We now report the advantageous use of "NRG-W41" host mice that are homozygous for the *NOD-Sirpa/Rag1*$^{-/-}$*/IL2Rγc*$^{-/-}$*/W*^{41} genotype but have an otherwise mixed genetic background. Initial studies showed that parental NRG mice given 900 cGy over 3 hrs survive for a year consistently and are similarly repopulated by human CD34^{+} cord blood (CB) cells as are NSG mice given 315 cGy. Kinetic studies of the levels of human cells regenerated from IV transplants of 5x10^{4} human CD34^{+} cord blood (CB) cells/mouse, showed that at 3 and 6 weeks post-transplant, more human WBCs and platelets were found in the blood of 900 cGy pretreated NRG mice vs unirradiated NRG-W41 mice (20-fold and 4-fold higher, respectively). However, these differences had disappeared 4 weeks later and remained unchanged at 20 weeks post-transplant. Moreover, when NRG-W41 mice were pretreated with 150 cGy (an estimated "equivalent" to the 900 cGy given to NRG mice), the numbers of human cells present at week 3 were already similar, and over time, the NRG-W41 mice acquired much higher levels of human (lymphoid and myeloid) cells (5-fold at week 20 in the bone marrow [BM] of the mice), with a parallel persistent increase in circulating human platelets (20-fold in irradiated NRG-W41 vs irradiated NRG mice at week 20). Similar effects were seen in comparisons of NRG and NRG-W41 mice transplanted with 10^{5} normal human CD34^{+} BM cells/mouse (5% & 0.5% human CD45^{+} cells in irradiated and non-irradiated NRG-W41 mice vs 0.04% in irradiated NRG at week 20). In addition, we have found that NRG-W41 mice crossed with NRG-human IL-3/GM-CSF/SF-producing mice to generate homozygous NRG-W41-3GS mice transplanted without irradiation support the production for at least 10 weeks of BCR-ABL^{+} B-lymphoid and myeloid cells from transplanted CD34^{+} cells obtained from a patient with chronic phase chronic myeloid leukemia (CML). In summary, our NRG-W41 mice support high outputs of human cells from CB, BM and CML samples without prior irradiation, but when sublethally irradiated show an increasing advantage with time over the previous "optimal" immunodeficient hosts.

T-1088

OSCILLATIONS IN HEMATOPOIETIC STEM CELL NUMBERS AND PROLIFERATION DURING RECOVERY FROM CYCLOPHOSPHAMIDE TREATMENT IN MURINE MODEL

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Cyclophosphamide (CY) is an antineoplastic drug widely used in clinical practice for treatment of leukemia and other malignancies. Besides immunosuppression and hematopoietic stem cell (HSC) mobilization, CY is also used in preconditioning regimens in recipients prior to HSC transplantation. It has been demonstrated that CY damaged bone marrow progenitors display a unique regenerative kinetics with rapid onset of regeneration followed by a secondary deep depression. Aim of the study was to describe in detail the HSCs regeneration kinetics in CY damaged murine bone marrow. Mice were injected with CY (135mg/kg b.w.). HSCs were determined in regenerating bone marrow and peripheral blood by flow cytometry as Lin- c-kit+ Sca-1+ CD48- CD150+ cells. HSCs proliferation was determined by 5-bromo-2-deoxyuridine (BrdU) incorporation into DNA in vitro and/or in vivo. HSCs apoptosis was

detected by Hoechst and propidium iodide staining. HSCs are slowly proliferating population with around 5% of cells in S-phase. After CY administration, HSCs were rapidly recruited into cell cycle with almost 20% in S phase 2 days after CY. Five days after CY there was a significant overshot of HSC numbers in bone marrow followed by almost entire proliferation quiescence. HSCs return to normal levels triggered second proliferation wave around day 10 (25% of HSCs in S phase). The secondary overshot of HSCs in the bone marrow occurred around day 14. HSC numbers thus oscillated in the bone marrow after CY treatment. Their first decline was accompanied by their vast mobilization to peripheral blood. In contrast, the second wave of HSC multiplication triggered a significant increase in their apoptosis. The dysregulation of cytokine production, mainly SCF and SDF-1, has been observed during regeneration process. The regulation of HSC numbers after cytostatic damage is a complex process resulting from HSC interaction with bone marrow microenvironment. Cyclophosphamide influence on microenvironment triggers unique oscillatory regeneration kinetics different from those observed after other cytostatics or radiation treatment.

T-1089

THE SPLICE FACTOR PTBP1 REGULATES HEMATOPOIETIC STEM CELL FUNCTION AND RED BLOOD CELL DEVELOPMENT

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The molecular networks that control stem-cell maintenance, lineage commitment and differentiation in hematopoiesis has been extensively studied. Still, alternative mRNA splicing represents a whole layer of gene regulation with potentially enormous impact on such processes that has not yet been carefully considered. The importance of mRNA splicing in regulation of hematopoiesis is further highlighted by the recent findings of numerous splice-factor mutations in several types of hematological malignancies. As a step towards elucidating the role of alternative mRNA splicing in hematopoietic stem cell (HSC) biology, we study the function of the splice-factor Polypyrimidine-binding protein 1 (PTBP1) in the hematopoietic system, by interferon-inducible, conditional deletion of PTBP1 in *Ptbp1* $^{flox/flox}$ *Mx1Cre* mice. Upon PTBP1 deletion, the numbers of peripheral red blood cells are decreased, followed by lowered hemoglobin levels and anemia. Detailed flow cytometric analyses of bone marrow cells revealed that upon PTBP1 loss, the frequencies of several types of erythroid progenitor cells are decreased. At the same time, the numbers of HSCs and multipotent progenitor cells are increased in both bone marrow and spleen. In addition, PTBP1 deficiency presents with extramedullary hematopoiesis in the form of splenomegaly and presence of immature cells in the spleen and peripheral blood, suggesting a situation of stress hematopoiesis. Although immunophenotypically defined HSCs are more abundant in PTBP1 deficient mice, HSC function is markedly impaired, as demonstrated by a reduced ability to competitively repopulate irradiated hosts. Furthermore, PTBP1 deficient HSCs display a gradual decrease in repopulation capacity in serial reconstitution experiments, suggesting impaired self-renewal potential. Our analyses clearly demonstrate that PTBP1 is involved in the regulation of stem-cell maintenance and differentiation decisions in hematopoiesis, likely by balancing the relative abundance of specific splice variants with distinct functions. We are now applying next-generation sequencing to highly purified HSCs and erythroid progenitors with the attempt to define specific mRNA isoforms that are involved in regulation of self-renewal and differentiation decisions in hematopoiesis.

T-1090

STAT5 REGULATED MICRORNA_193B CONTROLS HEMATOPOIETIC STEM AND PROGENITOR CELL EXPANSION BY MODULATING CYTOKINE RECEPTOR SIGNALING

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Hematopoietic stem cells (HSCs) require the right composition of microRNAs (miR), small non-coding RNAs with the ability to orchestrate complex gene expression networks, for life-long balanced blood cell regeneration. In this study, we show a regulatory circuit that prevents excessive HSC self-renewal by up-regulation of miR-193b upon self-renewal promoting Thrombopoietin-MPL-STAT5 signaling. In turn, miR-193b restricts cytokine signaling by targeting the tyrosine kinase c-KIT. We generated a miR-193b knockout-out mouse model to unravel the physiological function of miR-193b in hematopoiesis. MiR-193b-/- mice showed a selective gradual enrichment of functional long-term repopulating HSCs (LT-HSCs), which were fully competent in multilineage blood reconstitution upon transplantation without signs of exhaustion. The absence of miR-193b caused an accelerated expansion of LT-HSCs, without altering cell cycle or survival, but by decelerating differentiation. Conversely, ectopic miR-193b restricted LT-HSC expansion and blood reconstitution. MiR-193b-deficient hematopoietic stem and progenitor cells exhibited increased basal and cytokine-induced STAT5 and AKT signaling. Therefore, the STAT5-induced miR-193b provides a negative feedback for excessive signaling by directly targeting the receptor tyrosine kinase c-Kit to restrict uncontrolled LT-HSC expansion.

T-1091

THE EFFECT OF CYTOKINES ON ENGRAFTMENT AND INSERTIONAL MUTAGENESIS IN HEMATOPOIETIC GENE THERAPY

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Gene therapy of autologous hematopoietic stem cells (HSC) using retroviral vectors is a treatment option for rare inherited disorders of the immune system. To allow for efficient gene transfer with gamma- or lentiviral vectors, cells are prestimulated and transduced in xenofree media supplemented with certain cytokines. The medium usually contains 300 ng/ml SCF and FLT-3L, 100 ng/ml TPO as well as 20-60 ng/ml IL-3 and is used in most clinical trials today. While the role of cytokine combinations on engraftment of cultured HSC has partially been studied in the past, little is known about their effects on clonal diversity of the graft. The risk of insertional mutagenesis is still one of the main concerns regarding integrating vector technologies. Hence, both in vitro and in vivo genotoxicity studies are frequently used to assess the risk associated with HSC gene transfer. Our group investigated an important parameter influencing the conclusions we draw from these studies, which also has implications on the clinical use of cytokine conditions for further trials. We transduced murine HSC in standard cytokine conditions with or without IL-3. This growth factor is implicated in proliferation of HSC but was also described to induce differentiation towards the myeloid lineage. We monitored repopulation kinetics of lethally irradiated mice (n=65) by flow cytometry and found the early engraftment capabilities of HSC to be reduced in IL-3 containing cytokine conditions. We further marked the transplanted cells with a lentiviral barcode technology (16-N library) and analyzed the diversity with Ion Torrent sequencing. Bioinformatic analysis of 6,240,802 sequences revealed a reduction of clonal diversity in the stem cell pool when IL-3 was used during transduction. Lower numbers of repopulating stem cells ultimately increase the proliferative stress for the individual cell and hence the chance for secondary mutations. Coupled with the risk of insertional mutagenesis, this loss in clonal complexity might increase the risk for insertional mutants to succeed. We further found that the effect of enhancer mediated transformation of murine HSC in vitro was strictly linked to the presence of IL-3 in the medium. Together, our results show the impact of the early steps of gene therapy protocols on stem cell clonality and treatment safety.

T-1092

RARS (REFRACTORY ANEMIA) CLONAL EVOLUTION SUPPORTS THE HYPOTHESIS OF THE EXISTENCE OF MDS-STEM CELLS

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Myelodysplastic syndrome (MDS) is a heterogeneous disease, and thus despite the recent evidence of the existence of rare multipotent MDS stem cells (MDS-SCs) in 5q- MDS patients, it is unclear whether hematopietic stem cells (HSCs) could also be the initiating cells in other MDS subgroups. Refractory anemia with ring sideroblasts (RARS) is an MDS disease, whose pathogenesis frequently involves mutations of the pre-mRNA splicing gene SF3B1. Although it is known that these mutations occur in the hematopoietic compartment, it is still unclear whether they originate from hematopoietic progenitors or hematopoietic stem cells (HSCs). Here, we demonstrate that SF3B1 mutation(s) arise from HSCs and is the initiating mutation in these patients. Using xenotransplantation we reveals a persistent long-term engraftment restricted to myeloid lineage which was initiated only via the transplantation of HSCs. Remarkably, genetically diverse evolving multiple subclones of mutant SF3B1 were observed in mice, indicating a branching multi-clonal as well as ancestral evolutionary paradigm. We observed that subclonal evolution in mice was also mirrored in the clinical evolution in patients. Sequential bone marrow samplings from a patient in which MDS transformed into AML, unveiled the presence of a new mutation occurring in a myeloid progenitor level. This case underlies the switch from an HSC disease to a Progenitor disease.

T-1093

DETAILED GENE EXPRESSION KINETICS OF HEMATOPOIETIC STEM CELLS RE-ENTERING CELL CYCLE FROM G0 PHASE

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Hematopoietic stem cells (HSCs) are known to be in G0 phase of the cell-cycle in the most of time but re-enter the cell-cycle at intervals to give rise to stem cells themselves and/or downstream progenitor cells. The precise sequence of gene expression activites governing G0 and re-entery has not been elucidated yet. We investigated expression activity of entire genes in HSCs by Gene Expression Commons (https://gexc.stanford.edu) which enables objective gene expression profiling based on global-scale meta-analysis of large-scale data, rather than relative profiling of only selected differentiatly expressed genes. Purified mouse HSCs were stimulated in vitro with cytokines (SCF, TPO, Flt3l, IL-3, Epo and GM-CSF) in 10% serum. Under clonal observation, HSCs started thier first division at 24h after stimulation and completed this division until 48h. To focus on G0-to-G1 transition, we performed microarray analysis on HSCs in culture at times 0, 3, 6, 12 and 24h. At 0h, cell-cycle regulators were in a similar state as those in G1 phase, except for cyclin D. Cdk4 and Cdk6 were active, suppressor Cdkn1b(p27) was inactive, however cyclin D was not active. And interestingly Cdkn1c(p57) was highly active. From 0h to 3h, 1090 genes were activated and 2276 genes were inactivated. At 3h, Cdkn1c expression significantly decreased and Cyclin D expression increased, then at G1-to-S transition, suppressor Cdkn1a(p21) was significantly activated. Similarly, Gadd45a, b and c were significantly activated at 3h. Cdk4(G1) and Cdk2(G1-to-S) were once inactivated at 3h. Cdkn1a then decreased at 6h, and Cdk2(G1-to-S) was re-activated, while cyclin E is still inactive. In parallel with cell division kinetics, S-to-G2 regulators, cyclin A and Cdk1, and M-phase related cyclin B became active at 24h. There is controversy over whether G0 phase is an extended G1 phase or a distinct quiescent stage that occurs outside of the cell cycle. These results indicate that HSCs in bone marrow are in a phase distinct from G1 and not quiescent but actively expressing many genes. Upon stimulation, HSCs immediately exit G0 state and positively extend G1 phase by activating G1-to-S transition supplessor Cdkn1a(p21), not by Cdkn1b(p27).

T-1094

HEMATOPOIETIC STEM CELLS DEVELOP IN THE ABSENCE OF ENDOTHELIAL CADHERIN 5

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Rare endothelial cells in the aorta-gonad-mesonephros (AGM) transitions to become hematopoietic stem cells (HSCs) during embryonic development. Lineage tracing experiments have indicated that HSCs emerge from Cdh5 (VE-cadherin)+ precursors, and isolated populations of Cdh5+ CD45- cells from murine embryos and mouse ES cells can be differentiated into hematopoietic cells. Cdh5 is also implicated in hemogenic specification of endothelial cells as well as development of HSCs from hemogenic endothelial cells. However, the factors regulating this process and the hemogenic potential of tissues lacking Cdh5 expression remain largely unknown. To investigate this we used zebrafish genetic screens and cloned cdh5 from malbec (mlbbw306), a zebrafish mutant with circulation defects. Here we show that HSCs emerge from hemogenic endothelial cells devoid of Cdh5 expression and subsequently differentiate in adult hematopoietic lineages. mlb has normal primitive and definitive blood formation. We utilized confocal time-lapse imaging, parabiotic surgical pairing of zebrafish embryos and blastula transplantation assays to demonstrate that HSCs continuously emerge, migrate, engraft and differentiate in the absence of cdh5 expression. We also demonstrate that Cdh5 is dispensable for mammalian adult hematopoiesis. We developed Cdh5-/-GFP+ : Cdh5+/+GFP- chimeric mouse embryos and demonstrate that the Cdh5-/- GFP+HSCs emerging from e10.5 as well as e11.5 AGM or from e13.5 fetal liver not only differentiate into hematopoietic colonies but also they engraft and reconstitute multi-lineage adult hematopoiesis. Our demonstration that functional HSCs develop from Cdh5 deleted aortic hemogenic endothelial cells during zebrafish and mouse fetal development runs counter to the widely-held presumption that Cdh5, a documented marker of emerging HSCs in the AGM, is itself essential for the transition of hemogenic endothelium to HSCs. Our analysis illustrates the power of combining model organisms to investigate condundrums in developmental biology, and provides a refined view of molecular features of hemogenic endothelium.

T-1095

DEVELOPMENT OF A FUNCTIONAL HUMANIZED IMMUNE SYSTEM IN MICE BY TRANSPLANTATION OF HUMAN HEMATOPOETIC STEM CELLS - A MODEL FOR IMMUNO-ONCOLOGY RESEARCH

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The preclinical evaluation of novel immune checkpoint modulators is dependent on models with functional human immune cells (HIC). In previous experiments, we have demonstrated, that hematopoietic stem cells (HSC) can proliferate and differentiate in vivo to form a functional humanized immune system (HIS). However it has to be evaluated whether parallel xenotransplantation of patient-derived human tumors (f.e.melanoma, PDX) in these mice would allow to generate a panel of personalized models for immuno-oncology research. We designed a study to investigate the differentiation and function of HSC in immunodeficient mice in the presence of co-transplanted PDX. HSC, isolated from cord blood were transplanted intravenously into 3 week (w) old irradiated nod scid gamma mice. To evaluate lineage-specific differentiation of HIC, mice were treated with different cytokines after HSC transplantation (f.e.IL-2). After 4 and 8w, blood was collected and screened by FACS for HIC (huCD45+). Finally mice were sacrified after 12w and organs were analysed concerning lineage-specific differentiation of engrafted HIC. PDX were subcutaneously co-transplanted into these humanized mice and followed for growth and tolerance by the HIC. Functionality of the HIC was evaluated by treatment with the CTLA-4 checkpoint inhibitor ipilimumab. After 8w up to 50% of leucocytes in the blood were CD45+. A high percentage of B-cells was measured (up to 85%). The administration of cytokines did not significant induced lineage-specific differentiation of HSC. After 12w up to 20% of the HIC in the blood were T-cells. Engraftment in different organs has been detected, with up to 15% CD45+ cells in spleen and thymus and 50% in the bone marrow. PDX showed engraftment on the humanized mice. No difference was observed compared to the growth on immunodeficient mice demonstrating no innate immune response against the tumors. Treatment with ipilimumab led to a slight tumor growth delay and an increased percentage of T-cells in the blood and in the tumor. Transplantation of HSC into immunodeficient mice generates a HIS. PDX can be successfully transplanted into these humanized mice. Initially results revealed the function of our model as a tool to investigate the human hematopoiesis and the efficacy of immunotherapeutics.

T-1096

MESENCHYMAL STROMAL CELLS ENHANCE G-CSF-INDUCED MOBILIZATION OF HEMATOPOIETIC STEM- AND PROGENITOR CELLS

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Hematopoietic stem- and progenitor cell (HSPC) mobilization is a property of most hematopoietic growth factors, such as Granulocyte Colony Stimulating Factor (G-CSF). Mesenchymal stromal cells (MSC) are a cellular component of the hematopoietic stem cell niche and produce cytokines that are involved in HSPC mobilization. Therefore, we aimed to study the effect of MSC co-administration on G-CSF-induced HSPC mobilization. MSC were obtained from bone fragments and were expanded in alpha-MEM containing 10% fetal calf serum. MSC were administered intravenously for three days at a dose of 200 x10E3 cells per day to syngeneic C57BL/6 recipients that were simultaneously mobilized with G-CSF or PBS as a control. Co-injection of G-CSF and MSC lead to a 2-fold increase in HSPC mobilization compared to G-CSF alone (8,013 ± 3,521 vs 4,052 ± 1,759 CFU-C per ml peripheral blood respectively; n=20, p<0.01), while administration of MSC alone did not induce mobilization. Moreover, peripheral blood obtained after co-injection of MSC and G-CSF contained more HSC with long-term repopulating capacity following transplantation into lethally irradiated recipients, in comparison with G-CSF administration alone. Administration of MSC alone induced a significant decrease in the frequency of osteal macrophages (11.5 ± 2.8 vs 9.2 ± 1.9% in CD11b+ bone marrow cells for PBS vs MSC respectively; n=11, p<0.05) and osteoclasts (11,703 ± 2,379 vs. 4,308 ± 1,449 osteoclasts per femur; n=3, p=0.01). Furthermore, MSC

administration induced a downregulation of niche factors including CXCL12, SCF and VCAM in bone-lining cells. Following intravenous injection, MSC were detected in the lungs, but not in other organs. Moreover, intraperitoneal administration of G-CSF and MSC resulted in increased HSPC mobilization compared to G-CSF alone (10,178 ±3,039 vs 5,158 ± 2,436 CFU-C per ml peripheral blood; n=5-12). Together, these data point to an endocrine effect of MSC on G-CSF-induced HSPC mobilization. In conclusion, G-CSF-induced HSPC mobilization is enhanced by injection of MSC and coincides with downregulation of niche-associated factors. We hypothesize that the MSC-induced partial depletion of osteal macrophages and osteoclasts in the bone marrow are crucial factors involved in the enhancement of G-CSF-induced HSPC mobilization.

T-1097

NICOTINAMIDE RIBOSIDE IS A POTENT STIMULATOR OF HEMATOPOIETIC PROGENITOR COMPARTMENTS

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Hematopoietic stem cells (HSCs) constitute the most extensively used adult stem cells treatment. Nevertheless, standard allogeneic HSC therapy for hematological malignancies is still a very risky procedure with early mortality close to 40%, mostly due to infectious complications secondary to the lag of severe neutropenia subsequent to bone marrow ablation. The development of strategies to stimulate blood recovery during the immediate post transplant period is crucial to avoid severe infections and decrease morbid-mortality associated with HSC transplantation. Here we show that treatment of mice with the natural vitamin and NAD+ precursor nicotinamide riboside (NR), used as a nutritional supplement, dramatically improves survival and blood recovery after transplantation. The effect of NR is exerted through modulation of mitochondrial activity in the most primitive hematopoietic compartments, resulting in an expansion of hematopoietic progenitor cells while preventing HSC exhaustion. Our work demonstrates that NR, and possibly other NAD+ precursors, could be used as safe drug to improve outcome and to reduce hospitalization period of post transplanted patients.

T-1098

EXPLORING THE EMERGENCE OF FIRST BLOOD CELLS BY USING SINGLE CELL GENE EXPRESSION ANALYSIS

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During the vertebrate embryogenesis, the first blood cells are generated through a key developmental event called endothelial to hematopoietic transition (EHT). Inside the blood vessel wall, only a small subset of endothelial cells, called hemogenic endothelial (HE) cells, undergo the EHT. During the transition, those HE cells start to express hematopoietic markers and become pre-hematopoietic stem or progenitor (Pre-HSP) cells. Next, Pre-HSP cells reduce the expression of endothelial markers, detach from the endothelial layer, gain hematopoietic phenotype and eventually become either progenitor cell (HPC) with limited lineage potential or hematopoietic stem cell (HSC). Current knowledge on identities of HE and Pre-HSP cells allow us to isolate only enriched populations, which constitute functionally heterogeneous cells. It triggers the questions about the source of heterogeneity: What makes an endothelial cell hemogenic and why some EHT events yield HSC and others HPC? Until now these questions could not be addressed in cellular level due to the scarcity of HE and Pre-HSP cells. Here, we used single cell qRT-PCR technique to identify the gene expression signature of rare cells. We focused on the differences between EHT events in two tissues. The yolk sac (YS) is the tissue generating first hematopoietic cells and the aorta-gonad-mesonephros (AGM) is the tissue generating first HSCs. We investigated the expression of 96 genes of each cell derived from YS and AGM of mouse embryos at E9.0, E10.5 and E11.0. According to their gene expression profile we identified the sub-populations of Pre-HSP cells and the change of their frequency from E9.0 to E11.0 fits the expectation. We characterized different endothelial populations for AGM and YS, which might be related with their hematopoietic program differences. Our results indicate that the pre-HSP in AGM and YS are transcriptionally alike suggesting that the different hematopoietic program between AGM and YS could be due to the microenvironment. Surprisingly, we identified a small population with both endothelial and mature erythroid profile, which was not documented before. All together, our study on EHT with single cell qRT-PCR technique revealed a detailed roadmap of a highly dynamic developmental event and uncover a very rare endothelial-erythroid population.

T-1099

CD97 EXPRESSION IN ACUTE MYELOID LEUKEMIA IS ASSOCIATED WITH FLT3-ITD MUTATION AND MODULATES THE BONE MARROW STROMAL MICROENVIRONMENT

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Bone marrow niches are specialized microenvironments that facilitate homing and survival of normal hematopoietic stem and progenitor cells (HSPCs) as well as leukemic stem cells (LCSs). Targeting the niche is a new strategy to eliminate persistent and drug-resistant LSCs. However, the interactions and influence of acute myeloid leukemia (AML) cells with the microenvironment remain largely unexplored. We recently demonstrated that expression of the adhesion GPCR CD97 is elevated in blasts of FLT3-ITD positive AML patients. Therefore, we aim to investigate how CD97 expression influences normal and malignant stem cell characteristics and its impact on mesenchymal stromal cells (MSCs) as a main cellular component of the bone marrow niche. Real-time PCR and FACS analysis of AML cell lines with or without FLT3-ITD or NPM1 Mutation revealed 20- and 10-fold higher CD97 expression levels in cells carrying this mutation, as MV4-11 and OCI-AML3, compared to normal CD34+ HSPCs or EOL-1 cells, respectively. CD97 knock-down in MV4-11 cells was achieved by lentiviral transduction of plko1.6/shRNACD97 and had no effect on the viability and proliferation of the cells but resulted in significantly inhibited spontaneous trans-well migration to 25% as well as

decreased adhesion to a MSC layer. Treatment of MV4-11 cells with CT04 suppressed migration of both control and CD97 knock-down cells to the same baseline level, suggesting that the majority of CD97-dependent migration is RhoA-dependent. Moreover, the expression of FLT3 correlated with CD97 in the transduced cells confirming an association of the molecules. To test the impact of leukemic cells and their CD97 expression on the MSC phenotype, FACS analysis was performed after 3 days of MSC incubation with tumor cell conditioned medium. Interestingly, CD90 and CD146 expression levels were increased by about 50% by MV4-11 wildtype cell medium but declined to the basic level after incubation with conditioned medium of CD97 knock-down cells. In contrast, the expression levels of CD73 were increased by MV4-11 medium and even further elevated by CD97 knock-down cell medium. In summary, our data suggest a modulation of the bone marrow microenvironment by leukemic cells expressing CD97 and mutated FLT3. Therefore, CD97 may serve as a promising new diagnostic and therapeutic target.

T-1100

CONNEXIN 32 MAINTAINS STEMNESS OF HEMATOPOIESIS: MAINTAINING CELL CYCLING RATIO AND RECONSTITUTION CAPABILITY

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The role of connexin (Cx) 32 in steady-state hematopoiesis and leukemogenesis has been reported. Although young Cx32-knockout (KO) mice showed a larger number of primitive hematopoietic stem/progenitor cells (HSCs/HPCs) than wild-type (WT) mice, such a numerical difference decreased with age. In addition, cells in the lineage-negative, c-kit-positive, and Sca1-positive (LKS) fraction isolated from one-year-old Cx32-KO mice completely lost their capability to reconstitute the bone marrow (BM) in secondary recipients, as determined by serial transplantation assay. In this study, using young Cx32-KO mice, the functions of Cx32 in HSCs/HPCs in relation to the cell cycle in the steady state and the capability of these cells to reconstitute the BM, if it was starting to lose it, were evaluated. First, for cell cycle analysis, the bromodeoxyuridine (BrdUrd) incorporation ratio of cells in the LKS fraction from mice treated with BrdUrd added to drinking water was measured. As a result, a significantly higher ratio was observed in the Cx32-KO group than in the WT group after one month of treatment (Cx32-KO, 72.9 ± 0.40%: WT, 46.5 ± 0.88%, p=0.004). After three months of treatment, the ratio became the same between the two groups. Next, for serial transplantation assay, 500 cells in the LKS fraction isolated from Ly5.2 mice, WT, or Cx32-KO were transplanted into lethally irradiated Ly5.1 primary recipients. Although the Cx32-KO donor cells successfully reconstituted the primary recipients' BM at least after 2 months, interestingly, their capability to reconstitute the secondary recipients' BM was limited (ratio in the recipients, over 2.5% donor cells in the LKS fraction, 4 months after reconstitution; WT, 80%: Cx32-KO, 33%). In addition, in the primary recipients 8 months after transplantation, solely number of white blood cells of donor origin in Cx32-KO group showed significantly decreased (WT, 9,182 ± 4,537/microL: Cx32KO, 4,812 ± 1,651/microL, p=0.011). The above-mentioned findings in the present study, in addition to the previous findings, imply that Cx32 plays an essential role in maintaining self-renewal proliferation of primitive HSCs to prevent their exhaustion and also in simultaneously suppressing neoplastic alterations.

T-1101

CTS™ IMMUNE CELL SR FOR SERUM FREE CULTURE AND EXPANSION OF HUMAN T CELLS

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The manufacture of a majority of clinical T cell products for immunotherapy applications requires in vitro T cell culture and expansion. Commercialization of T cell manufacturing processes requires reagents that meet regulatory guidelines and ultimately help reduce manufacturing cost of goods. A key component in many T cell culture protocols is human serum, which is expensive and requires extensive testing prior to use for the manufacture of cGMP-compliant T cell therapies. To this end, we have developed a xeno-free serum replacement, CTS™ Immune Cell SR, with defined components that can be used in combination with multiple cell culture media to support in vitro expansion of functionally intact T cells. T cells activated and expanded with Dynabeads® CD3/CD28 CTSTM and cultured in CTS™ OpTmizer™ T cell Expansion SFM, X-VivoTM 15, or CTS™ AIM-V® supplemented with pooled human serum or serum free CTS™ Immune Cell SR showed similar growth kinetics, total fold expansion and transduction efficiency after 2 weeks in culture. Numbers of CD4+ and CD8+ T cells were comparable in cultures expanded with media containing human serum or CTS™ Immune Cell SR. T cells demonstrated efficacy when infused in an in vivo leukaemia mouse model. T cell engraftment and leukaemia control were similar between mice treated with T cells grown in media containing human serum or CTS™ Immune Cell SR. These studies demonstrate that human serum may be replaced by a xeno-free formulation in combination with several commonly used T cell culture media to support in vitro expansion and lentiviral transduction of polyclonal T cells. Culturing T cells in CTS™ Immune Cell SR facilitates a favourable culture profile and immune function. Serum free CTS™ Immune Cell SR contains only fully tested human-derived or human recombinant proteins which facilitates supply security for large-scale production of clinical and commercial therapies.

CARDIAC CELLS

T-1102

EXPANSION AND DEVELOPMENTAL PATTERNING OF PSC-DERIVED CARDIOVASCULAR PROGENITORS

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The inability of multipotent cardiovascular progenitor cells (CPCs) to undergo multiple divisions in culture has precluded stable expansion of precursors of (human) cardiomyocytes and vascular cells. This contrasts with neural progenitors, which can be expanded robustly and are a renewable source of their derivatives. Here, we used human pluripotent stem cells (hPSCs), in which CPCs are genetically marked, to show that regulated MYC expression enables their robust expansion with IGF-1 and a hedgehog pathway agonist. These CPCs can be patterned with morphogens, recreating features of heart field assignment, and controllably differentiated to relatively pure populations of pacemaker-like or ventricular-like cardiomyocytes with distinctive physiological characteristics. The cells are clonogenic and can be expanded over 40 population doublings yet still be directed to differentiate to cardiomyocytes as well as vascular cells. Together, this tractable model reveals the potential of hPSC-derived CPCs for precisely recreating elements of heart development in vitro.

T-1103

LAMININ DIRECTED GENERATION OF CARDIAC PROGENITORS FROM CARDIAC MESENCHYMAL STEM CELLS IMPROVE MYOCARDIAL FUNCTION

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The mammalian heart is derived from cardiac progenitors from the first and second heart fields, which in turn are defined by their expression of the transcription factors Tbx5, Nkx2.5 and Islet-1(Isl1) respectively. Furthermore, pro-epicardial progenitors expressing Tbx18 are also important for the heart development. We demonstrate that mesenchymal stem cells from rat and human embryonic hearts seem to represent quiescent intermediate stem cells that upon stimulation can be committed into multi-potent cardiac progenitor cells, expressing Isl1, Tbx5, Nkx2.5 and Tbx18, by using biologically relevant laminin (LN) molecules in combination with canonical Wnt/β-catenin stimulation. Under these culturing conditions, the multi-potent progenitor cells were spontaneously enriched and expanded rapidly without changing their phenotype. These cells could be directed to differentiate into spontaneously beating cardiomyocytes, smooth muscle cells and endothelial cells. When injected into a normal rat heart, the LN-derived cardiac progenitor cells migrated to the outflow tract where Isl1+ cells normally reside in the embryonic heart. Following myocardial infarction, the cardiac progenitors migrated into the ischemic region, where they inhibited the remodeling process and improved the myocardial function. These characteristics of cardiac MSCs may be of importance for the development of new treatment strategies for heart disease.

T-1104

HIGH-CONTENT ELECTROPHYSIOLOGICAL ANALYSIS OF HUMAN PLURIPOTENT STEM CELL-DERIVED VENTRICULAR CARDIOMYOCYTES

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Human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cell (iPSCs) have been considered as a potential unlimited cell source for replacement therapies and other applications for their ability to self-renew and differentiate into all lineages. Given that cardiotoxicity is a common leading cause for drug withdrawal, hPSC-derived cardiomyocytes (CMs) have been more recently exploited for drug discovery and toxicity screening platforms, serving as an alternative to non-cardiac aneuploidy cell lines such as the Chinese hamster ovary and human embryonic kidney cells heterologously modified to express single cardiac ion channel types (e.g. HERG) that are the current pharmaceutical standards. While conventional manual patch-clamp assays for studying electrical properties are widely accepted gold standards, the techniques are both time- and labour-intensive. Here we adapted hESC-derived ventricular (V) CMs differentiated using the highly efficient specification protocol that we recently reported for high-content robotic electrophysiological recordings. An automated planar patch-clamp system was employed for action potential (AP) and cardiac ion channels (I_{Na}, $I_{Ca,L}$, I_{kr}, I_{ks}, I_f, I_{to}, I_{K1}) profiling of hESC-VCMs from the HES2 and H7 lines. Ventricular-like APs were detected with the current-clamp mode and their AP parameters were analyzed. The functional expression of ion channels and their biophysical and pharmacological properties were examined and compared between cell lines. hESC-VCMs from both cell lines functionally expressed I_{Na}, $I_{Ca,L}$, I_{kr}, I_{ks}, I_f, *but not* I_{to}, I_{K1}. Our results showed that the two lines basically displayed largely identical electrophysiological traits although some quantitative differences existed. The effect of Flecainide, a class Ic antiarrhythmic agent, on the I_{Na} of the hESC-VCMs was also investigated as an example to illustrate the feasibility of this combined use of the high-content electrophysiology with the hESC-VCMs on drug-screening. Further development of such high-content assay may benefit the assessment of proarrhythmogenic/antiarrhythmogenic risks of candidate drugs, and also serve as a diagnostic tool when applied on patient-specific iPSC-derived CMs from channelopathy patients.

T-1105

NEUREGULIN-1 INFLUENCES DIFFERENTIATION EFFICIENCY AND SPECIFICITY OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO CARDIOMYOCYTES

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Due to cardiomyocytes' limited capability to proliferate, the degeneration of cardiac function due to cardiomyocyte death after myocardial infarction is difficult to halt and current clinical treatment does not reverse loss of heart function. Human induced pluripotent stem cells (hiPSCs) are a unique resource to engineer

cardiac tissue for heart regeneration, and the directed differentiation process can be manipulated in order to influence cardiomyocyte pheynotype. Neuregulin-1 (NRG), a signaling molecule prevalent in the heart, is necessary for cardiac muscular and conduction system development and is thus a prime candidate for such manipulation. It was observed that tissues engineered from human embryonic stem cell (hESC)-derived cardiomyocytes (hESC-CMs), when treated with NRG, metabolized media more quickly versus untreated controls, motivating an in depth study of the as of yet unreported effects of the timing of NRG treatment on differentiation. HESC-CMs treated with NRG for 5 days had a significant increase in mitochondrial content over control ($P<0.05$). HiPSCs were then differentiated in chemically defined conditions using Wnt signaling modulation and treated with NRG for 96-hour periods starting day 0, 1, 3, 5, 7, or 9 of the two-week differentiation process. FACS analysis revealed that NRG-treated cells showed a more immature phenotype with a trend towards a larger percentage of smooth muscle actin and cardiac troponin T double-positive cardiomyocytes, with a peak 28% increase with NRG at day 0-14 over control ($P=0.06$). HiPSCs treated at all time points presented an overall decrease in differentiation efficiency, indicating multiple, not only cardiogenic, downstream effects of NRG. Analysis of mRNA expression levels with qPCR revealed >40-fold increase in connexin 43 in cardiomyocytes treated during the second week of differentiation over control. This suggests the capability of NRG treatment, and in particular timing during differentiation, to influence cardiomyocyte subtype. Ongoing experiments assess these subtypes and examine markers of conduction system, pacemaker, and metabolic activity. Utilizing knowledge of NRG signaling involved in cardiac development in vivo, differentiation of hiPSCs can be manipulated to produce cardiomyocytes of a desired phenotype.

T-1106

CLONAL ANALYSIS OF CARDIAC CELL GENERATION DURING MURINE HEART DEVELOPMENT AND INJURY

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The mammalian heart possesses endogenous regenerative capacity that significantly decreases with age and it is not sufficient to replenish the lost myocardium following injury in the adult. Both resident cardiac stem/progenitor cells and mature cardiomyocytes have been proposed to contribute to cardiac tissue generation. Understanding the molecular and cellular mechanisms governing cardiac tissue generation is imperative towards the development of novel therapeutic strategies for cardiac regeneration. During embryonic and fetal development, cardiac growth occurs primarily through progenitor cells that can give rise to all four cardiac cell types and to a lesser extent through cardiomyocyte proliferation. We performed clonal analysis using a multicolor reporter system (Rainbow) that allows labeling of single cells with one of three fluorescent proteins and retrospective analysis of their progeny. Rainbow mice were crossed to βactinCreER, αMHCCreER, Nkx2.5CreER and Rosa26CreER mice. Tamoxifen was administered at E9.5 and analysis was performed at P1, P7, P15 and P30. We observed significant clonal expansion in βactinCreER;Rainbow hearts while αMHCCreER;Rainbow hearts exhibited clones of decreased size. Although αMHC-expressing cells maintain a limited proliferative capacity in the early stages of heart development, this ability is significantly reduced after E12.5. Interestingly, cells marked by NKX2.5 exhibited considerable clonal expansion at E9.5. We further identified clones consisting of four cardiac cell types suggesting the existence of multipotent cardiac progenitors (at E7.5) able to give rise to cardiomyocytes, fibroblasts, endothelial and smooth muscle cells. Finally, we demonstrated that αMHC positive cardiomyocyte proliferation is reactivated following myocardial injury soon after birth. Our data support that clonal dominance of progenitor cells promotes cardiac development, while cardiomyocyte proliferation contributes to a lesser extent early in development and postnataly.

T-1107

IN VIVO POTENTIAL OF HUMAN PLURIPOTENT STEM CELL-DERIVED EPICARDIUM

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The epicardium forms the outer layer of the wall of the heart. Recently it has been identified as a source of progenitors during heart development and in particular for the formation of the coronary vasculature. In humans its developmental potential is turned off during adult life. The embryonic epicardium is then a very interesting system to study per se for developmental biology but also an attractive tissue for the field of regenerative medicine. We have set up a chemically-defined protocol to produce epicardium in culture from human pluripotent stem cells (HPSCs). We showed that in vitro generated epicardium faithfully expressed a range of well-known molecular markers (such as WT1, TCF21, BNC1 and TBX18) as observed during epicardial development in vivo. In vitro derived epicardial cells exhibited morphological features and gene expression levels comparable to human fetal epicardial explants. We addressed the lineage commitment and functionality of our cells in vivo by injecting 500 to 1000 genetically tagged HPSC-derived epicardial cells into the circulation of Hamburger Hamilton (HH) stage 24 (day 4) chicken embryos and harvesting the hearts at HH stage 34 (day 8). Amongst the 39 injected embryos, 20 survived and 13 exhibited tagged cells in the heart. The engrafted cells (on average 50 per heart) were mostly found in the epicardium at the apex of the heart, in the sub-epicardial space or associated with the coronary vasculature. Interestingly, a small proportion expressed a smooth muscle phenotype as revealed by an anti α-smooth muscle actin labelling. HPSC-derived neural crest cells were also injected as a negative control and none of them were found in the epicardium, sub-epicardium or coronary vasculature. Studies are now underway using this system to further understand the developmental potential of in vitro generated epicardium. We are setting up methods to address the heterogeneity of this tissue in term of molecular signature and lineage potential. We are also using the HPSC-derived epicardium as a progenitor tissue for regenerative medicine and as a platform to identify and test drugs that could reactivate embryonic molecular pathways in the adult epicardium.

T-1108

SECRETED FRIZZLED RELATED PROTEIN 2 (SFRP2) PROMOTES THE DIFFERENTIATION OF MOUSE C-KIT+ CARDIAC PROGENITOR CELLS TO CARDIOMYOCYTES IN VIVO.

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Resident c-Kit+ cardiac progenitor cells (CPCs) in the mammalian heart are believed to differentiate into cardiomyocytes, smooth muscle cells, and endothelial cells. However these resident c-Kit+ CPCs rarely differentiate into cardiomyocytes in vivo. Using lineage tracing techniques we found that the Wnt signaling modulator Sfrp2 when delivered into damaged myocardium substantially increases the differentiation of c-Kit+ CPCs into cardiomyocytes. c-Kit-CreERT2/mTeG mice were used where the c-Kit promoter drives the expression of a tamoxifen-inducible Cre. Upon tamoxifen treatment Cre-mediated recombination at the LoxP sites allows expression of eGFP exclusively in c-Kit+ cells. We tested the validity of the c-Kit-CreERT2/mTeG model. Expression of the marker eGFP had no effect on cultured c-Kit+ CPC differentiation, proliferation or apoptosis. In vivo, recombination was found to be robust. In cells from tamoxifen treated mice, >90% of c-Kit+ cells were eGFP+ while no eGFP+ c-Kit+ cells were seen in hearts of vehicle treated mice. In the bone marrow, recombination was similarly efficient. Hematopoietic cells in the bone marrow derive from c-Kit+ precursors. Tamoxifen treatment did not inhibit c-Kit cell differentiation in vivo. Importantly, c-Kit negative cells in the heart, such as cardiomyocytes, endothelial cells, and smooth muscle cells, did not express eGFP following tamoxifen treatment. Adult mice were subjected to permanent ligation of the left anterior descending coronary artery. Two days later, mice were injected 0.5 µg of recombinant Sfrp2 or saline at the infarct border zone. Heart tissue was analyzed two months later for the expression of eGFP and cardiac markers by microscropy. In sham animals, eGFP was localized to small c-Kit+ cells. In the control MI group there were only a limited number of eGFP+ cardiomyocytes (<1% in the infarct border zone). In contrast, the number of eGFP+ cardiomyocytes was found to be significantly higher in the Sfrp2 treated group; approximately 15% of the cardiomyocytes in the infarct border zone were expressing eGFP. Co-localization of GFP with blood vessels or smooth muscle cells was rare. Using a c-Kit lineage-tracing mouse we have identified that Sfrp2 increases the rate of CPC differentiation into cardiomyocytes in vivo.

T-1109

CONDITIONS FOR HIGH QUALITY CARDIAC FIBROBLAST CO-CULTURE WITH CARDIOMYOCYTES

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Next to cardiomyocytes (CMs), the heart consists of more than 50% cardiac fibroblasts (CFs). More than providing a mechanical scaffold that keeps the heart intact, CFs secrete signalling molecules, growth factors and cytokines that affect CM function. To find a suitable platform to study the interaction between CMs and CFs, we tested different media compositions and determined cell quality. Human pluripotent stem cell (hPSC)-derived CMs and commercially available CFs were cultured alone or together (co-culture) using commercially available CF medium, RMPI with FBS, RPMI with B-27 serum free supplement (RPMI/B-27), and RPMI/B-27 with different concentration bFGF (0.1-10 ng/ml) for 72 hours. CF quality was determined by quantifying the number of cells expressing alpha-smooth muscle actin (a-SMA) using immunocytochemistry. The worst condition that caused 15±6% of CFs to express a-SMA was RPMI with 10% FBS. The best condition with <1% a-SMA positive cells was RPMI/B-27 with 1ng/ml bFGF. 1 ng/ml bFGF did not affect the transcription of core cardiac genes in CMs (incl. MYH6, RYR2 and KCNJ2). bFGF is necessary for CFs to maintain their physiological phenotype during in vitro culture. RPMI with B-27 serum free supplement and 1 ng/ml bFGF is a suitable medium for co-culturing CFs with hPSC-CM at a high quality.

T-1110

CREATION OF CARDIAC TISSUE-LIKE CONSTRUCTS FROM INDUCED PLURIPOTENT STEM CELLS

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Myocardium is composed of rod-shaped cardiomyocytes which are aligned in form of quasi-parallel and dense myofibers with coordinated gap junctions. Any damage of this particular architecture after myocardial infarction will disturb the electric and mechanic activities of the heart, causing problems such as arrhythmia. Human pluripotent stem cells (hPSCs) are attractive as reliable cell resources for injured cardiac repairing since they can be produced in large quantity and be differentiated to functional cardiomyocytes. Previously, we have shown that by using a small molecule based method, hPSCs could be differentiated into clinical grade cardiomyocytes with efficiency up to 98%. These hPSCs derived cardiac cells might be safe and reliable for therapy number of applications. To create cardiac tissue-like constructs for in-vitro evaluation, drug assessment and future applications in heart therapy, we used a patch-form scaffold made of aligned and biodegradable nanofibers for cardiac cell culture. As expected, we obtained high quality cardiac tissue-like constructs (CTLC) which bear morphological and electrophysiological resemblance to native myocardium and show anisotropic 3D-characterestics and spatiotemporally coordinated beating. When used for drug assessment, the CTLC could resist drugs of higher dosages comparing to the cell sheets cultured on a flat surface. These CTLCs could also be used for evaluation of electrical coupling between two tissue constructs. Both electrical signal and the optic observation confirmed that the hPSCs-derived CTLC could be rapidly coupled. As perspective, the hPSCs-derived CTLC hold high potential to improve the function of the infarcted heart and prevent the occurring of the arrhythmia activities. In addition, the easy-to-prepare and easy-to-handle feature of this type of CTLC might also greatly facilitate its clinical implementation.

T-1111

CONTROLLING MATURATION AND FUNCTION OF CARDIOMYOCYTES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS USING ALIGNED NANOFIBERS

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Human pluripotent stem cells (hPSC), including both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), hold high potential for large variety of applications due to their unique capability of infinite self-renewal and controlled differentiation to a number of types of target cells. One of the examples is to differentiate hPSCs to cardiac cells for therapy and drug-screening. Recently, we have successfully developed a small molecular based differentiation method, which can effectively promote cardiac differentiation of hPSCs up to 98% under defined, cytokine- and xeno-free conditions. This grade of cardiomyocytes (CMs) should be suitable for transplant. However, the most of the hPSC-derived CMs are still in their immature and low functional state. It is therefore important to develop effective methodologies to improve the cardiac maturation and function. In this study, we aimed at cardiac tissue-like constructs (CTLC), which mimic three-dimensional and anisotropic organization of cardiac cells in vivo, by using aligned nanofibers. The material of nanofiber is biodegradable, biocompatible and approved by Food and Drug Administration (FDA). As expected, aligned fibres led to the formation of cardiac tissue-like constructs with improved expression of maturation-related cardiac genes such as β-MHC, a marker of cardiac maturation. The electrophysiological measurement also revealed that our CTLC have higher maturation and better tissue function than that obtained by usual two-dimensional culture. Although now it is still not yet clear which stage of iPSC-derived CMs are suitable for transplant, our scaffold-based CTLC is applicable for various assays. On the other hand, they should also be useful for the new drug assessment. Besides, our CTLC can be easily obtained and handled, thereby providing a new perspective for both transplant and drug screening.

T-1112

COSTIMULATION BLOCKADE INDUCES ACCEPTANCE OF FETAL ISLET1+ CARDIAC MESENCHYMAL CELLS

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Heart failure is a major source of morbidity, mortality and healthcare expenditure. Cell therapy can potentially prevent the development of heart failure or alleviate heart failure when it has manifested. By culturing the mesenchymal fraction of human fetal hearts, islet1+ cardiac progenitor cells can be expanded in large numbers. Since the cell source is from an allogeneic donor then the recipient will reject the cell transplant unless immune modulation in some form is implemented. Chronic immunosuppression carries the side effects of increased risk of cancer, renal failure and opportunistic infections. In order to avoid the use of chronic immunosuppression, inhibition of costimulatory signals at the time of transplantation can be utilized to induce immunological tolerance. Previous studies have shown that by inhibiting costimulation at the time of transplantation tolerance to allografts, xenografts and human embryonic stem cells can be induced. Immunological tolerance is partially mediated by the presence of foxp3+ regulatory T cells around or in the grafts. In order to test if costimulation blockade could prevent rejection of islet1+ cells, recipients were treated with anti-LFA-1, CTLA4Ig and anti-CD40L at the time of intra-myocardial transplantation after myocardial infarction. Islet1+ cells were suspended in medium or Matrigel™ in order to test if engraftment could be improved. Recipients were wild-type NMRI mice as well as Nod/Scid/gamma (NSG) mice which were used as control. NMRI were given costimulation blockade at the day of transplantation and then every other day for one week. NSG were untreated. We found similar results in NMRI and NSG with engrafted cells after 4 weeks in all cases. Cells suspended in medium were found to be dispersed in the myocardium and mostly as single cells. Cells suspended in Matrigel™ on the other hand were in 50% of the animals found in more concentrated grafts in close contact with surrounding normal mouse myocardium. The islet1+ cells did not express Troponin T implying that at this time the cells did not attain a cardiomyocyte phenotype.

T-1113

DEVELOPMENT OF 3D ENGINEERED CARDIAC TISSUE WITH HUMAN IPS CELL-DERIVED CARDIOMYOCYTES AND ENDOTHELIAL CELLS

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Three-dimensional (3D) bioengineered tissues have been shown to be a robust modality for in vitro cell expansion, tissue maturation, and provide an excellent microenvironment for tissue transplantation and cardiac repair. We have generated cylindrical 3D engineered cardiac tissues (ECTs) using cardiovascular cell populations efficiently differentiated from human iPS cells (hiPSCs) in preparation for scale up to large animal preclinical studies. We employed a novel monolayer culture-based differentiation protocol adapted from our recent report (*Sci Rep* 2014) that uses ActivinA/Wnt3a/BMP4/bFGF/VEGF to induce cardiovascular cell populations from hiPSCs. By differentiation day 15 (n=14) this protocol yielded a distribution of cell lineages with $cTnT^+$-cardiomyocytes (CM, 63.8±7.1% of total cells), VE-$cadherin^+$-endothelial cells (EC, 19.8±7.1%), and a small percentage of $PDGFR\beta^+$-vascular mural cells (1.0±1.2%). We collected cells on day 15 and seeded them in our standard collagen/Matrigel mixture to form 3D linear shaped constructs. We harvested spontaneously beating ECTs after 14 days of culture. ECTs remodeled with 49% gel compaction occurring in the initial 4 days after seeding (final width: 0.96±0.39mm, n=7). We noted 48.5±8.7% of total cells were $cTnT^+$-CM and 9.8±3.3% of CM showed EdU^+ nuclei indicating CM proliferation and preferential alignment of CM and non-CM to the ECT long axis. We assessed ECT functional maturation via Force-Length (FL) Relations. Active FL relationship of ECTs yielded an R^2 value of 0.994 (100-120% length, 0.50±0.12mN/mm^2 at 120% length, n=3) analogous to Frank-Starling curves in the intact heart. The selective I_{Kr} blocker, E4031 (0.1µM), decreased the maximal electrically captured beat frequency (2.2±0.6 vs. 3.3±0.3Hz, $P<0.05$) and prolonged the 90% force relaxation time (303±32.5 vs, 230±21.5msec $P<0.05$) (n=3), indicating the presence of functional I_{Kr} channels. Human iPSC-derived CM and EC can generate functional 3D ECTs with histologic and contractile properties of functional myocardium relevant for scale-up to large animal pre-clinical studies and clinical translation.

T-1114

A METHIONINE-FREE CULTURE CONDITION IS USEFUL FOR ELIMINATING REMAINING UNDIFFERENTIATED IPS CELLS IN HUMAN CARDIAC TISSUE FOR TRANSPLANT

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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 have shown that a methionine-free culture condition in the process of cardiac cell sheet fabrication is quite useful and easy method to eliminate remaining iPS cells. When human iPS cells cultured on feeder cells or matrigel were cultured in a methionine-free culture condition, they did not survive without feeder cells and could not proliferate or form colonies on feeder cells at day1, suggesting that methionine is essential for survival and growth of human iPS cells. Next we examined the effects of a methionine-free condition for human iPS cells co-cultured with human iPS cell-derived cardiomyocytes and fibroblasts. High content confocal image analysis revealed that the number of Oct3/4 positive cells was significantly decreased in a methionine-free culture condition compared with other methionine-containing conditions at day1. Moreover the number of Oct3/4 positive cells was decreased in a methionine-free culture condition with the time dependent manner until day2. When iPS cell-derived cells after the cardiac differentiation were transiently cultured in the methionine-free condition, cell sheets were fabricated by lowering culture temperature. The spontaneous and synchronous beating was observed in whole area and the cardiac cell sheets were mainly composed of cardiomyocytes and fibroblasts. Finally we examined the function of human cardiac tissue in vivo. When triple layered human cardiac cell sheets were transplanted on the subcutaneous tissue of nude rats, cardiac tissues were engrafted over 1 year, and 300μm thick and dense cardiac tissues were fabricated without teratoma formation. These findings suggest that a methionine-free culture condition in cardiac cell sheet fabrication process might be useful for diminishing the risk of tumor formation upon transplantation.

T-1115

ISOLATION AND CHARACTERIZATION OF HCN4 POSITIVE CELLS DERIVED FROM HUMAN EMBRYONIC STEM CELLS

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All of the major cell types of heart is formed from two distinct progenitor pools in the first and second heart fields (FHF and SHF). Whereas SHF progenitors is marked specifically by Isl1 (Islet1) and give rise to the right ventricle, outflow tract and parts of atria, the FHF cells remain poorly studied because of the lack of exclusive markers. HCN4 (Hyperpolarization-activated cyclic nucleotide-gated channel 4) channel is the marker for the sino-atrial node (SAN) and is correlated with the funny current (If) which is responsible for pacemaking and heart rate control. Recently, several studies have provided evidence for HCN4 as an FHF marker that have the potential to differentiate into the left ventricle and parts of the atria. Here we provide a useful in vitro system to isolate and characterize both HCN4+ cardiac progenitors and sino-atrial pacemaker cells derived from human embryonic stem cells (hESCs). First, hESCs were introduced with human HCN4-BAC (Bacterial artificial chromosome) semi-knock-in vector, which replaced the part of HCN4 exon1 in BAC vector with eGFP. In established HCN4-BAC hESCs, the expression of eGFP was first observed weakly in early cardiac spheroids along with the onset of self-contraction and detected only at beating cells. Then, the expression aria and intensity of eGFP was increasing around two month of differentiation. HCN4+ cells isolated from two month old cardiac spheroids showed not only the expression of cardiac pacemaker markers but also the automaticity with If current. Currently, we are investigating the property and developmental potential of HCN4+ cells isolating from cardiac spheroids at multiple time points, especially at early phase of differentiation. Generation and isolation of HCN4+ cells from hESCs represents an attractive strategy for use in regenerative medicine for bradycardia and drug screening as well as in understanding the heart cell lineage diversification.

T-1116

TWO DIMENSIONAL ELECTROPHYSIOLOGICAL CHARACTERIZATION OF HUMAN EMBRYONIC STEM CELLS-DERIVED CARDIOMYOCYTE SYSTEM

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The ability of human pluripotent stem cells to self-renew indefinitely and differentiate into virtually any cell types makes them a valuable source for cell-based regenerative therapy, developmental studies, disease modeling, and drug discovery/assessment. With their natural counterparts hardly propagating in vivo or ex vivo, cardiomyocytes derived from human embryonic stem cell/induced pluripotent stem cell(hESC/iPSC-CMs) provide a particularly powerful biological tool. The differentiation protocols have evolved over the years to allow for large-scale induction of human cardiomyocytes. However, compared to the progress in the efficiency of cardiac induction, maturity of hESC/iPSC-CMs still remains fetal-type. As the maturity of the cardiomyocytes is relatively less reflected in gene expression pattern, the electrophysiological properties are critical parameters to monitor. Here, we present a model system that utilizes monolayer hESC/iPSC-CMs cultured on multielectrodearray (MEA) platform, which allows us to monitor electric signals over weeks directly from the cells in contact with electrodes and to perform computational analyses of localized voltage-type signal and position-dependent properties of electrical activities.

T-1117

ATOMIC FORCE MICROSCOPY AND HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES FORMS A BIOSENSOR OF PHYSIOLOGICAL CHANGES AND ADRENERGIC MODUALTION THROUGH DYNAMIC FORCE-CONTRACTION ANALYSIS

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A differentiation of human pluripotent stem cells (PSCs) into the functional cardiomyocytes presents a powerful tool for cardiac diseases modeling when coupled with sensitive and robust detection of physiological response of cardiac cells. Although the contractions of the cell clusters resemble heart functionality and the phenotype description of stem cell-derived cardiomyocytes (SC-CMs) is crucial parameter for understanding of physiology and further modeling of the disease, the analysis of drug effects and the environmental conditions on the maturity and functional characteristics of SC-CMs usually depends on the technologically challenging techniques, such as patch clamp or Ca2+ imaging disqualifying the method from the routine use. We thus developed a novel biosensor combining the atomic force microscope (AFM) with SC-CMs differentiated in vitro from human embryonic SCs and human induced PSCs. Methods: Defined number of undifferentiated single cells in AggreWell plates formed homogeneous spheroids. The differentiation process was achieved using defined growth factors (ActivinA, BMP4, IWR2, VEGF, FGF2) at different stages to enhance mesodermal differentiation and production of cardiac progenitors. Molecular and functional characterization confirmed the cardiac identity of the resulting cells. AFM experiments were performed in Tyrode's solution; different ion concentrations and temperature effects were tested, as well as drugs modulating the beta-adrenergic receptors were applied. Dynamics of uniform-sized and homogenous spheroid embryonic and induced PSCs-CMs were well measurable using the AFM-based technique. The beat rates were comparable among measured clusters and did not show high variability when thermal stability was assured. Spheroids responded comparably upon stimulation or inhibition of the beta-adrenergic pathway. Thermal and ionic dependency curves were obtained. Mechano-biological properties of homogenous beating spheroids, containing SC-CMs can be investigated by atomic force microscopy. The method allows testing of various physiological conditions as well as novel drugs on 3D homogeneous spheroid containing beating human cardiomyocytes.

T-1118

A NEWLY IDENTIFIED CARDIOMYOCYTE- COMMITTED PROGENITOR POPULATION FOR CARDIAC REGENERATION

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Cell surface markers such as CD13 and PDGFRa for progenitor populations that can give rise to cardiomyocytes (CMs) as well as other mesoderm derivatives are reported, however, a specific cell surface marker of cardiac progenitor cells committed to the CM lineage has been longed to be identified. Therefore, we tried to identify cell surface markers of CM-committed progenitor cells using a stepwise differentiation protocol for human iPSCs to CMs (PLoS One 2011). CD13+PDGFDRa+ mesoderm population appeared from differentiation day 2 (D2) and cardiac toroponin T (cTnT)+ CMs started to be observed from D7. To isolate CM-committed progenitor cells, we screened antibodies for human cell surface molecules using cells that appeared between mesoderm and CM stages. Then, we found a cell surface molecule successfully marked a cell population with features of CM-committed progenitors (we tentatively called the molecule as CCP). Purified CCP+ cells gave rise to CMs with more than 95% efficiency in vitro. To examine the engraftment efficiency and cardiogenic potential of CCP+ cells in vivo comparing with mesoderm cells and CMs, we injected five million cells of each population into sub-renal space of SCID mice. Fourteen days after transplantation, only CCP+ cells could successfully survive and differentiate into CMs with almost 100% efficiency. We succeeded in identifying a novel CM-committed progenitor population during human iPS cell differentiation with a cell surface marker. The CCP+ progenitor cells that efficiently and selectively differentiate into CMs both in vitro and in vivo would be a promising cell source for cardiac regeneration.

T-1119

MAPPING THE CELL SURFACE SIGNATURE OF THE DEVELOPING MOUSE HEART

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Well-defined populations, as those known from the hematopoietic system, are identified by the combined expression of surface molecules allowing their prospective isolation and functional characterization. The advances in discriminating discrete progenitor subsets contributed to the progression in our understanding of the molecular mechanisms underlying stem cell maintenance and tissue regeneration. Despite the identification of several proteins expressed on the surface of cardiomyocytes (SIRPα in humans, VCAM-1 in developing mice and caveolin-3 in postnatal mice and humans), as well as on fibroblasts (Thy-1 and Ddr-2), smooth muscle (PDGFrb) and endothelial (CD31) cells, a surface signature to identify the populations that compose the developing heart is not available. We approached this problem by combining flow cytometry analysis, transcriptional profiling (single cell multiplex qRT-PCR) and in situ immunofluorescence to distinguish all the cellular subsets engaged in cardiogenesis. Our characterization of the heart constituents, throughout development, enabled the identification of previously un-reported cardiac subsets. The populations were primarily defined by the combined expression of CD24, CD54, Sca-1 and CD90. Transcriptional profiling of single sorted cells contributed to the identification of cardiomyocytes by the expression of CD24, while differential expression of CD54, Sca-1 and CD90 defined the cardiac stroma. The identified subsets exhibited specific distributions in different anatomical regions, i.e. atria, auriculo-ventricular junction and ventricles. We have thus identified a panel of surface markers, some of which novel in the cardiac context, that allows the discrimination of the main cellular components in the heart and their

prospective isolation, along development. This is the foundation for comprehensive studies on the role of different cell fractions of the heart in differing physio-pathological conditions.

T-1120

THE APPLICATION OF PERICARDIAL EFFUSION STEM CELLS (PESCS) HARVESTED FROM PEDIATRIC OPEN HEART SURGERY DRAINAGE FLUID IN A IN A MOUSE MODEL OF MYOCARDIAL INFARCTION

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Stem cell mobilization is the naturally process after the open heart surgery. Drainage of blood and tissue fluid from mediastinum and pleural space is a routine practice. In this study, we isolated the stem cells from the drainage fluid and applied these cells in a mouse model of myocardial infarction. We collected the drain fluid in the congenital heart disease patients after surgery. We completed the isolation and expansion of stem cells from open heart surgery drain fluids by using the flow cytometry. We also performed the immunocytochemistry study to stem cell markers on pericardial effusion stem cells (PESCs). Adult male SCID-beige mice aged 10 to 20 weeks were used. The left main coronary artery was ligated to mimic myocardial infarction. Expanded cells were characterized with cell surface markers and were transduced with luciferase gene as tracer after injected into ischemic heart in mice. Echocardiography assessment, bioluminescence imaging, and histological analysis were performed. We had collected the drain fluid in 37 congenital heart disease children after surgery were. The isolation and expansion of stem cells from open heart surgery drain fluids by using the flow cytometry were done. Spindle-like cells spread out on the culture dish on 6-7 days-in-vitro (DIV). Spindle-like cells became confluent with cardiospheres grew on top on 14 DIV. Immunofluorescence staining of CD117 (c-kit) also revealed positive in the cardiospheres, indicating the presence of cardiac stem cells. Echocardiograph revealed a significant improvement of ejection fraction in mice 3 weeks after PESCs injections compared to the control. Trichrome stain showed that the injured area in the PESC-injected hearts was significantly smaller compared to that of the PBS or fibroblast-injected hearts. The in vivo luciferase signals declined from 3 days to 15 days and became undetectable at 22 days post-surgery. The present study has provided a practical method for obtaining autologous stem cells. These results suggest that the therapeutic advantage of the transplanted PESCs may not be directly contributed to enhanced differentiation of PESCs in vivo, but by other alternative mechanisms.

T-1121

CTGF-D4/LRP6 SIGNALING PROMOTES ADULT EPICARDIAL CELL GRAFTS AFTER MI

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Low survival and engraftment with transplanted stem/progenitor cells is a formidable barrier to clinical application of many promising cell-based therapies. Effective tissue regeneration depends strongly on initial graft success with donor cells. Upon transplantation cells must successfully adhere, migrate and differentiate. Notably, however, cultured adult stem/progenitor cells and derivatives from ES and iPS cells typically exhibit poor survival in vivo. This is especially true for grafting to treat ischemic tissue injuries such as myocardial infarction (MI, heart attack). Here we report a novel strategy to enhance graft success with primary adult epicardial cells, a promising cell therapy candidate for cardiac repair, based on Connective Tissue Growth Factor (CTGF). CTGF is composed of four distinct domains. The 4th domain of CTGF (CTGF-D4) is known to interact with the LRP6 (Wingless [Wnt] co-receptor). To improve graft success, we "primed" adult rat epicardial cells by incubating them in a defined combination of CTGF-D4 and Insulin prior to transplanting them into rats with myocardial infarction (MI). Compared with controls that exhibited poor cell engraftment, the CTGF-D4/Insulin-primed cells successfully grafted into sub-epicardial tissue, proliferated and migrated into the myocardium over 1 week. The primed cells persisted at 1 month after transplantation and differentiated into vascular cells. Notably, antibody mediated blockade of LRP6 during priming prevented epicardial cells from grafting after MI. In further studies, we determined that CTGF-D4 signaled through LRP6 to increase the expression of vascular, smooth muscle and fibroblast differentiation markers. Of special interest, CTGF-D4/LRP6 signaling also induced Endothelin Receptor Type B and controlled epicardial cell migration into the injured myocardium. Our grafting studies identify LRP6 as a critical determinant of graft success after MI, and a ligand receptor pair (CTGF-D4/LRP6) that can be harnessed to improve graft success for the heart and perhaps other organs and tissues.

MUSCLE CELLS

T-1122

TRANSGENIC MICE WITH DEFICIENCY OF TID1 GENE SPECIFIC IN MUSCULAR TISSUE AS A CLINICAL DISEASE MODEL

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Tid1, a mitochondrial cochaperone protein, is also a mammalian homolog of the Drosophila tumor suppressor Tid56, therefore; we suspect that Tid1 may be involved in regulating cell differentiation in mammals. Mice with loss of Tid1 lead to embryonic lethality as early as E4.5 that precludes investigating Tid1's role in most cell types. To further study the physiological function of Tid1 during muscle homeostasis, we generated HSA-Tid1f/f, and MCK-Tid1f/f, mice strains where Tid1 was deleted mainly in skeletal and/or heart muscle cells, respectively. However, we observed that the HSA-Tid f/f and MCK-Tid1f/f mice became lethal at postnatal day (P) 3 to P10, respectively. To further elucidate the effect of Tid1 deletion on muscle cells development, we plan to carry on the specific aims of this project as the followings. (1) Generation of mouse mutant strains (HSA-CreER-Tid1f/f and Pax7-Tid1f/f,) with Tid1 deletion specifically in skeletal, heart muscle cells or satellite cells, respectively. (2) With the different muscular tissue specific Tid1 deletion mice (HSA-CreER-Tid1f/f and Pax7-Tid1f/f,), we can elucidate the effect

of Tid1 deletion during the different stages of muscular homeostasis. The differential expression profile of genes, such as AMPK, PGC-1 and oxidative phosphorylation complexes, involved in regulating ATP synthesis, will be studied. In addition, the differentiation ability and mitochondria biogenesis of the mutant muscular cells will be further studied after the deletion of Tid1. Last, the physiological behavior between mutant and wild type mice will be analyzed to understand whether deletion of Tid1 causes abnormal physiological behavior in mutant mice. (3) We observed that expression of Tid1 protein was upregulated in in vitro induced differentiation of C2C12 cell line. Consequently, we want to elucidate the effect of Tid1 deletion on muscle cells development in vitro. (4) With the mice strains where Tid1 is deleted conditionally in muscular tissues such as skeletal muscle cells on different stages, generated through this research, we will be able to understand whether single deletion of Tid1 can cause sarcoma spontaneously. The significance of this project will provide the more complete spectrum of the function of Tid1 during normal muscular homeostasis.

T-1123

SEEDING DECELLULARIZED MUSCLE SCAFOLDS WITH MYOBLASTS AND STEM CELLS

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Decellularization of tissues preserves well stroma as well as original complex microstructure of the extracellular matrix (ECM) incl. its natural niches. For decellularization of anterior tibial muscle (ATM) obtained from C57Bl/6 mice we used a protocol based on combination of osmotic shock with SDS extraction followed by peracetic acid to sterilize the bioscaffold. DNA was removed with DNase. After a thorough washing with PBS buffer the scaffolds were collected for chemical and microscopic characterization or for recellularization. Light microscopy of paraffin-embedded sections proved absence of cell nuclei and cytoplasmic components in decellularized ATM. Transmission electron microscopy of the scaffolds revealed well preserved general microarchitectonics including basal laminas and transversely striated collagen fibrils. Immunohistochemical analysis confirmed preservation of small pericellular proteoglycans such as decorin and adhesive glycoproteins such as laminins and fibronectin in decellularized ATM scaffolds matrix, which are so important for ECM bioactivity. The scaffolds were recellularized with murine lin-Sca-1+ bone marrow cells, ES-D3 cells, muscle-derived stem cells or C2C12 myoblasts and cultured in vitro for 3, 6, 9 and 12 days in vitro. Histological examination confirmed cytocompatibility of the scaffolds as these were successfully reseeded with cells demonstrating the ability of cells to adhere, grow and migrate through this ECM without affecting the scaffold structure. Bone marrow cells and undifferentiated ES cells colonized stromal components like the perimysium and remained to express the undifferentiated cell markers. On the contrary C2C12 cells and muscle-derived stem cells were able to migrate in the endomysium and fuse into myotubes expressing myogenic markers. Our results confirm decellularized ATM can serve as suitable scaffolds that preserve a host environment for guiding and spatially organizing myogenic cell differentiation and represent an alternative platform for rebuilding the skeletal muscle organ. This work was supported by a grant from GACR No. 15-09161S.

T-1124

TRANSCRIPTIONAL SIGNATURE OF HUMAN FETAL MESOANGIOBLASTS

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A subset of vessel associated stem cells, named mesoangioblasts (MABs), isolated from muscle biopsies of mice, dogs and humans has been recently characterized and shown to participate to the myogenic program. A first phase I clinical trial has just been completed in children with Duchenne Muscular Dystrophy (DMD). Presently, the study of human fetal MABs remain to be addressed, in particular whether MABs from different organs or tissues possess specific differentiation capacities according to the niche in which they reside. To address this, we compared the phenotype and functional characteristics of fetal human MABs in order to define their potential and appropriate therapeutical use. We sorted MABs from fetal aorta, heart and skeletal muscle and assessed their known gene expression patterns by Q-PCR. Specifically, we evaluated the expression of surface markers and analysed early and late muscle transcription factors. We specifically analyzed their ability to proliferate and differentiate in skeletal and cardiac muscle, as well as in other mesodermal cell types (such as cells of bone, fat, and cartilage). Given the found differences, we deeply investigated the different gene expression profiles by comparative transcriptome analysis using RNA-seq. Our ongoing analysis indicates that the four cell populations have subtle but distinct expression signatures that correlated with their specific functional properties. We will present potential GO biological processes and KEGG pathways that could account for their distinct differentiation potentials. In vitro differentiation studies in 2D vs 3D microtissues point toward a role of specific ECM proteins and adhesion receptors in directing cardiac or skeletal phenotypes. The isolation of MABs in xeno-free media and GMP conditions will enable their validation in transplantation tests using a mouse model of Limb-Girdle Muscular Dystrophy (alpha-sarcoglycan knock-out mouse).

T-1125

SCREENING FOR MUSCLE REGENERATIVE DRUGS USING PAX7+ MUSCLE PROGENITORS DERIVED FROM HUMAN EMBRYONIC STEM CELLS

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Muscle wasting disorders that include muscular dystrophy, cachexia, disuse atrophy, aging and many others have different etiology but all feature excessive loss of muscle mass due to the decrease in

muscle regenerative potential. Enhancing muscle regeneration by treatment with small molecules drugs that stimulate myofiber regeneration can be beneficial in treating muscle-wasting diseases regardless of their cause. Here we describe a development of high content screening platform for muscle regenerative drugs using Pax7+ myogenic progenitors derived from human embryonic stem cells (hES). Novel serum-free differentiation protocols for generation of Pax7+ myogenic progenitors from hES cells were identified using a bead-based combinatorial technology, termed Combicult®, which is capable of multiplexing large numbers of cell differentiation media to identify critical combinations that result in high efficiency differentiation to a given phenotype. Pax7+ skeletal muscle progenitors were further characterized for the expression of satellite cell-specific nuclear and cell surface markers and tested for their ability to differentiate into fusion-competent myoblasts. hES derived Pax7+ progenitor cells were further expanded using selective culture conditions and tested for their suitability for drug screening. A high content screening system based on the expression of mature skeletal muscle markers was developed and optimized. Screening of chemical libraries including FDA approved drugs revealed a number of hit compounds that promote myogenesis in vitro. Target analysis revealed a number of putative drug targets and lead compounds for further development. Identified targets were further validated using siRNA mediated knock-downs. The regenerative properties of myogenic drugs discovered by the screen will be further evaluated in the established murine model of volumetric muscle loss.

T-1126

DIRECT REPROGRAMMING OF ADULT MUSCLE SATELLITE CELLS TO PERICYTE-LIKE CELLS VIA NOTCH AND PDGF SIGNALLING

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Satellite stem cells are responsible for post-natal skeletal muscle regeneration; upon activation they proliferate as transient amplifying myoblasts, most of which fuse into regenerating myofibres. Despite the encouraging outcomes obtained with myoblasts transplantation in dystrophic animals and in patients with localised forms of muscular dystrophy, results achieved in clinical trials with more severe forms of muscle diseases, such as Duchenne muscular dystrophy, showed limited efficacy. Pericyte-derived mesoangioblasts can as well contribute to muscle regeneration and give rise to satellite cells; when injected systemically they migrate through the vascular endothelium, circumventing the limitation of local intra-muscular injections. These cells have also recently undergone clinical experimentation in a phase I/II first-in-human trial in five Duchenne patients . We hypothesised that by modulating Notch and PDFG signalling, involved in pericyte specification in the embryo, we might reprogram adult satellite cells into pericyte-like cells. Here we show that a skeletal-to-smooth muscle lineage reprogramming can be induced by exposing adult satellite cells to Dll4 and PDGF-BB. These reprogrammed cells acquire perivascular markers and functional properties, such as stabilisation of capillary networks. Importantly, preliminary data show increased engraftment of reprogrammed cells upon both intramuscular and intra-arterial delivery in dystrophic mice. Interestingly, treated satellite cells also show upregulation of Pax7, a marker normally found in quiescent satellite cells. The acquisition of an intermediate phenotype between satellite cells and pericytes may result in a more clinically-relevant stem cell population for cell therapies of muscular dystrophies. These results extend our understanding of smooth/skeletal muscle lineage choice and provide evidence of a druggable pathway that might have a clinically relevant potential, allowing myoblast systemic delivery in muscle diseases.

T-1127

MUSCLE NICHE CHARACTERISTICS MODULATE THE DIFFERENTIATION/SELF-RENEWAL BALANCE IN HUMAN SKELETAL MUSCLE PROGENITOR CELLS

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Duchenne Muscular Dystrophy (DMD), a genetic disorder caused by mutations in dystrophin, results in sarcolemmal instability and skeletal muscle deterioration. Subsequent hyperactivation of CD45+NCAM+ skeletal muscle progenitors (SMPs) to repair degeneration depletes them at an elevated rate, leading to limited muscle renewal capacity. SMPs are limited in both number and expansion capabilities, but they can temporarily retain their ability to proliferate in vitro and engraft in vivo when cultured in niche of physiological (~11 kiloPascal, kPa) but not DMD-like stiffness (>25 kPa). However, a more complete niche including growth factors, tissue-matched substrate stiffness, and muscle-mimetic extracellular matrix (ECM) proteins may maintain the SMP phenotype. To establish which matrix components comprise normal and DMD muscle so that they can be mimicked in vitro, we compared ECM composition using liquid chromatography coupled with tandem mass spectroscopy (LC-MS/MS). DMD muscle up-regulated collagens I, II, III and VI and proteins involved in collagen fibrillogenesis (eg, decorin and mimecan), whereas basement membrane proteins, including laminins, nidogen, perlecan, and collagen type IV, were expressed more in normal muscle. Polyacrylamide (PA) gels of physiological (11 kPa) or DMD stiffness (34 kPa) were created with ECM protein combinations mimicking either healthy or DMD ECM to create permissive or non-permissive environments, respectively. Human SMPs were then culture-expanded and subsequently differentiated in myogenic medium (5% horse serum, 10 µg/ml insulin) to assess the ability of these niches to promote expansion without phenotype loss. Experiments suggest that while the population-doubling times of human SMPs remain consistent in all niches, the addition of certain growth factors (eg, FGF2) will improve proliferation at higher passage numbers. Together these data suggest that ECM composition in conjunction with growth factors and ECM stiffness can expand SMPs without limiting their self-renewal and differentiation capacity, thus making it possible to use them in therapeutic applications for muscle.

T-1128 see abstract in EPIDERMAL CELLS (NOT SKIN) before T-1156

KIDNEY CELLS

T-1129

THE POLYCOMB REPRESSOR COMPLEX 2 AND STEM CELL-SPECIFIC MIR-302 CLUSTER COLLABORATE TO INTERPRET THE TGF-BETA SIGNALING DURING FIBROSIS AND REPROGRAMMING

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Developmental cell fate decisions are controlled by the interplay of transcription factors, miRNAs and epigenetic modifiers, which together determine cellular identity. During the progression of diabetic complications, cells within affected tissues undergo a process of reprogramming, evoking gene expression profiles reminiscent of ontogenesis. Several studies have identified members of the TGF-β family as central to these processes and demonstrated the involvement of histone lysine methylation in the regulation of key fibrotic genes related to Diabetic Nephropathy (DN). We have identified a context dependent switch enhancing complex comprising Smad3 and Polycomb Repressor 2 (PRC2) that regulates cell fate during embryonic differentiation and is reactivated during fibrotic processes. Our data suggests that the H3K27 methylating PRC2 regulates the silencing of TGF-β activated genes during cell differentiation and commitment. Gene expression profiles of renal cells undergoing TGF-β mediated differentiation has revealed a number of transcription factors that co-segregate with Smad3 including Oct4, while differential expression of these factors in response to TGF-β was demonstrated by western analysis of chromatin associated proteins. Moreover, we have recently demonstrated increased expression of miR-302 cluster, in epithelial cells undergoing dedifferentiation. Intriguingly in the context of nephropathy, the primary validated target of miR-302s is the Type II TGF-β receptor. Critically, miRNAs have been shown to be essential for regulating cell fate and pluripotency and in particular the miR-302 cluster has been demonstrated to be involved in iPSCs generation. Human renal mesangial cells transduced with miR-302 undergo profound changes in morphology and gene expression indicative of acquired pluripotency. We have devised a protocol for miR-302 reprogrammed cells comprising an inhibitor of TGF-β signaling and an inhibitor of PRC2 activity. Our data show that mir302-transduced cells start to express E-cadherin and Oct4 and acquire epithelial characteristics. These data demonstrate for the first time a functional association between miR-302, Smad signaling and the PRC2 in the context of cell reprogramming, with a potential for therapeutic manipulation during the progression of DN.

T-1130

UNDERSTANDING PODOCYTE RENEWAL IN ADULT MOUSE KIDNEY

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Glomerular diseases account for 90% of end-stage kidney disease in western countries. Several studies have pointed to a unified concept of glomerular diseases in which podocyte dysfunction, injury or loss is a common factor. Podocytes serve a crucial role in supporting and maintaining the integrity of the glomerular filtration barrier by extending numerous foot processes that surround the glomerular capillary. As a consequence of their high degree of differentiation, mature podocytes are thought to have a severely limited capacity for renewal, and the mechanisms by which podocytes are maintained during life remain very poorly understood. Despite the prevailing paradigm that kidney podocytes do not renew, Shkreli et al. showed in 2012 that these cells possess significant regenerative capacity, a potential revealed by activation of a telomerase signaling pathway. Indeed, the use of double transgenic mice in which expression of telomerase can be switched on in adult tissues demonstrated that transient induction of telomerase overexpression causes robust podocyte dedifferentiation and proliferation. Strikingly, silencing transgenic telomerase expression result in regeneration of quiescent and fully functional podocytes. The aim of this study is to determine whether podocyte renewal occurs in this system through simple duplication of mature podocytes, or through activation of podocyte progenitor cells. To address this question, we used a murine model that allows podocyte lineage tracing during the regeneration process. In those mice, Cre-mediated DNA excision lead to a permanent eGFP mark of matures podocytes and their progeny. Fate of marked podocytes was determined after induction of podocytes renewal. Immunohistological analysis of kidney sections showed a significant reduction of eGFP+ podocytes after renewal in 70% of glomeruli. Moreover, those glomeruli showing a low eGFP signal expressed normal amounts of podocyte specific markers. These results suggest that podocyte renewal in adult kidney occurs through the recruitment of a progenitor cell population that is able to generate fully differentiated podocytes. Further lineage tracing studies and flow cytometry analyses currently in progress will allow to track podocyte progenitors during the regeneration process.

PANCREATIC, LIVER, LUNG OR INTESTINAL/GUT CELLS

T-1131

IMMORTALIZED HEPATOCYTE-LIKE CELLS DERIVED FROM HUMAN MESENCHYMAL STEM CELL ALLOW COMPLETE PROPAGATION OF HEPATITIS C VIRUS

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Hepatitis C virus is a major human pathogen causing chronic liver diseases and hepatocellular carcinoma. A workable cell culture model for HCV life cycle was established in 2005 using Huh7.5 derived hepatocyte cell line and JFH-1 (genotype 2a) recombinant genome that have been widely employed for infection. However, human hepatic Huh 7.5 cell lines could not support complete HCV propagation after being infected with HCV from patient serum that

limit the HCV cell culture model only to genotype 2a. In this study, immortalized human hepatocyte-like cells (iHLC) derived from hMSC transduced with Bmi-1 plus hTERT genes were evaluated for HCV associated receptors, HCV production from JFH-1 system, and HCVcc infectability after challenging with HCV from patient serum. The HCV receptors, for example; claudin-1, occludin, CD81 and SR-BI, were highly expressed in iHLC using immunofluorescence and real-time qPCR. The iHLCs permit not only replication of HCV RNA but also produce HCV particles after infection with HCVcc. Moreover, iHLCs exhibited higher susceptibility for propagation of HCVcc derived from JFH-1 stain than did Huh7.5 cells. Transfected JFH-1 and infected iHLCs with HCV from patient serum were also express HCV core antigen and HCV negative-stand RNA that confirmed fresh HCV protein synthesis and replicability. The conditioned medium from transfected iHLC could infect naïve iHLC and Huh 7.5 cell in a Transwell system. Treating infected iHLC with INF-α could decrease HCV RNA in both intracellular fraction and culture medium. This robust cell culture model for HCV using iHLC provides remarkable system for studying HCV life cycle, the development of HCV associated hepatocellular carcinoma, and an efficacy model for novel anti HCV medications.

T-1132

SERUM-FREE CULTURE OF HUMAN INTESTINAL STEM CELLS BY LIPOSOME-MEDIATED STABILIZATION OF WNT LIGANDS

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The in vitro expansion of adult human organ stem cells in organoids offers novel opportunities for regenerative medicine and gene therapy. These stem cells are dependent on Wnt signals provided in the form of a serum-containing medium conditioned by a Wnt3a-producing cell line. Stem cell culture conditions suitable for clinical applications should include not only all the factors that are required for self-renewal, but also eliminate differentiation-inducing and undefined components. For clinical applications the use of conditioned medium and serum is therefore undesirable. Moreover, serum batches vary in their capability of establishing and maintaining human organoid cultures. We noticed however that purified Wnt3a protein proved less efficient at maintaining organoids than Wnt3a-conditioned medium containing serum. We found that this was due to the rapid loss of Wnt3a activity in the absence of serum, combined with the presence of the detergent CHAPS in Wnt3a protein preparations, which becomes cytotoxic at higher concentrations. We show here that stabilization of Wnt3a protein by association with liposomes obviates the need for CHAPS to maintain Wnt activity and enables higher Wnt3a concentrations. Moreover, liposome-associated Wnt3a shows increased stability in serum-free media and supports the derivation and self-renewal of embryonic and human intestinal stem cells in serum-free conditions, removing a major obstacle impeding the clinical application of human adult stem cells.

T-1133

YAP/TAZ INCORPORATION IN THE BETA CATENIN DESTRUCTION COMPLEX ORCHESTRATES THE WNT RESPONSE

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Organ growth, tissue regeneration, and tissue replenishment are remarkable processes requiring the concerted activation and restriction of distinct gene-expression programs. How this is achieved by just a handful of signaling cascades is a central question in cell biology. Wnt growth factors play prominent and pleiotropic roles in cell-cell communication, including control of cell fate, proliferation, and stem cell maintenance. A main effector of Wnt signaling is nuclear b-catenin, which regulates the transcription of Wnt target genes by binding to TCF/Lef transcription factors. Wnt causes functional inactivation of the destruction complex, with ensuing escape of b-catenin from degradation, resulting into b-catenin accumulation and nuclear entry . The mechanisms by which Wnt inhibits the destruction complex are only partially understood, but recent work indicated that a key step regulated by Wnt is the dissociation of b-TrCP from the complex . How this step takes place remains unknown. Indeed, we provide biochemical, functional, and genetic evidence that YAP and TAZ, closely related proteins, well known for being nuclear effectors of the Hippo signaling cascade and of mechanical cues, are integral components of the b-catenin destruction complex that serves as cytoplasmic sink for YAP/TAZ. In Wnt-ON cells, YAP/TAZ are physically dislodged from the destruction complex, allowing their nuclear accumulation and activation of Wnt/YAP/TAZ-dependent biological effects. YAP/TAZ are required for intestinal crypt overgrowth induced by APC deficiency and for crypt regeneration ex vivo. In Wnt-OFF cells, YAP/TAZ are essential for b-TrCP recruitment to the complex and b-catenin inactivation. In Wnt-ON cells, release of YAP/TAZ from the complex is instrumental for Wnt/b-catenin signaling. In line, the b-catenin-dependent maintenance of ES cells in an undifferentiated state is sustained by loss of YAP/TAZ. This work reveals an unprecedented signaling framework relevant for organ size control, regeneration, and tumor suppression.

T-1134

HEPATOCYTES DERIVED FROM HUMAN VAL9 EMBRYONIC STEM CELLS PROTECT MICE FROM LIVER INJURY AFTER TRANSPLANTATION

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Although the initial proof of concept with hepatocyte transplantation has been demonstrated by a number of researchers, wide application of this technology has been hindered by the inability to secure a reliable and well-characterized cell source and by the challenges of successful and sustained engraftment and expansion of transplanted cells in vivo. Human embryonic stem cells (hESCs)

may provide a suitable source for functional hepatocytes for use in clinical applications and drug development. We utilized a new generation of hESC, VAL9 cells which were generated in traceable conditions easily transposable to GMP compatible conditions. By using sequential modulation of different signalling pathways involved in induction of the distinct developmental stages, we demonstrate that VAL9 hESCs can be efficiently differentiated into endoderm, hepatic progenitors (hepatoblasts) and functional hepatocyte-like cells, recapitulating key stages of liver development. Moreover, hepatoblasts could be successfully differentiated into precursors of cholangiocytes. The hepatocyte-like cells exhibited different functions associated with mature hepatocytes, including upregulation of hepatic-specific transcripts (HNF4-α, ornithine transcarbamylase, UDP glucuronosyltransferase 1A1) indocyanin green uptake and excretion, cytochrome P450 enzyme and UGT1A1 activity, secretion of urea and albumin, and glycogen storage capability. Importantly, when transplanted into a mouse model of acetaminophen-induced acute liver failure, differentiated VAL9 hESCs were able to rescue the mice. Lentivirally transduced VAL9 cells expressing GFP safely engrafted and repopulated up to 10% of the liver of transplanted mice and produced a significant decrease of AST and ALT within 2 weeks after transplantation. Importantly, we did not observe any sign of tumorigenicity in transplanted mice nor the presence of these cells in other tissues as assessed by histology data. These results suggest the potential utility of differentiated VAL9 cells for cell therapy for liver injury.

T-1136

LUNG/THYROID CONVERSION OF MOUSE ESC-DERIVED ANTERIOR FOREGUT THROUGH TRANSIENT OVER EXPRESSION OF NKX2-1

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Lung/thyroid progenitors 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 (anteriorization), followed by FGF2/BMP4 treatment. These progenitors are characterized by expression of Nkx2-1, a homeodomain transcription factor expressed in the developing lung, thyroid, and forebrain. To investigate if transient Nkx2-1 expression can increase the efficiency of Nkx2-1+ lung/thyroid progenitor specification, we utilized a mESC line double knock-in GFP-T/hCD4-Foxa2 with a Tet-On Nkx2-1 system. Activation of the Nkx2-1 transgene by addition of doxycycline for 24 hours at the anterior foregut endoderm stage (post-anteriorization) induces and maintains high levels of endogenous Nkx2-1 as well as both lung and thyroid specific markers at later stages in our protocol. Outside this narrow window, cells have reduced competence to respond to exogenous Nkx2-1. In addition, we sorted several post-anteriorization populations based on hCD4-Foxa2 expression and subsequently over-expressed Nkx2-1 for 24 hours. Cells varied in differentiation propensity with Foxa2neg/low cells yielding increased thyroid progeny as witnessed by high levels of Pax8 and thyroglobulin. To study the mechanisms of this conversion, we are integrating Nkx2-1 ChIP-Seq and RNA-Seq data sets acquired from relevant stages during lung/thyroid directed differentiation to identify potential binding targets of Nkx2-1 and changes in global gene expression. This analysis may also provide insights into how the lung and thyroid domains are initially specified from the foregut endoderm in vivo. The results demonstrate that Nkx2-1 can act as a stage-specific inductive signal during directed differentiation of mESCs and exemplify the potential of a more efficient system for deriving and studying Nkx2-1+ lung/thyroid progenitors.

T-1137

EVIDENCE OF GENE EXPRESSION SYNCHRONEITY OF LINEAGE-SPECIFIC KEY GENES DURING IN VITRO DIFFERENTIATION OF SEVERAL HUMAN PLURIPOTENT STEM CELL LINES TO HEPATOCYTE-LIKE CELLS

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Hepatocytes differentiated in vitro from human pluripotent stem cells (hPSCs) have the potential to replace other human hepatic models in drug discovery and regenerative medicine. However, the generation of fully functional hepatocytes in vitro is still a challenge. In order to gain better understanding of the maturation process, we have differentiated five hPSC lines to hepatocytes using one standardized multistage differentiation protocol specifically focusing on definitive endoderm (DE), ventral foregut and hepatoblast to fetal hepatocyte-like cells and finally mature hepatocyte-like cells. Applying RT-qPCR we have profiled the mRNA levels, each day throughout the protocol, of lineage-specific key genes of the hepatic differentiation in order to define the time points where the cell lines reach the different developmental stages. We monitored the following markers: OCT4 and NANOG for pluripotency; SOX17, CXCR4, and CER1 as DE markers; hHEX as ventral foregut marker; TBX3, PROX1, and HNF6 as hepatoblast markers; AFP, HNF4a, and HNFa1 as fetal hepatocyte markers; ALB, AAT, CYP3A4, and ASGR1 as mature hepatocyte markers. The results demonstrated that the expression of these key markers is highly synchronized among all investigated cell lines. The expression of DE markers started on day 3 after initiation of differentiation, when the pluripotency markers were almost undetectable, and reached highest level on day 5. Notably, the shift from DE medium to progenitor medium on day 7 rapidly resulted in the induction of TBX3 on day 8. In addition, the shift from progenitor medium to the first maturation medium on day 14 upregulated PROX1 and downregulated HNF6 from day 15. Subsequently, the shift to the second maturation medium on day 21 resulted in the expression of CYP3A4 from day 22. Furthermore, the maturation of the hepatocytes marked by the expression of ALB, coincided with the downregulation of AFP on day 25. In conclusion, the cell lines appear highly synchronized with regard to the expression of key genes during in vitro hepatic differentiation, enabling further in-depth investigations of the differentiation process across multiple cell lines.

T-1138

GENERATION OF A TALEN-ENGINEERED STEM CELL MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE TO STUDY ABERRANT LIPID METABOLISM IN HEPATOCYTES CARRYING A PNPLA3 POLYMORPHISM.

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Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in adults and children and is a complex disease with both environmental and genetic components. Genome-wide association studies have identified a polymorphism in the gene PNPLA3 with a strong association with risk and severity of NAFLD. The variant allele is associated with more severe biochemical and histological abnormalities. The protein product of PNPLA3 is involved in lipid metabolism, but its exact function in humans is unclear. Using TAL effector nuclease (TALEN) technology, I have designed TALENs specific to the PNPLA3 SNP and generated isogenic lines of human induced pluripotent cells (IPSCs) from a known genetic background with the variant (var) and wildtype (wt) homozygous alleles of PNPLA3. To our knowledge, this is the first set of isogenic lines of IPSCs designed specifically with the PNPLA3 wt and var alleles. The IPSCs are then differentiated to hepatocyte like cells (HLC) with which I can test the hypothesis that the var PNPLA3 allele confers its risk in NAFLD due to aberrant lipid metabolism resulting in lipotoxicity as a potential early pathogenic event in NAFLD. I am comparing intracellular lipid accumulation by nile red triglyceride (TG) staining of HLC using immunofluorescence and flow cytometry and expression of genes involved in lipid metabolism. Preliminary results show that palmitic acid stress induces significant lipid droplet formation in wt but not var hepatocytes, suggesting that the var hepatocytes were unable to accumulate TG perhaps triggering toxic effects leading to poor hepatocyte health. Additionally, results demonstrate that mRNA expression of genes involved in lipolysis (PPARa and PNPLA2) is upregulated in the var compared to wt indicating aberrant lipid metabolism. I am studying downstream effects of abnormal lipid processing that may affect hepatocyte function and viability, including autophagy, mitochondrial function and apoptosis. If these experiments reveal that the risk variant of PNPLA3 drives hepatocyte phenotypes that correlate with a higher likelihood of progressing to severe NAFLD, then this work will open the door to a new range of experimentation in elucidating the mechanism underlying this association, predictive diagnostics and therapeutic discovery.

T-1139

A NOVEL PROTOCOL ENABLES THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELL DERIVED BIPOTENTIAL HEPATOBLASTS INTO HEPATOCYTE OR CHOLANGIOCYTE LIKE CELLS

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The liver is the organ in the human body that is responsible for metabolic coordination and for detoxification of a wide variety of drugs. It has an immense capability of self-renewal. Damaged cells are reliably replaced and even after partial hepatectomy the organ is reconstructed to its fully original size and it regains all its former functions. This is particularly challenging as liver lobules are highly vascularized structures consisting not only of hepatocytes. Cholangiocytes, epithelial cells which line the intra-hepatic bile ducts, are of major importance for the structure and function of the liver. Up to now it is not known exactly how liver regeneration takes place. Residual stem/progenitor cells could differentiate to replace the lost cells, normally quiescent hepatocytes could start proliferating or cholangiocytes could transdifferentiate in order to give rise to functional hepatocytes. Therefore, we decided to investigate the differentiation potential of hepatic progenitor cells in more detail. While there exist several elaborate protocols for the in vitro differentiation of human pluripotent stem cells (hPSCs) into hepatocytes, the differentiation into cholangiocytes has been only marginally investigated and the existing protocols are rather complicated. Here we present an easy and straightforward method for the differentiation of hPSCs into bipotential hepatoblasts that have the capability to differentiate into hepatocyte (HLCs) or cholangiocyte (CLCs) like cells. In our protocol the cell fate decision between HLCs and CLCs is made at the stage of the last common progenitor which closely mirrors the in vivo situation. It depends highly on cell density and is influenced by NOTCH signalling. Immunestainings and global transcription analyses revealed gene expression patterns characteristic for the two different cell types. HLCs express for example Albumin and HNF4a while CLCs are positive for EpCAM and CK19. Additionally, differentially expressed receptors and transcription factors clearly separate the two cell types and give hints for the further optimization of the differentiation protocol. Thus, our new protocol enables us to study the differentiation of hepatic cells in a scenario close to the in vivo situation.

T-1140

INFECTION OF STEM CELL-DERIVED HEPATOCYTES WITH THE HEPATITIS E VIRUS

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Every year 20 million people become infected with the hepatitis E virus resulting in over 3 million cases of acute hepatitis. Although hepatitis E is usually an acute self-limiting form of hepatitis, severe cases of fulminant hepatitis and chronic infections have been reported, resulting in an estimated 56.000 deaths per year. Current in vitro cell culture models rely on the use of hepatoma cell lines and human primary hepatocytes which both have conceivable limitations. Alternative sources of human hepatocytes are human embryonic and induced pluripotent stem cell derived hepatocyte-like cells. hESC and iPSC have numerous advantages compared to primary cells such as the ability to indefinitely self-renew, the potential to differentiate in any given cell type and the feasibility to be genetically modified. Previous studies already demonstrated the infection of stem cell-derived hepatocytes with the hepatitis C virus and the hepatitis B virus but to our knowledge there is no evidence yet that human stem cell-derived hepatocytes can as well be infected with

the hepatitis E virus. hESC and hiPSC were differentiated toward hepatocytes during a 20 day protocol and subsequently infected with the Kernow C1 hepatitis E virus strain. Viral replication was analyzed by RT-qPCR and could be inhibited by ribavirin and alpha interferon treatment. RNA FISH was used to demonstrate the presence of intracellular viral HEV RNA while a strand specific RT-PCR was used to specifically detect negative strand RNA, a proof of viral replication. In addition we demonstrated that pluripotent stem cells differentiated toward mesoderm or neural progenitor cells did not support HEV replication confirming the hepatotropism of HEV. Moreover, a previously identified mutation in the RNA-dependent RNA polymerase enhanced viral replication. Our studies demonstrate that pluripotent stem cell-derived hepatocytes are a promising model to study infection with various hepatotropic viruses and, can be considered a novel test system for antiviral drugs.

T-1141

HYPOXIA-INDUCED STEM CELL CHARACTERISTICS ARE RELATED WITH CXCR4 REACTIVATION BY ITS PROMOTER DEMETHYLATION

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As a cancer stem cell marker, CXCR4 has been known to be closely associated with tumor progression, angiogenesis, metastasis and poor prognosis. Increased expression of CXCR4 by hypoxic condition has been implicated in several types of cancers. Previous study suggests that CXCR4 expression is regulated by its promoter methylation. In the current study, we investigated whether hypoxic stress could induce CXCR4 expression by its promoter demethylation in lung cancer. Normal lung cell (BEAS-2B) and lung cancer cell lines (A549, H292, H226 and H460) were incubated under hypoxic condition. To analyze CXCR4 expression, real-time RT-PCR, FACS and western blot were performed. To determine whether CXCR4 expression was reactivated, cell lines were treated with a DNA methyltransferase inhibitor (5-azacytidine, AZA). Hypoxia-induced DNA demethylation was identified by methylation-specific PCR and bisulfite sequencing. The functional role of CXCR4 was examined using CXCR4 gene and siRNA transfection. Stem cell characteristics were assessed by sphere formation assay, wound healing assay, Matrigel invasion assay and in vivo mice tumor model. CXCR4 was increased more in hypoxic condition than in normoxic condition. CXCR4 was reactivated by treatment with AZA. Methylation-specific PCR showed decreased CXCR4 promoter methylation in hypoxic condition compared with normoxic condition, which was further validated by bisulfite sequencing. Functional stem cell assay indicated that hypoxic stress and CXCR4 increased sphere formation, wound healing, Matrigel invasion and in vivo mice tumor formation. These results suggest that hypoxia induces stem cell characteristics which are related with CXCR4 reactivation by its promoter demethylation.
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T-1142

CHARACTERIZATION OF THE NOVEL HUMAN PANCREATIC PROGENITOR CELLS

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Congenital hyperinsulinism (CHI) results from loss of function mutations in genes regulating insulin secretion from pancreatic β-cells. Disturbances in secretion of insulin hormone result in severe and persistent hypoglycemia which can lead to brain injuries. There are two main histopathological forms of the disease that have been described: a diffuse form that affects the entire pancreas and a focal form which is restricted to a single region of the pancreas. Cell lines established from CHI patients undergoing pancreatectomy represent a novel and unique source of human pancreatic progenitor cells. They may be an important tool to study hyperinsulinemic conditions but can also be used to study human pancreatic progenitor to beta-cell differentiation. Recently we have established several cell lines and are currently characterizing growth conditions, phenotype and functional features of the two of them. We have been able to successfully propagate NES64 and NES159 cell lines established from resected pancreata of young patients suffering from diffuse and focal form of CHI respectively. Optimising growth conditions showed that within 7 days of culture we had 40-50% more NES64 and NES159 cells when using E8 and MesenPRO RSTM media in comparison to regular RPMI medium. Similarly, E8 and MesenPRO RSTM media facilitated formation of bigger clusters using low-adhesion plates and Erlenmeyer flasks for both cell lines. Time required to form clusters was also reduced for both cell lines when using E8 and MesenPRO RSTM media. Immunohistochemical analysis show that both NES64 and NES159 express several markers characteristic to mesenchymal cells (vimentin, CD73 and CD105) suggesting that the cells most likely underwent epithelial-mesenchymal transition. Interestingly, using qPCR we observed low but detectable expression of several endodermal and pancreatic progenitor markers (Sox17, Pax6, Isl1 and MafB). In contrast to that, we could not detect any of the mature beta-cell markers (Insulin, MafA, Nkx6.1). These results suggest that CHI-derived NES cells could be a useful model to study human pancreas progenitor cell function and subsequently their differentiation to beta-cell. In addition, CHI-derived beta-cells could be used for drug screening purposes both in vitro and in vivo in a humanised mouse transplant model.

T-1143

ESTABLISHMENT OF A TRANSCRIPTION FACTOR-BASED PROTOCOL FOR THE TRANSDIFFERENTIATION OF INTESTINAL STEM CELLS INTO PANCREATIC LINEAGE CELLS

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Currently, one attractive therapeutic approach to treat Diabetes Mellitus type 1 is the transplantation of insulin-secreting β-cells. However, β-cells derived from an allogenic donor face serious problems such as immunological rejection and low amounts of donor tissue available. Therefore, alternative sources for the derivation of functional β-cells are needed. As pancreatic cells and intestinal stem cells (ISCs) share a similar developmental origin, ISCs represent an interesting cell source for the in vitro generation of functional β-cells. Consequently, the goal of this study is to transdifferentiate ISCs towards insulin-secreting β-cells using the transcription factor Ptf1α that plays an important role during pancreatic lineage differentiation. To this aim, we transduced ISCs derived from the duodenum of an $Lgr5^+$-eGFP transgenic mouse line with recombinant PTF1α protein. The capacity of Ptf1α to induce pancreatic lineage differentiation from ISCs was assessed by analyzing intestine- and pancreatic-specific gene expression patterns. 24-48 hrs post transduction an up-regulation of pancreatic-specific genes and down-regulation of intestinal-associated genes could be observed. However, the number of viable cells was extremely low. Therefore, we developed alternative strategies like liposomes and mRNA transfection to deliver Ptf1α into ISCs. The liposomal strategy was tested in diverse cell lines as well as in crypt-derived organoids and single cells isolated from those organoids. Efficiencies ranged between 70-80% in all cell types and up to 90% in organoids. Our future work will focus on *Ptf1a*-specific mRNA transfection of ISCs. In addition, further pancreatic-specific transcription factors like Pdx1 or Ngn3 will be tested in these transdifferentiation approaches hypothesizing an improved generation of functional β-cells from ISCs. Taken together, we describe here a new pancreatic-lineage differentiation protocol based on the transdifferentiation of ISCs and assume that this protocol will improve the in vitro generation of functional β-cells. As the maturity of functional β-cells is a major limitation of current monolayer differentiation protocols we finally plan to perform these transdifferentiation approaches in a bioreactor system on vascularized scaffolds (BioVaSc®).

T-1144

DIFFERENTIATION OF LUNG STEM CELLS IN MOUSE AND HUMAN EMBRYONIC LUNGS

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Human iPS cells have been used for directed differentiation to mature lung cells. Several independent differentiation protocols have now been published, but more work is required to achieve fully mature and functional lung cells. We aim to characterise and differentiate non-iPS derived embryonic lung stem cells in both mouse and human embryonic lungs. During lung branching morphogenesis in mouse, the distal tip Sox9+ epithelial cells are multipotent stem cells. Initially (~E11-15), they make bronchiolar descendants and at E16 switch to producing alveolar descendants. What mechanisms control this competence switch? Is it conserved between mouse and human embryos? Using mouse lungs, we have performed heterochronic grafting experiments in which tip cells are transplanted into a lung of a different developmental age. Grafts grow and integrate into host lungs and fate analysis revealed that progenitor competence is determined both intrinsically and by the host. The intrinsic component is stronger at E16 than at E12. Further experiments showed that addition of synthetic glucocorticoids was sufficient to initiate the competence switch, both in grafted and un-manipulated mouse lungs. Do the same mechanisms function in developing human lungs? Preliminary analysis shows that there is some conservation of the role of glucocorticoid signalling, but that additional mechanisms are also involved. Directed differentiation of human iPS cells towards maturity has been based on mouse experiments. Do human embryonic lungs provide the missing link to achieve full maturity?

T-1145

CHARACTERIZING THE EFFECTS OF A COMMON HUMAN MUTATION IN THE ALDEHYDE DEHYDROGENSASE-2 GENE ON THE LUNG

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Aldehyde dehydrogenase (ALDH) enzymes play a major role in detoxification of aldehydes. The lung is continuously exposed to endogenous and exogenous aldehydes from lipid peroxidation and air pollution. High levels of ALDH expression are observed in stem cells of many organs. A common mutation in the ALDH2 gene (ALDH2*2, affects 40% of East Asians) results in catalytic inactivation of the enzyme. This mutation is associated with alcohol flushing syndrome and an increased risk for Alzheimer's and cardiovascular diseases as well as some cancers. The effects of the ALDH2 mutation on the lung have not been examined. Lungs from ALDH2 knockout or ALDH2*2 transgenic mice were compared with their wild type (WT) control for the presence of structural phenotypes at birth, middle (8-30 W) and old (60-100 W) ages. We found that at middle age, airway epithelial thickness and number of basal stem cells in ALDH2-disturbed mice were significantly lower than WT mice. Similar changes have been previously observed to accompany aging of WT mice. However, there were no significant differences between mice groups when exposed to acute airway or chronic lung injuries with polidocanol or cigarette smoke, respectively. When both proximal airway and distal lung epithelial cells were compared in the 3D organoid stem cell assay, no significant differences were detected. Treating cells in culture with reactive oxygen species and/or an ALDH2 agonist produced no differential effects. We also examined primary human bronchial and distal lung epithelium obtained from fresh surgical samples for their in vitro sphere formation efficiency (SFE). Both bronchial and distal lung epithelium carrying the ALDH2*2 mutant allele showed a lower SFE compared to WT. Lastly, using genotyping data sets of large cohorts of asthma and COPD patients, and individuals with normal lung function, we

found that the ALDH2*2 allele was associated with lower FEV1 and FEV1/FVC in general population but not with development of asthma or COPD. In conclusion, ALDH2 mutation in mice resulted in signs suggestive of premature aging of airway epithelium. There were no detectable functional impairments in the mouse in vitro and in vivo assays examined, but human airway and lung epithelium showed lower SFE. ALDH2*2 allele was associated with lower FEV1 and FEV1/FVC in the general population.

T-1146

EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO HEPATOCYTES

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Secondary liver toxicity is a leading cause of commercial pharmaceutical drug recalls and compound failures during drug development. Traditional models for studying liver toxicity use hepatocytes isolated from donor livers. These primary hepatocytes can be difficult to culture, have limited proliferative capacity, and often lose functionality quickly in vitro. Hepatocyte-like cells derived from a renewable source, such as human pluripotent stem cells, are therefore a valuable resource for drug discovery research. In this study, we introduce our StemXVivo™ Hepatocyte Differentiation Kit, which efficiently directs human pluripotent stem cells through the cell fate decisions that are required for the development of functional hepatocyte-like cells. Differentiated hepatocyte-like cells were initially characterized using quantitative PCR for hepatic gene expression. We found a significant and sequential upregulation in RNA expression for genes that encode both early and late hepatic proteins, such as Hepatocyte Nuclear Factor 4 alpha (HNF-4 alpha), alpha-Fetoprotein (AFP), Glutathione S Transferase pi 1 (GSTP1), Phosphoenolpyruvate carboxykinase 1 (PCK1), Transthyretin (TTR), Serpin A1 (AAT), and Albumin. Expression of differentiation stage-specific and hepatocyte-specific markers was confirmed at the protein level by immunocytochemistry and flow cytometry. Finally, pluripotent stem cell-derived hepatocyte-like cells were functionally characterized by quantitative analysis of albumin and urea secretion. These results demonstrate that the StemXVivo Hepatocyte Differentiation Kit easily and reproducibly generates hepatocyte-like cells from multiple pluripotent stem cell lines and can be used to provide a renewable cell source for high throughput drug discovery and screening.

T-1147

CHANGES IN THE STEM CELL NICHE ARE PARTLY RESPONSIBLE FOR THE REDUCED REGENERATION OF THE AGING INTESTINE

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Adult stem cells maintain tissues for the life of an organism by balancing regeneration with tissue damage and turnover. However, stem cells eventually fail to regenerate tissues at the required rate, and the resulting decline in function manifests as aging. Niche cells provide stem cells with factors regulating proliferation and differentiation, but whether age-related changes in the niche itself moderate stem cell function is unclear. Interestingly, the prolongevity treatment Calorie Restriction (CR) increases intestinal stem cell activity via the Paneth cell niche. The slower degeneration of tissues during CR could therefore be at least partly due to the more functional niche. We have discovered that the old epithelial stem cell niche possesses reduced growth and regenerative capacity. More specifically, we note that the Paneth cells of the intestinal stem cell niche loose their stem cell promoting function with age. To further characterize the age-dependent changes in the intercellular communication, we are performing trancriptome analysis of the old Paneth cells. Our results indicate that the aging of the tissue results partly from alteration in the niche, and suggest that interactions between stem cells and their niche could provide points for intervention in aging related diseases.

T-1148

HIGHLY EFFICIENT AND REPRODUCIBLE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO PANCREATIC PROGENITORS USING A NOVEL SERUM-FREE MEDIUM

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A human pluripotent stem cell (hPSC)-derived regenerative medicine approach to treating diabetes is highly coveted. Current state-of-the art in vitro protocols generate immature pancreatic precursor cells (PPCs) that can be further matured to endocrine cells in vivo. These hPSC-derived PPCs are a useful tool in studying pancreas development and disease and are being used in clinical trials for treating type 1 diabetes. While several protocols exist to generate PPCs from hPSCs, efficiency and reproducibility across hPSC lines varies. To standardize generation of hPSC-derived PPCs, we developed the STEMdiff™ Pancreatic Precursor Differentiation Kit, a complete and defined medium and supplements that supports efficient generation of PDX1+/NKX6.1+ PPCs from multiple hPSC lines. hPSCs maintained under defined, feeder-free conditions on Matrigel® in mTeSRTM1 were seeded as single cells at 2.1x105 cells/cm2. Definitive endoderm (DE) was generated using the STEMdiff™ Definitive Endoderm Kit (Stage 1), which consistently provides >85% CXCR4+SOX17+ DE cells for downstream differentiation. These DE-enriched cells were then differentiated through 3 additional stages (Stages 2 - 4) over 11 days with daily full medium exchanges. At the end of Stage 4, expression of key pancreatic precursor transcription factors was assessed by flow cytometry (FC) and qPCR. By FC, PDX1 expression was: H1, 82.5 ± 2.3% (n=2); H9, 91.6 ± 2.4% (n=2) and WLS-4D1, 91.1 ± 4.8% (n=3; mean±SD). Co-expression of PDX1 and NKX6.1 was: H1, 72.5 ± 4.5%; H9, 85.3 ± 3.1% and WLS-4D1, 80.6 ± 9.4%. Essentially all NKX6.1+ cells were PDX1+. Cell yields of 1.3-1.7 x 107 cells/cm2 were high relative to previously published data, indicating a 6.5 to 8-fold expansion of total cells. This equates to approximately 4.5 to 11.4 PPCs per input hPSC. Furthermore, PPCs generated with this novel medium show upregulation of NKX6.1, NEUROD1, NGN3 and PTF1α mRNA. Insulin and glucagon mRNA was also upregulated at the end of Stage 4. We have developed a complete, defined and serum-free medium that promotes highly efficient differentiation of hPSCs to PDX1+NKX6.1+ pancreatic precursor cells. The protocol described here is robust across multiple hPSC lines and can be reliably used in studies aimed at further in vitro β-cell maturation or at understanding pancreatic development.

T-1149

CHANGE OF ISLET CELL IDENTITY AND INCREASED EXPRESSION OF THE MESENCHYMAL CELL MARKER VIMENTIN IN TYPE 2 DIABETES

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Pancreatic β-cell dedifferentiation has been proposed as a mechanism of β-cell dysfunction in murine models of diabetes. Human β-cells can undergo epithelial-to-mesenchymal transition (EMT) with expression of vimentin when cultured as a monolayer ex vivo but the role of such conversion in islet (patho)physiology in vivo is unclear. The aim was to determine if islet cells express mesenchymal markers in humans in vivo and whether this changes in type 2 diabetes (T2DM). Pancreatic tissue was obtained post-mortem from subjects, including organ donors, diagnosed with T2DM (n=28; age 34-86, BMI 22-44 kg/m2) or without diabetes (ND) (n=36; age 27-78, BMI 19-38 kg/m2). Formalin-fixed paraffin-embedded tissue sections were triple immunolabelled for the mesenchymal marker vimentin, insulin (for β-cells) or glucagon (for α-cells) and β-cell transcription factors Nkx6.1 or Pdx1. Quantitative morphometry was made on confocal images with the investigator blinded to donor status; double and triple positive cell proportions in relation to the total number of glucagon- or insulin-positive cells were determined. The proportion of insulin+/vimentin+ cells was higher in the T2DM group (median [IQR] ND 0.98 [0.29-1.42] % vs. T2DM 1.43 [0.74-2.32] %; $p<0.05$). A higher proportion of vimentin+/glucagon+ cells was observed in both groups with significantly more cells in the T2DM group (ND 2.53 [1.39-5.71] % vs. 4.53 [2.78-9.11] %; $p<0.05$). Triple positive cells were rare in ND and T2DM (vimentin+/ insulin+/ Nkx6.1+ cells < 0.1%, vimentin+/ insulin+/PDX1+ cells < 0.3%, vimentin+/ insulin+/ glucagon+ cells < 0.1%). The proportion of vimentin+ islet cells was not related to age or BMI. T2DM is associated with a higher proportion of islet cells expressing the mesenchymal marker vimentin. These cells rarely express Pdx1 or Nkx6.1, indicating that they are likely to be dysfunctional. We suggest that mesenchymal gene expression could be triggered by diabetes in a small proportion of islet cells leading to islet cell dysfunction in T2DM. Understanding the mechanisms by which adult β-cells maintain or change their identity may have important implications for islet pathophysiology and protective and regenerative strategies in diabetes.

ENDOTHELIAL CELLS/HEMANGIOBLASTS

T-1150

ENHANCEMENT OF AUTOPHAGY WITH HYPOXIC PRECONDITIONING IMPROVES SURVIVAL OF TRANSPLANTED ENDOTHELIAL PROGENITOR CELLS

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Recent clinical studies have suggested that endothelial progenitor cell (EPC) transplantation provides a modest benefit for treatment of limb ischemia and myocardial infarction. The engrafted EPCs play an angiogenic role in repairing the ischemic tissue by differentiation towards endothelial cells and production of cytokines. However, several approaches to cell-based therapies have been limited by poor survival of the engrafted cells. This investigation was designed to examine effects of autophagy induced with hypoxic preconditioning on survival of the engrafted EPCs in rat ischemic hind-limb models. For determining of optimal hypoxic preconditioning, $CD34^{+}VEGFR\text{-}2^{+}$ EPCs isolated from rat bone marrow were incubated in hypoxic condition (1% O_2) for 1, 2, 4 h respectively, then autophagy and apoptosis of the hypoxic cells were examined. The apoptotic cells increased significantly after the hypoxic cells were treated with autophagy inhibitor 3-MA. The cells treated with hypoxia for 2 h were used for in vivo experiments. To examine survival of the cells after treatment with hypoxia, cell sheets prepared with fibrin glue were implanted into the abdominal wall of rats. Comparing the control and 3-MA groups, number of the survived cells and density of microvessels in the sheets were greater in the hypoxic group. To evaluate efficiency of the engrafted cells preconditioned with hypoxia in repairing the ischemic tissue, rat hind-limb ischemia models were prepared with ligature of the femur artery of rats. The cells were injected into the ischemic muscles. Angiogenesis and blood perfusion in the ischemic hind-limb was analyzed with laser Doppler-based perfusion measurement, the density of microvessels in the histological sections and collateral growth in angiograms. Comparing the control and 3-MA groups, angiogenesis and blood perfusion in the hypoxic group were improved significantly. These results demonstrate that enhancing autophagy with hypoxic preconditioning is an optimizing strategy for EPC therapy of limb ischemia.

T-1151

HUMAN IN VITRO OXIDATIVE STRESS MODEL: COMPARISON BETWEEN HUMAN ENDOTHELIAL AND EMBRYONIC STEM CELLS AFTER EXPOSURE TO HYDROGEN PEROXIDE

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Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell. Hydrogen peroxide is generated in numerous biological processes, it is associated with the initiation and progression of oxidative stress-linked diseases and it is also implicated in redox

signaling. Given that, in this study the cytotoxic effect of hydrogen peroxide was evaluated in two different cell lines: HUVEC (Human Endothelial cells) and HUES3 (Human Embryonic Stem Cells). HUVEC cells were cultured in M-200 medium, while embryonic stem cells were cultured in feeder-free maintenance medium mTeSR1TM. After having established the optimal cell concentration to induce and detect a dose-response effect, the cells were exposed to hydrogen peroxide from 24h post-plating, at concentrations varying from 2 to 1024uM, for 72h with daily change of medium. At the end of the exposure the cell viability was evaluated with the Alamar blue assay (Life Technologies), the ROS levels with the 2, 7-dichlorodihydrofluorescein diacetate (H2DCF-DA) fluorescent probe (Invitrogen) and the lipid peroxidation cell levels with the Image-iT Lipid Peroxidation Kit (Life Technologies). In HUES3 loss of viability was gradually observed at concentrations ranging from 32uM to 64uM, while in HUVEC the concentration range was from 256uM to 512uM, demonstrating a higher resistance to the hydrogen peroxide of the endothelial cell line. Concerning ROS, significantly higher levels were observed in both cell lines in the not cytotoxic concentrations and the same trend was observed for the lipid peroxidation, with higher levels between 8uM and 16uM for HUES3 and between 8uM and 64uM for HUVEC. The transcription factor NFKB was also investigated by immunocytochemistry after 2 hours of treatment with hydrogen peroxide and its translocation into the nucleus it was observed in both cell lines, indicating the activation of a process that leads to the expression of several inducible oxidative stress genes and factors. These results pave the way for the in vitro study of oxidative stress effects and its long-term consequences on cell viability, stem cells pluripotency and ability to differentiate in multiple cell types. *This study was funded by EpiHealthNet project n. 317146. FP7-PEOPLE-2012-ITN.*

T-1152

THE EFFECTS OF MESENCHYMAL STEM CELL-DERIVED CYTOKINES ON THE FUNCTIONAL PROPERTIES OF ENDOTHELIAL PROGENITOR CELLS

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Mesenchymal stem cell (MSC) has been considered a potential source for cell transplantation therapy. Although, it is well-known that one of the mechanisms which MSCs use to promote tissue repair involves the production and secretion of pro-angiogenic cytokines, the interaction between those MSC-derived cytokines and the circulating endothelial progenitor cells (EPCs) which play important roles in tissue neovascularization is still poorly characterized. The present study aims to investigate the effects of cytokines released from bone marrow- and gestational tissue-derived MSCs on the functional properties of EPCs, using an in vitro model. MSCs derived from bone marrow, amnion, placenta, Wharton's jelly and umbilical cord were co-cultured with umbilical cord blood-derived EPCs using a transwell culture system. After co-cultured with various MSC sources, the proliferation, migration, extracellular matrix invasion and vessel forming capacity of EPCs were determined. The MSC-derived factors which play critical roles on the various aspects of EPC functionality were also identified by qRT-PCR and functional assays. The results show that the migratory capacity of EPCs co-cultured with placenta-derived MSCs (PL-MSCs) were three times higher that those co-cultured with other MSC sources. In contrast to EPC migration, the extracellular matrix invasion and vessel-forming capacity of EPCs co-cultured with bone marrow-derived MSCs (BM-MSCs) were significantly higher than those co-cultured with other MSC sources. The results also demonstrated that the migratory-inducing effect of PL-MSCs was mediated by secreted PDGF-β while the positive effects of BM-MSCs on invasiveness and vessel-forming capacity of EPCs were mediated by secreted IGF1 and SDF-1. There is no difference in the proliferative capacity among EPCs co-cultured with five distinct MSC sources examined in this study. In conclusion, we have demonstrated that PL-MSCs and BM-MSCs released angiogenic factors which have positive effects on migration, extracellular matrix invasion and vessel-forming capacity of EPCs. The knowledge gained from this study might enhance the therapeutic potential of MSCs as sources of angiogenic cytokines for inducing neovascularization in injured/ischemic tissues in the future.

T-1153

EFFECT OF IMMUNOSPPRESSION AND CO-TRANSPLANTATION WITH VARIOUS MESENCHYMAL STEM CELLS ON VASCULAR NETWORK FORMATION BY PLACENTAL DERIVED ENDOTHELIAL COLONY FORMING CELLS

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Human endothelial colony forming cells (ECFC) have vasculogenic potential that is limited because of poor survival in vivo upon implantation. To evaluate the effect of host immunological system on cell's functions, cells were implanted into the wild type (WT) (C57BL/6) and immunodeficient (Rag1(-/-)C57BL/6) mice. We also investigated whether co-transplantation of ECFC with mesenchymal stem cells (MSC) could improve their viability and function. Lentivirally-tagged ECFC implantation in matrigel plugs showed cells undergoing death 1 day after implantation with very few cells surviving for 7 days. Implantation in WT mice resulted in 5 fold less cell engraftment compared to implantation in RAG1(-/-) mice ($P < 0.05$). Co-implantation with MSCs was next tested in both strains. In RAG mice, co-implantation with adult bone marrow (AdBM-MSC), fetal bone marrow (fBM-MSC), fetal placental (fPL-MSC), or adult placental (AdPL-MSC) resulted in a significant increase in cell engraftment reaching from 96-205 fold. fPL-MSCs had a slightly higher potential to promote ECFC engraftment (non significant when compared to other MSCs). In WT immunocompetent hosts, MSC co-implantation also had a significant but smaller effect on ECFC engraftment ranging from 0.2-11 fold. Adult-bone marrow MSCs had the best potential to promote ECFC engraftment. In addition to number of cell engrafted, co-implantation favoured increased human vessel density and enlarged vessel areas in implantation area. Culture experiments recapitulated these findings. Majority of ECFCs (>92%) died under serum starvation condition after 2 days. However, ECFC co-culture showed up to 6 fold improved ECFC survival in the presence of MSC populations with the exception of fBM-MSCs. ECFC survival upon implantation is dependent on the host adaptive immune system. Co-implantation of MSCs with PL-ECFCs resulted in

improved survival in both immunocompetent and immunodeficient hosts and resulted in improved vascular network formation. The modulation of neo-vessel formation and ECFC engraftment depends on the MSC source. Together, MSC and ECFC co-implantation approach provides an essential step towards clinical application of ECFCs in allogeneic recipients.

T-1154

INVESTIGATING THE ANGIOGENIC POTENTIAL OF PHILIPPINE MEDICINAL PLANTS USING HUMAN UMBILICAL CORD VEIN ENDOTHELIAL CELLS

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Plant parts and extracts have been used in Philippine traditional medicine to heal wounds, as well as treat various injuries and ailments. Angiogenesis is one of the events associated with wound healing and is a response to compensate for decreased blood supply in ischemic conditions. Tissue engineering approaches to regeneration of bone and other tissues also face the challenge of providing adequate supply to tissue grafts and constructs. On the other hand, angiogenesis is also implicated in tumor growth and progression, as well as in macular degeneration. In the quest to discover new small molecule modulators of angiogenesis, we investigated the potential angiogenic or antiangiogenic activity of approximately fifty (50) Philippine medicinal plants, starting with those associated with wound healing and healing of skeletal injuries. Aqueous ethanolic extracts of Philippine medicinal plants were used in the study. Preliminary phytochemical screening using spray reagents and toxicity testing using brine shrimp (*Artemia salina*) nauplii were initially done. The angiogenic potential of the plant extracts was evaluated by their effects on proliferation of human umbilical cord vein endothelial cells (HUVECs) as shown by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay and by their effect on tube formation of HUVECs in a matrix. Phorbol myristate (PMA), a known pro-angiogenic compound, was used as positive control. The in vitro tube formation assay confirmed pro-angiogenic properties of *Mangifera indica* L., as previously reported by several groups, but also revealed potential pro- and anti-angiogenic properties of several other medicinal plants, suggesting starting points for more detailed analysis.

T-1155

LONG-TERM RECONSTITUTING ENDOTHELIAL CELL ACTIVITY FROM FETAL LIVER CELLS

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Transplantation of tissue vascular progenitor cells with potential to engraft and generate long-term and functional endothelial vascular beds in multiple organs was proposed as an important strategy for genetic correction of vascular diseases, vascular regeneration and manipulation of vascular mediated organ homeostasis and growth. However, reconstitution of long term surviving vascular networks has been proved to be a difficult task. We previously showed that SCL-PLAP+ fetal liver (FL) cells derived from SCL-3'Enh-PLAP transgenic mice, expressing the human placental alkaline phosphatase (PLAP) reporter under the regulation of the Stem Cell Leukaemia (SCL) gene 3´enhancer, contained hematopoietic stem cells (HSCs) and also presented superior long-term reconstituting endothelial cell (LTR-EC) activity when transplanted into the blood stream of busulfan-treated newborn mice (1). In the current study, we characterized the in vitro and in vivo endothelial potential of cell subsets within the SCL-PLAP+ population from FL at day 12 of gestation (E12 FL), isolating the SCL-PLAP+Ve-cad+CD45- cell subset, a strongly committed, endothelial population with stable multi-organ endothelial reconstitution potential. Moreover, the spatial/temporal mapping of LTR-EC activity during development showed its ascription to the E11-E14 FL stages, despite the ubiquitous presence of the SCL-PLAP+Ve-cad+CD45- cells, including the Lyve1+ subset, indicating that location and developmental time are critical factors shaping the cell subset competence to contribute to vasculature in busulfan-treated newborn recipients. We will also present results on the quantification of the relative vascular grafted area at different times post transplant and the induction of hepatocyte proliferation by the endothelial graft, related to functional assessment of the graft. To our knowledge, this FL derived cell population represents the first strongly committed endothelial cell subset endowed with extensive and stable multi-organ vascular repopulation potential described to date.

EPIDERMAL CELLS

T-1128 *This poster board is located in a different topic area in the poster hall*

LONG-TERM MAINTENANCE AND PROLIFERATION OF MURINE SKIN EPITHELIAL STEM CELLS BY WNT-3A IN VITRO

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Wnt signaling is critical for regulation of a number of basic cell functions and deeply involved in stem cell maintenance. To elucidate the role of Wnt-3a in stem cell maintenance in skin, we prepared CD49f+CD34+ cells, a skin epithelial stem cell (EpSC)-rich population, from adult mouse skin and examined the effects of Wnt-3a on them using sequential cultures in vitro as well as hair follicle reconstitution in vivo. CD34+CD49f+ cells were collected using fluorescence-activated cell sorting and cultured for 10 days in the presence of Wnt-3a. At the end of that culture period, CD34+CD49f+ cells were sorted and subjected to a second 10-day culture with Wnt-3a. Using the same procedure, sequential cultures were repeated a total of 15 times. Cells showed proliferation of approximately 1000-fold by day 10 and CD34 expression was retained in about 10% of them. CD49f+CD34+ cells sorted on day 10 retained canonical Wnt responsiveness, proliferated markedly in the presence of Wnt-3a, maintained undifferentiated epithelial cell

marker expression, and promoted hair follicle development in vivo. Furthermore, CD49f+CD34+ cells obtained from each subsequent culture retained the same EpSC characteristics. CD34+ and CD34- cells were found to produce Wnt-3a and Wnt/β-catenin inhibitors, respectively. Also, CD34+ cells were seen residing as small cellular clusters surrounded by a large amount of CD34- cells. Exogenous Wnt-3a delayed the conversion of CD34+ to CD34- cells and suppressed the production of Wnt/ β-catenin inhibitors by CD34- cells. Our results suggest that Wnt-3a plays an important role in maintenance of epithelial stem cells in skin.

T-1156

PERICYTES, A MICROENVIRONMENTAL PLAYER IN SKIN TISSUE REGENERATION AND SKIN AGEING

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The microenvironment of human skin dermis plays a crucial role in epidermal regeneration and skin ageing. The best studied mesenchymal dermal cells to date known to maintain and regulate epithelial renewal are the 'fibroblasts'. We have identified a VLA-1 (integrin α1) bright population in human skin dermis that exhibits phenotypic and functional properties of MSC-like pericytes. Our previous studies have demonstrated that the inclusion of primary uncultured VLA-1^{bright} pericytes in addition to fibroblasts (VLA-1^{dim}) into dermal equivalents (DE), promotes epithelial reconstitution in 3D organotypic cultures. We now further characterize the role of VLA-1^{bright} pericytes in tissue regeneration and skin ageing. In organotypic cultures, *cultured* pericytes retain the capacity to promote epidermal regeneration. Moreover, DE populated solely with pericytes, induced a polarized basal keratinocyte morphology, with a densely packed and well-organized stratum basale that highly resembled normal skin. Our data also show, for the first time, that pericyte populated DEs restore basal expression of keratin 15, present in normal healthy skin but absent from in vitro reconstituted, psoriatic and wounded skin. We also explored the effects of different dermal microenvironments on the orientation of mitoses in the epidermal basal layer. Our initial data suggest that DEs populated solely with pericytes resulted in a greater proportion of mitoses parallel to the DE (symmetric division - giving rise to two basal cells) than perpendicular mitoses (asymmetric division - giving rise to a basal and a suprabasal cell). We propose that pericytes may promote epidermal cell regeneration by promoting more symmetric divisions within the basal layer and therefore maintain the epidermal progenitor state of these cells during tissue renewal. Other data also show that, the proportion of VLA-1^{bright} pericytes decreases in human skin dermis with increasing age, concomitant with a decrease in their in vitro growth rate. Nonetheless, their functional ability to promote epidermal regeneration remains unimpaired despite ageing Taken together, we propose that pericytes are critical a microenvironmental regulator of epidermal regeneration, and have untapped potential to improve ex vivo skin regeneration for autologous transplantation.

T-1157

EPIDERMAL STEM CELLS SYNTHESISE LESS PROTEINS THAN THEIR DIFFERENTIATED PROGENY

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Regulation of both genes expression and protein translation are key steps for stem cell fate decisions. While the transcriptional regulation of epidermal stem cell populations is increasingly understood, the regulatory importance of protein translation rates on stem cell fate remains unknown. The lack of reliable techniques to measure protein synthesis in vivo has made it difficult to study global protein synthesis in tissue stem cells. Here, we make use of the recently developed technique to quantify protein translation rate in mouse epidermal stem cells and their progeny in vivo by measuring O-Propargyl-puromycin (OP-puro) incorporation into nascent peptides. We find that distinct hair follicle stem cell populations marked by keratin 19 (quiescent stem cells) or Lgr5 (activated stem cells) synthesize the same amount of protein. The distinct protein translation rate is unaffected by the hair cycle state and remains comparable both when the two populations are isolated at telogen (resting) and anagen (growing) phase of the hair cycle. While cell cycle entry influence translation rate, it plays only a minor role in controlling global protein synthesis in skin stem cells. In contrast, we observed that hair follicle stem cells produce less protein than their differentiating progeny and cells exhibiting the highest protein synthesis rates were non-dividing cells committed to undergo terminal differentiation. In conclusion, regulatory mechanisms controlling global protein synthesis in skin stem cells act largely independent of cell division and are determined by the differentiation programme.

T-1158

DOMINANT NEGATIVE MUTATION OF SOX18 ALTERS THE FORMATION/MAINTENANCE OF DERMAL CONDENSATES DURING FETAL DEVELOPMENT AND AFFECTS HAIR FOLLICLE DEVELOPMENT

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The hair follicle is a unique skin appendage, with the ability to continually regenerate from a population of multipotent epidermal stem cells relying on signals from the dermal papilla, a condensed region of dermal cells at its base. Although Sox18 has been reported to be important for the specification of the most frequent murine and human scalp hair types, very little is known about its expression and activity during hair development. Using classical murine models, the RaOP mouse, with a dominant negative mutation in the Sox18 gene, and a Sox18-GFP reporter, we investigated this role. Sox18 was expressed specifically in the hair follicle dermal papilla and not in any other epidermal compartment from birth to P21. Lymphatic vessels were also seen to be occasionally expressing Sox18. In RaOp mice, it was possible to observe hair follicles of guard and awl/auchene types that depended on Sox2 expression in their dermal papilla. However, many epidermal buds did not develop into full follicles despite having the classical epidermal stem cell markers such as Sox9 or Keratin15. Labelling of dermal papilla using alkaline phosphatase demonstrated that these structures did not have a dermal papilla, suggesting the importance of Sox18 in this process. Similarly, integrin alpha 8, CD133 and other dermal papilla markers confirmed these findings. We demonstrated in hair regeneration assays that keratinocytes from RaOp mice could form

hair follicles if combined with dermal spheroids derived from WT mice. In contrast, dermal cells, or dermally-derived spheroids from RaOp mice could not induce hair follicle regeneration even when associated with WT keratinocytes. To understand further the role of Sox18, gene expression arrays were performed on WT and RaOp dermal spheroids and revealed about 600 differentially expressed genes, some known to be regulators of hair follicle development and cycling such as Wnt5a, MMP9 or retinoic acid. In conclusion, the Sox18/RaOP mutation results in the inhibition of dermal papilla differentiation and prevents the mesenchymal-epidermal cross talk for the development of hair follicles.

T-1159

THE SIGNIFCANT EFFECTS OF EPIDERMAL STEM CELL NICHE-LIKE MICROENVIROMENT ON MODULATING HUMAN EMBRYONIC STEM CELLS EPIDERMAL COMMITEMNET KINETIC AND SUBSEQUENT PROLIFERATIVE POTENTIAL

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A wealth of published data shed a light on differentiation pathways of hESC toward various epidermal lineages, amid at different population purity and expansion ability. Currently, epidermal progenitors derived in feeder-free differentiation systems show only 5-8 population doubling. The limited replicative potential of hESC-derived keratinocytes pose a significant barrier in wide application of tissue engineered skin constructs populated with hESC-derived epidermal cells for in vitro and clinical applications. There is a necessity to elucidate and evaluate the limited replicative potential of hESC-keratinocyte in order to devise efficient and robust differentiation systems for their wide applications. The aim of this study is to elucidate the reason underlying this limited proliferative capacity of derived keratinocytes in feeder free systems in defined culture milieu and subsequently improving their replicative potential though establishment of epidermal stem cell niche-like microenvironment. hESCs committed to keratinocytes in two differentiation systems. One using novel decellularized fibroblast extracellular matrix (ECM) microenvironment in defined medium with growth ability of 8 population doublings during 50 days. In another system the same ECM microenvironment was utilized with addition of macromolecular crowding (MMC) reagent to induce deposition of basement membrane ECMs proteins and establish epidermal stem cell-like niche during course of differentiation. The keratinocytes derived under MMC condition showed 14 population doublings with enhanced culturing ability until 70 days. Our results demonstrated that MMC condition can induce formation of significant stem cells population of epidermal progenitor cells and the keratinocytes derived under this system showed lower telomere length erosion rate and lower expression of p16 and p21. By seeding the epidermal cells on top of collagen scaffold they formed stratified epithelia after 2 weeks exposure to air-liquid interface.

T-1160

MIR-184 REPRESSES K15 AND INDUCES CORNEAL AND EPIDERMAL STEM CELL DIFFERENTIATION

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The skin and the cornea are situated at the surface of our body and eye, providing us protection against external insults such as microorganisms, toxicants and physical injury. These tissues share many features including structure, function and common molecular pathways in health and disease. MicroRNAs (miRNAs) play a role in various physiological processes including embryogenesis, tissue regeneration and diseases. Here, we report that miR-184, a highly evolutionary conserved miRNA, regulates epithelial homeostasis. In situ hybridization analysis revealed that miR-184 is predominantly expressed in the adult mouse cornea, the developing lens, epidermis and hair follicles. The expression of miR-184 was restricted to progenitors or early differentiated cells in vivo and in vitro but was absent in the stem or terminally differentiated cell compartment. We further demonstrated that miR-184 is repressing the stem cell marker cytokeratin 15 (K15), cell proliferation and induce differentiation. This was correlated with an increase in Wnt/β-catenin and Notch pathways, which play a key role in the activation of epithelial stem cells and their differentiation. Interestingly, in corneal pannus collected from patients that suffer from severe limbal stem cell deficiency, we observed an uncontrolled increase in miR-184 expression that was coupled with a decrease in K15. Indeed, ectopic expression of miR- 184 in limbal stem cell-enriched culture resulted in a dramatic decrease in clonogenic potential. Altogether, these data indicate that miR-184 induces an escape from 'stemness' state while abnormal expression of miR-184 may lead to a decline in epithelial stem cell reservoir.

T-1161

THE ORIGIN AND REGULATION OF CORNEAL EPITHELIAL STEM CELLS

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Accumulating evidence supports the dogma that the corneal epithelium is regenerated by stem cells located exclusively in the limbal niche, at the corneal periphery. Moreover, limbal stem cell (LSC) loss leads to corneal opacity and blindness while limbal grafting restores patients' vision. However, contradicting data suggested that the limbus does not participate in corneal homeostasis and that the cornea itself contains stem cells. Here, we aimed to identify the origin, fate and regulation of stem cells of the corneal epithelium. We performed lineage tracing experiments using R26R-Confetti mice to follow K14+ limbal/corneal epithelial progenitors under homeostasis and injury. We found out that the limbus is the major resource of corneal regeneration under homeostasis, while rare corneal clones that did not migrate centripetally, but survived for over 4 months, were detected. Interestingly, centripetal migration of limbal cells was slower than expected as shown by radial limbal stripes that reached the corneal center within 4-5 months. Under wounding conditions, corneal progenitors significantly contributed to tissue repair while large limbal streaks appeared within a week following severe injury that coincided with partial loss of corneal transparency. Altogether, this data, which is in line with a back-to back publication by Di Girolamo and Lyons, suggests that the limbus plays a major role in corneal renewal, while the cornea has a long-term self-maintenance capability. In a second set of experiments we explored the regulation

of LSC by microRNAs. Previously, we identified miR-450b and miR-184 as regulators of corneal epithelial fate. Here, we showed that these miRNAs were not expressed by LSC but by their progeny in vitro and in vivo. In line, both miRNAs enhanced calcium-induced differentiation and reduced clonogenicity of limbal cells. Interestingly, miR-450b targets and inhibits Pax6, a key transcription factor that is considered to be essential for the maintenance of LSC pool while we further identified miR-184 is an inhibitor of the stem cell marker cytokeratin 15. Indeed, point mutations in MIR184 were recently linked with blinding eye dystrophy affecting the cornea and the lens. In future studies the role of these miRNAs in LSC migration/regeneration may be examined using confetti mice.

EPITHELIAL CELLS (NOT SKIN)

T-1162

LGR6 EXPRESSING CELLS IN MAMMARY GLAND DEVELOPMENT AND TUMORIGENESIS

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Different breast cancer subtypes are thought to arise from specific breast epithelial stem or progenitor cells. A refined characterization of the cellular hierarchy of mammary gland development is crucial to identify the cells-of-origin or cancer stem cells (CSC) that underlie various tumour types. In recent years, leucine-rich repeat-containing G-protein coupled receptors (LGR) have emerged as markers of tissue stem cells. The most prominent member, the Wnt target gene Lgr5, serves as a stem cell marker in intestine and colon, stomach, hair follicle, ovary and liver, whereas Lgr6 marks certain skin and hair follicle stem cells. Furthermore, Lgr5 has been described to mark CSC in intestinal adenomas. Due to the fact that Lgr5 expressing epithelial cells and Wnt-activated stem cells can be found in the murine mammary gland, we speculated that Lgr6 could similarly mark stem cells or progenitor cells in normal mammary gland development and CSCs in murine mammary tumors. We have applied genetic lineage tracing to follow the fate of rare Lgr6 expressing epithelial cells in the mammary gland in vivo. Thus, we could uncover distinct contributions of Lgr6+ mammary epithelial cells and their progeny to mammary gland development, remodelling in pregnancy and to the maintenance of homeostasis. To address a potential role of Lgr6 expressing cells and their progeny in mammary tumour development we traced the fate of the Lgr6 expressing cell population in mouse models presenting with basal- or luminal-type tumours.

T-1163

ASYMMETRIC CELL DIVISION AND STEM CELL ACTIVITY IN MAMMARY EPITHELIAL CELLS IS REGULATED BY EXTRACELLULAR MATRIX ATTACHMENT

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Adult stem cells maintain tissue homeostasis and integrate various signals from the surrounding niche. The stem cell niche consists of supporting cells and extracellular matrix (ECM) component, which together modulate stem cell functions, including asymmetric cell division. While advances have been made in the characterization of core properties of niche-stem cell interactions, the precise role of ECM or its alterations in regulation of asymmetric cell division or maintaining stem cell activity is unclear. Previous studies suggest that ECM has a pivotal role in sustaining tissue homeostasis and it is known to change considerably during the processes of aging and cancer. Therefore, determining how age and parity related changes in ECM affect stem cell maintenance of the mammary epithelium will be important not only for stem cell biology but also for understanding cancer initiation. Our preliminary results show that while young and old primary mouse mammary epithelial cells differ only slightly in their capability to grow as mammospheres, they may differ significantly in their ability to produce ECM constituents, as well as to respond to stemness maintaining cues from the ECM. Furthermore, our results using a cell culture model of human mammary stem-like cells suggest that different ECM constituents modulate stem cell maintenance by affecting their ability to undergo asymmetric cell division. In conclusion, our results imply an important role for ECM attachment in regulation of asymmetric cell division and stem cell activity and will further provide new insight in to the role of ECM in aging and cancer.

T-1164

OVOL2 AND PAX6 REGULATE TRANSCRIPTIONAL PROGRAMS OF ECTODERMAL LINEAGE

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In development, embryonic ectoderm differentiates into three lineages that are neuroectoderm, epidermis and corneal epithelium, although the mechanism distinguishing these lineages remains largely unclear. Here we report on master TFs regulating the differential transcriptional programs between the three lineages. By the functional screening which assays TF's interference with dedifferentiation (PNAS 110:6412-6417, 2013), we identified OVOL2 and PAX6 as master transcription factor(TF)s in corneal epithelial cells (CECs). The 6TFs including OVOL2 and PAX6 were able to induce the transcriptional profile of CECs when overexpressed in fibroblasts, of which lineage was presumably distant from ectoderm. In CECs, PAX6 repressed epidermis-specific genes, whereas OVOL2 repressed neuroectoderm-specific genes. Moreover, OVOL2 alone was able to induce the transcriptional profile of CECs in neural progenitors. This process involved regulation of epithelial-to-mesenchymal transition (EMT), a regulatory mechanism of the gene set in a modular manner. Our data suggest that PAX6 and OVOL2 combinatorially regulate the transcriptional programs of the three lineages and provide an evidence for modular mechanism to regulate transcriptional programs of distant lineages.

T-1165

AUTOPHAGY MAINTAINS STEM CELL SURVIVAL BY REGULATING REDOX BALANCE IN UVA-IRRADIATED CORNEAL STEM CELLS

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Ultraviolet-A (UVA), as the most common environmental radiation causing oxidative stress to our eyes, is often associated with acute corneal injury, such as photokeratitis, and chronic ocular surface disorders, such as pterygium and keratinizing squamous metaplasia. Limbal stem cells (LSC) are tissue reservoir cells responsible for maintaining corneal homeostasis at resting state and repairing tissue upon UVA-induced photo-oxidative tissue damage. Autophagy, a lysosomal degradative system for removal of damaged organelles and clearance of differentiation-impairing proteins, has been linked to the function of several types of stem cells for maintaining tissue integrity in response to variable exogenous stressors, including UVA-induced reactive oxygen species (ROS). To investigate whether autophagy plays a role in LSC's stress response to ROS, we created an LSC-specific autophagy-deficient transgenic mouse model, Map1lc3GFP:Krt14Cre:Atg7f/f. Mice were subjected to UVA irradiation at various doses (0-80 J/cm2), followed by fibronectin-based rapid adhesion LSC enrichment. Results demonstrated that UVA irradiation activated autophagy, as evidenced by dense perinuclear LC3-GFP punctae observed in 42.86 + 5.64% of Atg7f/f LSCs vs. 15.57 + 1.88% in Krt14Cre:Atg7f/f LSC colonies ($p<.05$). In addition, an LC3I-to-LC3II band conversion in Western blot (WB) was detected in Atg7f/f LSCs but not in Krt14Cre:Atg7f/f LSCs. Moreover, intracellular ROS levels revealed by CM-H2DCFDA live staining were ~35% higher in irradiated Krt14Cre:Atg7f/f LSCs, compared to the Atg7f/f counterparts. UVA-induced ROS levels were rescued by antioxidant pre-treatment by a 84.89% decrease in autophagy-competent LSCs vs. a 3.78% decrease in autophagy-deficient LSCs. Compared to unirradiated LSCs, flow cytometry showed a 21.99% increase of apoptotic cells in Atg7f/f LSC and a 79.46% increase in Krt14Cre:Atg7f/f LSCs upon UVA irradiation. With antioxidant rescue, the apoptotic rate dropped to a near baseline level both in Atg7f/f and Krt14Cre:Atg7f/f LSCs (0.33-fold and 0.46-fold, respectively). Collectively, our data reveal a pivotal role of autophagy in LSC survival by balancing intracellular UVA irradiation-induced ROS levels.

T-1166

KIT+ EPITHELIAL PROGENITORS REQUIRE SOX10 FOR THEIR MAINTENANCE

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Irradiation damage to salivary glands during cancer treatment often leads to a permanent loss of saliva production. Regeneration of irradiated submandibular glands (SMGs) in mice can occur after transplantation of SMG KIT+ epithelial progenitors. We previously demonstrated that KIT+ progenitor expansion involves combined KIT/FGFR2b signaling, which rapidly up-regulates a core set of transcription factors, including SOX10. Neuronal progenitors are known to express SOX10 where it regulates their maintenance. However, the function of SOX10 in epithelial KIT+ progenitors is not well understood. We aim to understand the function of SOX10 in KIT+ progenitors, and hypothesize its involvement in KIT+ cell expansion. By using a Cre-Flox mouse system, we specifically deleted SOX10 protein and mRNA in proliferating epithelial KIT+ cells. Neuronal cells, on the other hand, still expressed SOX10. Morphologically, SOX10-depleted SMGs formed less distal endbuds during development so that by E16 the glands were 50% smaller than control glands. qPCR and protein analysis both revealed a reduction in KIT-expressing cells as well as in CCND1-mediated proliferation. Other progenitor-markers, such as Krt14 and Krt5, and their progeny remained unchanged. Similarly, transcriptional and morphological analysis of surrounding nerves and blood vessels were unaffected. These data indicate that SMG epithelial KIT+ progenitors require SOX10 for their maintenance and proliferation, which is important for proper organ development.

T-1167

REGULATION OF STEMNESS OF DENTAL EPITHELIAL STEM CELLS BY RHO SIGNALING

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Rodent incisors grow continuously throughout life. This growth is supported by the division of dental epithelial stem cells that reside in the cervical loop region (apical bud). We have previously shown that dental epithelial stem cells divide rarely and asymmetrically, and one daughter cell remains as an undifferentiated stem cell in the cervical loop whereas the other cell give rise to actively proliferating transit amplifying (TA) cells which finally differentiate into ameloblasts. The dental epithelial stem cells with mesenchymal cell-shaped morphology are maintained in stellate reticulum (SR) region of apical bud, and once differentiate into TA cells, they move to the incisal end and turn into square-shaped cells. We have recently found that Rho regulated epithelial-mesenchymal transition (EMT) through the maintenance of the cell polarity and cell-cell adhesion. In this study, we examined the function of Rho signaling in dental epithelial stem cells. After inhibition of ROCK (downstream Rho effectors), the shape of dental epithelial cells isolated from mouse incisor apical bud (mHAT 9d) changed from epithelial phenotype to mesenchymal-like cells. qPCR showed that ROCK inhibitor decreased expression of epithelial cell markers, and increased mesenchymal markers, suggesting an induction of EMT. In the treated cells, mRNA expressions of slug (EMT markers), and Sox2 (dental epithelial stem cell marker) also increased and the intense nuclear accumulation was observed. Further, immunohistochemistry of mouse incisors showed Yes-associated protein (YAP) transcriptional coactivator was strongly expressed in actively proliferating TA cells, whereas not expressed in dental epithelial stem cells. Treatment of Rho activator promoted YAP transcriptional activity in mHAT9d, as assayed by luciferase reporter assays. Conversely, inhibition of ROCK inhibited YAP transcriptional activity, suggesting that Rho regulated cell proliferation through YAP. Together, these results indicated that Rho signaling was multifunctional pathway that control stemness of dental epithelial stem cells, and rodent incisor was an excellent model for analyzing various aspects of stem cell regulation and function.

T-1168

ON THE WAY TO TOOTH BIOENGINEERING: FROM SOX2 GENE TO SOX2+ CELLS

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The embryonic formation and adult homeostasis of organs is of interest to scientists at the molecular, cellular and evolutionary levels. The mouse continuously growing front tooth serves as a valuable model to approach these issues. We analysed the genetic network involved in epithelial stem cell (SC) segregation during tooth development, and their maintenance in adult incisor renewal. We succeeded to reveal the importance of Sox2 during incisor formation and the role of Sox2+ cells in incisor renewal. In order to understand the role of the Sox2+ cell population during the incisor renewal, we selectively removed this cell population by the transitory expression of DTA in Sox2+ cells. Strikingly, 4 to 6 weeks after the ablation, the tooth did not exhibit any renewal defect in the stem cell niche. Therefore, we studied the function of Sox2 transcription factor during the tooth formation and renewal, we generated Sox2 conditional knock-out (cKO) mouse and analysed the tooth phenotype. While the incisors were able to form, the tooth orientation and cell differentiation were largely impaired. Altogether, our results suggested that while Sox2 expression seems to not be necessary for tooth formation, it is required for subsequent cell differentiation. Moreover, the Sox2+ SC population can be replaced in case of ablation, reflecting a possible co-existence of different stem cell populations in the continuously growing incisor. Also, we will try to generate tooth-like structures in vitro. So far we have managed to generate organoids from SC from the mouse CL and we aim to reproduce this experiment using more naïve cells as a starting material. Once this will be accomplished we will differentiate the SC into dental epithelium cells and will combine them with dental mesenchyme in order to differentiate them into a complete tooth.

T-1169

IDENTIFICATION AND CHARACTERIZATION OF CD133-POSITIVE CELLS FROM MOUSE SALIVARY GLAND

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Hypofunction of salivary glands due to Sjögren's syndrome or radiation therapy for head and neck cancers can promote various oral diseases and aspiration pneumonia in severe cases. However, there is no effective therapy for the patients with damaged salivary glands. Stem cell-based therapy is a promising treatment for these patients. Here, to examine whether CD133+cells have stem cell-like property, CD133+cells were isolated from mouse salivary glands and also characterized. Submandibular gland cells were dispersed by enzymatic digestion and stained with anti-CD133-APC and anti-Lin-FITC antibodies. CD133+Lin-cells were sorted using flow cytometry. The analysis confirmed the presence of CD133+Lin- cells, whose percentages were 3.0%. In vitro, CD133+Lin- cells showed significantly higher abilities of colony and sphere formations as compared to CD133-Lin-cells, suggesting that CD133+ cells have progenitor/stem cell-like potential. We found that CD133+ cells showed higher expression of CK18 than CD133- cells. Consistent with this, CD133 was localized in ductal cells. Furthermore, to clarify whether CD133+Lin- cells have tissue reconstitution ability, CD133+Lin- cells isolated from transgenic mice expressing GFP were transplanted into the partially excised submandibular gland of wild type mice. The CD133+Lin-GFP+ cells differentiated into ductal and acinar cells. The CD133+Lin-- transplanted cells were able to efficiently reconstitute the excised glands. In addition, the differential gene expression patterns of the CD133+Lin- and CD133-Lin- cells were analyzed using RNA sequence. The analysis revealed that c-kit and Sox9 were highly expressed in CD133+Lin- cells. These findings suggest that CD133+Lin- cells of the adult salivary glands have progenitor/stem cell-like ability.

EYE OR RETINAL CELLS

T-1170

UNLEASHING THE POTENTIAL OF ADULT MOUSE RETINAL STEM CELLS IN VIVO

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In lower vertebrates, Müller glia and Retinal Stem Cells (RSCs) located in a peripheral region of the retina named the ciliary margin zone (CMZ) have the potential to generate new neurons throughout life, and regenerate damaged retinas after injury. In mammals, however, endogenous repair is not observed. Müller glia can only produce a very limited number of new neurons in vivo, and although the ciliary body (CB) - which develops from the CMZ - was proposed to contain adult RSCs, the existence of these cells in vivo remains controversial, and thus far, they were only shown to produce neurons when grown in culture. What prevents adult RSCs from generating new neurons in vivo is unknown. Here we show that the endocytic adaptor Numb, an antagonist of Notch signalling, is expressed in the developing and adult CB. Conditional inactivation of Numb specifically in the peripheral retinal and CMZ results in a massive expansion of the adult CB that contains several types of differentiated neurons, suggesting that Numb plays a part in maintaining RSCs quiescence and/or in CB cell fate specification. In neurosphere assay, we also found that Numb knockout spheres are significantly larger than controls, suggesting that Numb inhibition releases proliferation of adult neurospheres-forming cells. To ask whether the cellular expansion observed was due to loss of Numb function in the CMZ, we used a tamoxifen-inducible Cre mouse line inactivating Numb specifically in the developing CMZ from embryonic day 14 (cKO). Strikingly, cell-lineage tracing in both controls and Numb cKO revealed the presence of cell clusters composed of all retinal cell types in the peripheral retina of postnatal mice, but, strikingly, significantly more clusters were detected in the Numb cKO. These data indicate that cells from the CMZ normally contribute to neurogenesis in the peripheral retina, and that Numb is required to maintain RSCs quiescence and/or promote the ciliary epithelium fate at the expense of the neural cell fate.

T-1171

DIFFERENTIATION AND ENRICHMENT OF RETINAL GANGLION CELLS FROM HUMAN EMBRYONIC STEM CELLS

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Pluripotent stem cells (PSCs) provide an invaluable tool for disease modelling in vitro. Diseases such as glaucoma, a heterogeneous group of optic neuropathies that culminate in retinal ganglion cell (RGCs) degeneration, are difficult to study due to limited access to retinal tissue pre-mortem and complex etiology. Here, we report the differentiation of human embryonic stem cells (hESCs) into retinal lineages and enrichment using magnetic-activated cell sorting (MACS) for the isolation of RGCs. RGCs were differentiated from hESCs into embryoid bodies formation and plated down as adherent culture on Matrigel matrix with DKK-1, IGF-1, noggin and bFGF. Cells were differentiated for 30 days followed by enrichment with MACS for RGCs positive for the surface antigen THY1.1/CD90.1 and then cultured for another 15 days. Quantitative RT-PCR (qRT-PCR), immunocytochemistry and whole-cell patch-clamp electrophysiology were used to characterize hESC-derived RGCs in vitro. Our results demonstrated successful hESC differentiation into RGCs with long axons. Gene expression analysis revealed the increased expression of retinal progenitor cell markers (*PAX6, RAX, ATOH7, MITF*) and RGC markers *(BRN3B, ISLET-1)* during 30 days of differentiation. Immunocytochemistry analysis revealed the presence of hESC-derived RGCs that were positive for common RGC markers within the retina (BRN3A, VGLUT1, βIII-TUBULIN, HU C/D). Further, electrophysiological testing revealed the presence of functional neurons with active sodium channels and capable of generating mature action potentials. These results indicate that hESCs can be differentiated into the retinal lineage and MACS can successfully isolate hESC-derived RGCs. This study describes a method to generate an enriched population of RGCs from hESCs, providing a necessary step for future optic neuropathic disease modelling studies using PSCs.

T-1172

EFFICIENT XENO-FREE AND FEEDER-FREE CULTURE SYSTEM FOR THE GENERATION OF RETINAL CELLS FROM CONFLUENT HUMAN IPS CELLS

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For retinal cell therapy based on human induced pluripotent stem (iPS) cells, one of the major challenges is to develop essential culture conditions for the use of these cells for future clinical purposes. Until recently, iPS cell culture (maintenance and/or differentiation) has been carried out using feeder cells and/or culture media that contain animal products. Here, we adapted our new retinal differentiation method using confluent human iPS cells, bypassing cell clumps or embryoid body formation and in absence of Matrigel or serum , in a well defined xeno-free (XF) / feeder-free (FF) system. Integration-free iPS cells cultured on mouse embryonic fibroblasts were transferred onto vitronectin-coating plates and cultured with XF medium. Confluent iPS cells obtained in these XF/FF conditions were directed toward a retinal lineage in a XF proneural medium. In less than one month, confluent iPS cells are able to generate simultaneously retinal pigmented epithelial (RPE) cells and self-forming neural retina (NR)-like structures containing multipotent retinal progenitor cells (RPCs). Emergent NR-like structures were isolated and cultured in floating conditions for their maturation in a XF proneural medium. Immunohistochemistry and qRT-PCR analysis triggering specific retinal markers confirmed that RPCs were able to differentiate into all types of retinal cells. Early-born retinal cells (i.e. ganglion, amacrine and horizontal cells) were identified after one month in culture, and late-born retinal cells (i.e. photoreceptors, Muller glial and bipolar cells) started to appear after two months. Human iPS-derived RPE cells can be amplified in XF conditions while retaining their phenotype close to their in vivo state. These data demonstrate that human iPS cell lines can be maintained and directed to differentiate into retinal cell types under XF/FF conditions that are required for translation to clinical applications. In this context the reliable generation of retinal ganglion cells, photoreceptor precursors or RPE cells could find important applications in regenerative medicine.

T-1173

TOWARD IN VIVO RETINAL STEM CELL ACTIVATION: INHIBITION OF BMP AND SFRP2 PROTEINS IN THE ADULT MOUSE EYE INDUCES CILIARY BODY-SPECIFIC PROLIFERATION AND EXPANDS THE RETINAL STEM CELL POPULATION

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Adult retinal stem cells (RSCs) are rare cells that reside in the pigmented ciliary epithelium (CE) of the mammalian eye. Once dissociated from the CE, RSCs readily proliferate to form clonal, free floating spheres after 7 days, with the capacity to self-renew and differentiate into all of the cell types of the neural retina and retinal pigmented epithelium. Despite having the capacity to proliferate in vitro, 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. We injected noggin or α-sFRP2 antibody intravitreally into the right eye 3 times, once every 24hrs, at 2 doses (1.5mg/mL or 2.5mg/mL). The left eye was injected with PBS as a control. During the injection period, all mice received EdU in their drinking water, but one group received no injections to serve as naïve controls. Mice were euthanized at either 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 treatment did not affect the number of EdU+ cells in NR 24hrs or 4 weeks post-injection. 24hrs post-injection, there were more EdU+ cells in the CB of the 2.5mg/mL α-sFRP2 treated eyes than the controls and the 1.5mg/mL dose. Both the 1.5mg/mL and 2.5mg/mL noggin treated eyes had increased EdU+ cells in the CB compared to the controls. 4 weeks post-injection, α-sFRP2 treated eyes had more EdU+ cells than both controls in the CB. Noggin treated eyes also had more EdU+ cells in the CB than naïve control but did not reach significance compared to PBS control. This indicates that α-sFRP2 and noggin may have different efficacies or mechanisms to induce proliferation, or may be acting on different cells. To investigate this further, we performed a clonal sphere assay 7 days after injection

of noggin or α-sFRP2. We found that α-sFRP2 doubled the number of primary RSC spheres, whereas the increase in noggin treated eyes was not significantly different from PBS control. These results establish that inhibition of BMP and sFRP2 signaling can cause CB-specific proliferation within the adult mouse eye. Further, inhibiting sFRP2 appears to expand the retinal stem cell pool.

T-1174

GENERATION OF AN IMMORTALISED HUMAN RETINAL GANGLION CELL (RGC) LINE FROM PLURIPOTENT STEM CELLS

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Retinal ganglion cells (RGCs) are specialised neurons which relay visual information obtained by the retina for processing in the brain. Currently there are no good human RGC lines available and studies of RGCs have relied heavily on the use of primary cells and tissues obtained from animals, as it is difficult to obtain adequate samples from the human eye. Having sufficient samples will allow studies of RGC physiology and to model pathological mechanisms in disease, highlighting the importance and urgency for a true cellular model of RGC. One approach to acquire human RGCs samples non-invasively, is to differentiate RGCs from human pluripotent stem cells. However, current RGC differentiation protocols generate a heterogeneous population of cells which can add significant experimental variation. In order to obtain cells with consistent characteristics, we propose to generate immortalised cell lines from human pluripotent stem cell-derived RGCs. The immortalised cell lines generated from this project will be made available to the scientific community as a cellular model to study the biology of RGCs. Future studies using this immortalisation approach can be extended to model neuro-retinal pathologies (such as glaucoma and LHON) where immortalised diseased cell lines can be used to study the pathology and to test for potential therapeutics via high throughput drug screens.

T-1175

INVESTIGATION OF EXTRACELLULAR MATRIX (ECM) COMPONENT EXPRESSION IN DEVELOPING RETINA

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In Europe, approximately 3.5% of the population experience sight loss and 75% of those are unemployed, representing a social and economic burden which will likely increase as the population ages. Diseases affecting the retina account for approximately 26% of blindness globally and 70% of blindness in the UK. Recent ground-breaking work has shown that neural tissue containing each of the retinal phenotypes and closely resembling the developing human retina can be made in vitro from human pluripotent stem cells (hPSCs). No treatments currently exist to restore vision in many retinal diseases and therefore this lab-generated retina represents an excellent platform for the study of human retinal development and biology, disease modelling, and could provide an unlimited source of functional cells for transplantation. The ability to produce retina from stem cells is in its infancy and requires further optimisation. The production of light sensitive photoreceptors, for example, is an extremely rare event. We hypothesise that ECM plays an important role in retinal histogenesis and insights learnt from this process may influence the differentiation process of hPSCs. Therefore, we studied the tempo-spatial expression profile of key ECM molecules in the developing human eye to understand potential involvement in human retinal development and maturation. Expression of these ECM components was also investigated in post mortem adult human eyes and compared with other species (mouse & monkey) to understand whether the distribution was highly conserved and therefore likely to be important for both retinal histogenesis and tissue maintenance. Our results to-date show that laminin, aggrecan, fibronectin and collagen IV are strongly expressed in Bruch's membrane (BrM) and inner limiting membrane of the adult human eye. Versican is most abundant around the photoreceptor outer segments; hyaluronic acid was most strongly expressed in the ganglion cell layer (GCL) and the inner nuclear layers (INL), whilst neurocan was most abundant in the inner and outer plexiform layers (IPL and OPL). SPACRC was found to be most strongly expressed in the INL and GCL. At 12 weeks post conception, laminin is abundant in the INL, the interphotoreceptor matrix and BrM although laminin sub-types shown distinct expression patterns and potential roles.

T-1176

DIFFERENTIATION OF ADULT RETINAL STEM CELLS INTO CONE PHOTORECEPTOR-SPECIFIC PROGENITORS

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Adult retinal stem cells (RSCs), rare pigmented cells in the ciliary epithelium of many mammals, are capable of giving rise to retinal neurons including photoreceptors. Here we report the induction of an enriched population of cone photoreceptors from RSCs, and the cell biological mechanisms underlying this enrichment. Using coco (a multifunctional TGFβ, Wnt and BMP antagonist) during differentiation of RSC progeny, we found that nearly 45% and 55% of RSC progeny treated with coco (in addition to pan-retinal FBS differentiation conditions) were positive for S-opsin and cone arrestin (markers of mature cones), respectively. In contrast, in pan-retinal differentiation conditions alone RSC progeny produced only 1% cones. The coco induced cone arrestin+ cells did not express the rod marker, rhodopsin, suggesting coco is not inducing rods to ectopically express cone arrestin. The S-opsin+/cone arrestin+ cells were also negative for RPE65, a RPE cell marker. To investigate the cell biological mechanisms underlying the effects of coco on RSC differentiation, we sorted undifferentiated RSC progeny into single non-pigmented (NP) or pigmented (P) progenitors, plated them at a single-cell-per-well and differentiated the clones in coco or pan-retinal control conditions for 28 days. We found that 50 % and 45% of P and NP clones survived in coco conditions, respectively. However, less than 40% of P and 30% of NP clones survived in pan-retinal conditions suggesting a possible survival role for coco. Most important, only clones from NP progenitors in coco were cone arrestin+.

Interestingly, smaller sized clones (<100 cells, n=10 clones) were 100% cone arrestin+. The larger clones, in contrast, were between 86%-96% cone arrestin+ (n=37 clones). These data indicate that coco acts only in NP progenitors, possibly in an instructive manner. However, coco must be present continuously throughout the 28-day differentiation period in order to see an enrichment of cone differentiation, suggesting a potential selective survival effect of coco. These data reveal that RSC NP progeny differentiated into cone photoreceptors may provide a source for targeted cell therapy/ transplantation.

T-1177

LOW OXYGEN CAN INDUCE IN VITRO DIFFERENTIATION OF HUMAN FETAL RPE PROGENITORS INTO NEURON-LIKE CELLS

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It has been shown that RPE cells can differentiate into neuronal-like cells in vitro. In this study we investigated the role of environmental oxygen concentration on the induction of neuronal markers during maturation of RPE progenitors in tissue culture. Human fetal eyes (18 weeks gestation age) were obtained from Advanced Bioscience Recourses (ABR) Inc. Primary cells were propagated in low (5%) O2 conditions in MEM medium containing 5% of FBS until passage 3. The expression of RPE specific markers was tested by flow cytometry. For differentiation purposes, expanded RPE progenitors were cultivated in transwell permeable inserts under normal or low O2 conditions in RPE medium containing 1% FBS. Barrier function was assessed by measuring transepithelial resistance (TER). The cell morphology and expression of neuronal markers such as beta - III tubulin and RPE markers such as ZO1 and Na/K ATPase were evaluated by immunocytochemistry. Prior to differentiation, RPE progenitors were ≥ 90 % positive for pancytokeratin, ZO1, and RPE65. As reported previously, RPE progenitors differentiated under normal oxygen conditions showed signs of mature RPE cells such as increased TER, hexagonal morphology, re-pigmentation, well-developed tight junctions, clear presence of ZO-1 and Na/K ATPase. The expression of beta - III tubulin was insignificant or not detectable. In contrast, RPE cells differentiated in low oxygen did not upregulate the TER values. The cells appeared heterogeneous with no signs of pigmentation and some of them morphologically resembled neural cells. On day 10 in tissue culture, cells showed high level of nestin and low beta - III tubulin expression. On day 20, however, beta - III tubulin marker was acquired while nestin expression was down regulated. These data suggest that low oxygen concentration promotes induction of neuron-like cells from RPE progenitors during their maturation in vitro.

T-1178

HIGH-RESOLUTION TRANSCRIPTIONAL MAPPING OF PURIFIED, HUMAN STEM CELL-DERIVED LENS-LIKE CELLS BY RNA-SEQ SHOWS STRONG IDENTITY TO PRIMARY HUMAN LENS EPITHELIAL CELLS

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The ocular lens has been an important model for defining developmental mechanisms for over a century, including Spemann's and Waddington's seminal works on embryonic organisers and lens transdifferentiation in the 1920s and 1930s. However, modern lens research has been severely hampered by poor access to human lens cells. Accordingly, a reproducible and large-scale supply of human lens epithelial cells (LECs) would enable valuable new insights into fundamental developmental mechanisms, the molecular events that drive cataract formation, as well as identification of candidate anti-cataract agents. We previously demonstrated that human LEC-like cells can be purified efficiently, reproducibly and in large numbers from differentiating pluripotent stem cells using a magnetic cell separation approach. Initial flow cytometry, PCR and Western blot analyses indicated the purified cells express high levels of primary human LEC markers and low levels of lens fibre cell markers. We hypothesized that more detailed characterization would provide additional support that the purified cells represent a vital new source of human LECs. To this end we used RNA-seq to generate high-resolution mRNA and miRNA profiles of the purified cells. Detailed analyses of these extensive datasets identified key groups of genes known to be required for lens development and function. This includes over 100 genes shown previously to be involved in lens and/or human congenital cataract development. These analyses also showed the purified cells do not express key marker genes associated with pluripotent cells (OCT4, NANOG, ZIC3, ESRRB, etc), endodermal cells (GATA4, GDF3, VWF, etc), mesodermal cells (T, GSC, CD34, CXCR3, etc) or non-lens ectodermal cells (NEUROD1, CHAT, RPE65, etc). Comparison of the RNA-seq datasets with published mouse and human lens cell transcriptional data shows the purified cells are more similar to human LECs than lens fibre cells, and more similar to embryonic than adult LECs. Our combined morphological, immunological, protein and transcriptional data demonstrate a novel, much-needed and highly useful abundant source of purified human LECs. These cells will provide new information on broadly-relevant developmental processes, as well as molecular insights and new drug candidates for cataract research.

T-1179

NOVEL TRANSCRIPTIONAL MECHANISMS REGULATE VISUAL PATHWAY DEVELOPMENT

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What pathways specify retinal ganglion cell (RGC) fate in the developing retina? RGCs are born from multipotent retinal progenitor cells (RPCs) but little is known about the cell-autonomous mechanisms and environmental signals that specify RGC fate. Using an in vitro differentiation model of RPCs, we screened and identified transcription factors important for RGC fate. Using knockout mice models, we further studied the mechanism of RGC specification in retina flat mounts by immunofluorescence and in human embryonic stem cells and induced pluripotent cell cultures assaying for RGC differentiation. We found a new molecular pathway involving Sox4/Sox11 is required for RGC differentiation from retinal progenitor cells (RPCs) and for optic nerve formation in mice in vivo, and is sufficient to differentiate human induced pluripotent stem

cells into electrophysiologically active RGC-like cells. The previously described inhibitor of RGC differentiation, REST, depended on suppression of Sox4 expression. A novel soluble regulator for RGC differentiation, TGFβ superfamily member GDF-15, acted through Sox4 to induce RGC differentiation from progenitor cells. Sox4 and Sox11 interacted such that the normal SUMOylation of Sox11, which decreased its nuclear localization and suppressed its pro-RGC activity, was decreased in the absence of Sox4, allowing Sox11 to compensate for Sox4 absence. These data define novel regulatory mechanisms for this SoxC molecular network, and suggest pro-RGC molecular manipulations with potential promise for cell replacement-based therapies for glaucoma and other optic neuropathies.

T-1180

GENERATION OF MOUSE-ESC-DERIVED RETINA ORGANOIDS TO STUDY RETINA DAMAGE AND REPAIR

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The differentiation of pluripotent stem cells into retinal cell types opens up new possibilities for studies on retinogenesis, retinal degeneration and regeneration. We developed a protocol for 3D retina organoid differentiation from mouse embryonic stem cells (mESC) independent of a transgenic eyefield reporter. We efficiently generated (87 ±3 SD % of aggregates, N=4) big, stratified retinal tissue reminiscent of early postnatal retina in vivo. Using this protocol we proposed to develop (1) a protocol for the generation of photoreceptor-enriched organoids, e.g. as a cell source for cell replacement therapies; and (2) an experimental model to study reactive gliosis, a response frequently associated with neuronal damage and diseases. Therefore, we used Notch signaling inhibition to force cell differentiation at defined timepoints during retina organoidogenesis. Further, we tested the effect of damage-related signaling molecule TNF on retina organoids. Notch inhibition by DAPT treatment culture time-dependently increased Crx+ photoreceptors, reduced numbers of progenitors and prevented the differentiation of retinal bipolar cells and Müller glia. Strikingly, if DAPT was applied at early stages of the protocol cone photoreceptor related markers were upregulated (RxRy, TRß2, S/M-Opsin). Next, we observed that radial Müller glia in retina organoids, in contrast to mouse retina in organotypic culture, do not express glial fibrillary acidic protein (GFAP), hallmark of reactive gliosis, even at late stages of the protocol (after D20). However, application of TNF to retina organoids from day 20 onwards induced upregulation of GFAP in Müller glia. I summary, with our protocol 3D retina organoids can efficiently be generated from wildtype mESC. Inhibition of Notch signaling pathways at defined stages of the protocol, leads to rod and cone photoreceptor-enriched organoids and could thus facilitate e.g. photoreceptor transplantation studies. Further, our results upon application of damage-related signaling molecule TNF suggest reactive gliosis response can be induced in retina organoids. We conclude that mESC-derived retina organoids open up new approaches in retina research.

NEURAL CELLS

T-1181

IDENTIFYING REGULATORS OF NEUROEPITHELIAL STEM CELL STATE

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During the development of the brain various stem and progenitor cell types (neuroepithelial cells-NES, radial glia cells-RG and transit amplifying cells-TA) can exist together with differentiated cells. The regulation of different cell states and the transition between these states is not known. Studying the regulation of cell fate decisions in the early mammalian embryo is highly challenging, therefore in vitro stem cell cultures could provide a complementary system to understand the biology of early neural stem cells. We have successfully isolated and maintained NES cells with cortical potential representing an early stage of development. The cerebral cortex specified neuroepithelial cells (cNES) can self-renew independently of NOTCH or EGF receptor activity if 4 signaling pathways (4F) are under control. Neural stem cells present during the neurogenic period on the other hand depend on NOTCH signaling for self-renewal and EGF/TGFa signaling can enhance their proliferation. Here we analyse the acquisition of NOTCH and EGF dependent state in cNES cells. First we used bioinformatics analysis of whole genome RNA-SEQ datasets to identify candidate regulators of cNES cell state. The bioinformatics analysis of genome wide gene expression profiles of human pluripotent stem cells, cortical neuroepithelial cells, cortical neuronal cultures and in vivo gene expression data identified protein coding genes, miRNAs and lincRNAs that are enriched in neuroepithelial cells and can regulate self-renewal of cNES cells. Using an in vitro cNES differentiation assay we are testing if the candidate genes can maintain the NOTCH and EGF independent cNES cell state.

T-1182

PBX HOMEOBOX GENES CONTROL THE DEVELOPMENT AND SURVIVAL OF MIDBRAIN DOPAMINERGIC NEURONS AND THEIR PROTEIN LEVELS ARE REDUCED IN PARKINSON'S DISEASE PATIENTS

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Understanding the molecular mechanisms that regulate the development and maintenance of midbrain dopaminergic (mDA) neurons is a prerequisite for the advancement of stem cell therapies for Parkinson's disease (PD). In order to identify novel molecular players in mDA neuron development, we performed RNA sequencing and found that Pbx homeobox genes are

enriched in the midbrain floorplate. Pbx genes are members of the TALE (three-amino acid loop extension) superclass of homeobox transcription factor genes, and they are known to regulate basic developmental processes such as hematopoiesis, organogenesis, skeletal morphogenesis, but their function in mDA neuron development, stem cells and Parkinson's disease is largely unknown. Here we show that Pbx homeobox genes are required for mDA neuron development and survival, as assessed in single, double and conditional mutant mice. PBX proteins are present in mouse and human mDA neuroblasts and neurons, and regulate the expression of several previously unknown target genes, including a transcription factor controlling several mechanisms impaired in PD such as mitochondrial function, proteasomal degradation, oxidative stress response and neurotoxicity protection. Notably, the levels of this transcription factor, as well as PBX proteins, are reduced in PD patients compared to healthy donors, suggesting a dysfunction of this pathway in PD. Furthermore, overexpression of Pbx genes in human neural stem cells increases the generation of DA neurons in vitro. Together these results identify PBX proteins as novel molecular determinants of mDA neuron development and survival. As such, Pbx genes may be useful for the development of cell replacement therapies for Parkinson's disease.

T-1183

COMPREHENSIVE IDENTIFICATION OF LONG NONCODING RNAS IN PURIFIED CELL TYPES FROM BRAIN REVEALS NOVEL FUNCTIONAL LNCRNA IN OPC FATE DETERMINATION

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Emerging evidences suggest that long noncoding RNAs (lncRNA; >200bp) play important roles in cell fate determination. It is challenging to build a comprehensive lncRNA catalog, since lncRNAs are often expressed at lower levels than protein-coding genes. It is difficult to detect and assemble these transcripts, especially if the lncRNAs are expressed in the minor cell types within a tissue. We have broadened lncRNA collection by ab initio transcriptome reconstruction using purified cell populations from mouse cortex. We generated a more comprehensive lncRNA annotation database by combining the lncRNAs that we identified and multiple sources including GENCODE, RefSeq, Ensembl, lncRNAdb as well as lncRNAs recently identified by several other groups. More than 5,000 lncRNAs were detected in nine brain cell types studied. Predicting lncRNAs' function using cell type specific data revealed their potential roles in CNS. Moreover, we utilized ENCODE DNase I digital footprint data to infer transcription factor (TF) bindings proximal to the lncRNAs. This comprehensive brain cell type specific lncRNA database integrated with TFs binding and predicted functional information provides a powerful framework for systematically identifying lncRNAs that are essential for brain cell fate determination. Because oligodendrocyte precursor cells (OPCs) are crucial in myelination, and understanding the OPCs' fate determination is critical for harnessing their potential for cell-based therapies, we chose to investigate lncRNAs that may have essential functions in OPC fate determination to demonstrate the utility of this resource. Based on our integrative analysis, the top candidate linc-OPC shows highly specific expression in OPCs and remarkable sequence conservation among placental mammals. Furthermore, Loss-of-function experiments confirmed linc-OPC plays functional role in OPC genesis. Our results substantiated the role of lncRNA in OPC fate determination and provided an unprecedented resource and a powerful analysis framework. We present our datasets and analysis results as an online resource freely available to the research community. We anticipate that these resources will advance the knowledge of this major new class of non-coding genes and their potential roles in neurological development and diseases.

T-1184

HEPARAN SULFATE-DEPENDENT PROLIFERATION AND NEURAL DIFFERENTIATION OF EMBRYONIC STEM CELLS

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Heparan sulfate (HS) proteglycans, present on cell surfaces and in the extracellular matrix, interact with growth factors and morphogens to influence cell growth and differentiation. Depletion of HS leads to embryonic lethality, and deficiency in embryonic stem cell differentiation to cells of the neural lineage in vitro. The NDST (glucosaminyl N-deacetylase/N-sulfotransferase) enzymes have a key role in HS biosynthesis, greatly influencing total sulfation of the HS chains. Our previous study shows that embryonic stem cells (ESCs) lacking both NDST1 and 2, can only take initial steps towards neural differentiation. HS mediated signaling is thus an early and necessary step in the commitment to the neural lineage. The cleavage of HS side chains is mediated by heparanase (Hpse) that releases HS-bound bioactive molecules thereby affecting proliferation and differentiation. ESCs were generated from mice overexpressing Hpse (Hpse-Tg) and compared to those of wild type (wt) mice. The ESCs were confirmed for their pluripotency and a normal karyotype. Hpse-Tg ESCs proliferated faster than wt, and when injected into immuno-deficient mice, they formed larger teratomas. ESCs from both genotypes could be differentiated into neural stem/progenitor cells (NSPC) while Hpse-Tg ESCs exhibited a higher proliferation rate. This suggests that HS size affects progenitor proliferation in generating NSPCs from pluripotent ESCs. NSPCs from Hpse-Tg readily generated neurons and astrocytes by mitogen withdrawal. In addition, Hpse-Tg NSPCs generated 5-10% oligodendrocytes. There was no reduction of astrocytes, but fewer neurons as a consequence of elevated Hpse levels. In wt ES-derived NSPCs, Hpse levels declined during the formation of neurons and glia. Thus, a continuous high level of Hpse in the transgenic NSPCs coincided with the observed shift in differentiation potential to oligodendrocytes. The generation of oligodendrocytes in Hpse-Tg ESCs was in contrast to wt, which only rarely formed. Much effort has been made to promote oligodendrocyte differentiation from ESCs but so far, the protocols require complex culture conditions. Our data suggest that continuously high Hpse overcomes a rate-limiting step in oligodendrocyte differentiation and reveal mechanistic insight to the role of HS in oligodendrogenesis.

T-1185

THALAMIC STIMULATION IN AWAKE RATS INDUCES NEUROGENESIS IN THE HIPPOCAMPAL FORMATION

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Deep brain stimulation provides substantial clinical benefits for a variety of movement disorders, and it lately started to emerge as a treatment of cognitive and mood disorders. Although the molecular mechanisms mediating these effects remain elusive, the regulation of adult hippocampal neurogenesis may be a promising one. The present study investigates the effects of unilateral anteromedial thalamic nucleus stimulation on adult hippocampal neurogenesis in awake and unrestrained rats. Several groups of adult rats received each one of the following treatments: *Group 1 and 2:* adult female (n=4) and male rats (n=6) were subjected to unilateral stimulation in the right anteromedial thalamic nucleus (AMN). *Group 3*: adult female (n=4) and male (n=4) sham rats were subjected to electrode implantation with no current delivery. *Group 4:* adult male rats (n=4) received unilateral stimulation in the right ventral posterolateral thalamic nucleus (VPL). All rats received 4 injections (50mg/Kg/injection) of 5'-bromo-2'-deoxyuridine (BrdU) 3 days after surgery and were euthanized 24 h later. Confocal immunofluorescent analysis of BrdU, GFAP and NeuN was performed. Stereological counting of positive cells was done in the dentate gyrus and hilar zone of the hippocampal formation, using the fractionator method. Stimulation of the right AMN induced focal neurogenesis in the ipsilateral (right) dentate gyrus. Stimulation-induced effects were gender-independent and were translated by an increase in proliferation of amplifying neural progenitors. Importantly, increased-neurogenesis was most prominent at the caudal region of the dentategyrus. Furthermore, this increase was specific for the AMN and not the VPL stimulation. Neurogenesis at the level of the hilar and the subventricular zones was not affected by AMN stimulation. Our results suggest a role for hippocampal neurogenesis in the mechanisms underlying the effects of deep brain stimulation. The exclusivity of these effects to AMN stimulation denotes the importance of the components of Papez circuitry in the treatment of cognitive and behavioral disorders. The behavioral implications of short and long term deep brain stimulation can constitute a subject for further investigation in awake and unrestrained animals.

T-1186

THE MECHANISTIC BASIS BY WHICH THE BONE MORPHOGENETIC PROTEINS DIRECT SENSORY INTERNEURON IDENTITY IN THE DORSAL SPINAL CORD

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Inductive signaling by the Bone Morphogenetic Protein (BMP) family is reiteratively required for many aspects of nervous system development, including cell fate specification, axon guidance and synaptogenesis. We are studying the mechanistic basis by which the BMPs direct such diverse cellular processes using the vertebrate spinal cord as a model system. The developing spinal cord has a relatively simple architecture: discrete classes of neurons arise along the dorsal-ventral axis, including the sensory and motor neurons that permit individuals to perceive sensory stimuli and execute motor responses. The roof plate (RP), located at the dorsal midline of the spinal cord, secretes many BMPs, including BMP4, BMP5, BMP6, BMP7 and Growth/Differentiation Factor 7 (GDF7), which are required for the specification of the dorsal-most classes of sensory interneurons (INs). Surprisingly, the mode by which the BMPs direct IN identity remains unresolved. Previous studies have suggested that the BMPs act as concentration-dependent morphogens to specify dorsal IN identity, largely by analogy with the gradient of Sonic hedgehog (Shh) that patterns the ventral spinal cord. However, unlike Shh, there are many BMPs present in the RP making it unclear how they would cooperate to establish a single morphogen gradient. Our recent studies have suggested an alternative model: that individual BMPs direct the specification of dorsal spinal identity in a signal-specific manner. Using pluripotent mouse embryonic stem cells, as well as complementary in vivo methods, we have found no evidence that the BMPs act as concentration dependent morphogens, rather the RP-resident BMPs appear to have distinct activities specifying spinal IN populations. In further studies, we are evaluating how the canonical BMP second messengers, Smad1 and Smad5, translate the activity of the different BMPs into the specification of particular neural identities. Understanding the mechanisms that establish distinct spinal IN identities is a critical step towards using stem cell therapies to replace spinal sensory neurons lost as a result of injury or disease.

T-1187

CEND1 AND NEUROGENIN2 REPROGRAM MOUSE ASTROCYTES AND EMBRYONIC FIBROBLASTS TO INDUCED NEURAL PRECURSOR CELLS AND DIFFERENTIATED NEURONS

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Recent studies demonstrate that astroglia from non-neurogenic brain regions can be reprogrammed into functional neurons through forced expression of neurogenic factors. Based on our previous work demonstrating the neurogenic potential of Cend1 and its activation by genes of the neurogenin family, we explored the combined effect of Cend1 and Neurogenin-2 (Neurog2) on reprogramming of mouse cortical astrocytes. Forced expression of Cend1, Neurog2 or both resulted in acquisition of induced neuronal cells expressing βIII tubulin and the subtype-specific markers GABA, glutamate or tyrosine hydroxylase. Cell tracking analysis following 1-week live cell imaging indicated that, while Cend1-transduced astrocytes undergo one or two divisions prior to neuronal differentiation, Neurog2+ astrocytes directly trans-differentiate to neurons without passing from a proliferative stage. Surprisingly, a subpopulation of Cend1/Neurog2-double transduced astrocytes formed highly proliferative spheres exhibiting neural stem cell-like properties and multipotentiality. Furthermore, knock-down of endogenous Cend1 mRNA demonstrated that it is a key downstream mediator of Neurog2-induced neuronal reprogramming of astrocytes. To address whether Cend1 and Neurog2 possess a broader neurogenic potential being capable of trans-differentiating

more distant in lineage cell types, we forced their expression in mouse embryonic fibroblasts (MEFs). Ectopic expression of Cend1, Neurog2 or both resulted in reprogramming of MEFs towards neural precursors which could be further differentiated towards subtype-specific neurons, in percentages reaching 60% of the total cell population. Our data suggest that common reprogramming mechanisms exist driving the conversion of in lineage unrelated somatic cell types towards neuronal identity and reveal a critical role for Cend1 in Neurog2-driven astrocytic reprogramming. To shed light into these mechanisms, we are currently performing PCR-arrays for genes involved in neuronal differentiation pathways. Additionally, whole-cell patch-clamp recordings are in progress to functionally characterize induced neuronal cells.

T-1188

INDUCED HUMAN NEURAL CELLS FORM MATURE FUNCTIONAL NEURONS IN RODENT HIPPOCAMPUS

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Recent findings that human fibroblasts can be directly reprogrammed to induced neurons (iNs), opened new opportunities for cell replacement therapies using lineage specific transcription factors in a number of neurological diseases, including epilepsy. This devastating multifactorial chronic neurological disorder characterized by recurrent seizures, affects at least 50 million people worldwide and available pharmacological treatments are only symptomatic, often with severe side effects and fail to adequately control seizures in one third of patients. The biggest portion of the drug refractory cases comes on the mesial temporal lobe epilepsy, originating in the hippocampus. In this study we transplanted iNs, derived from human fetal lung fibroblasts, using three transcription factors: Ascl1, Brn2a and Myt1L, into the adult rat hippocampus and observed their development throughout a six month period post transplantation. Immunohistochemical and electrophysiological characterization of the transplanted cells was carried out at one, three and six months post grafting. The transplanted cells show temporal morphological differentiation and maturation, with more developed arborisation and neurites stretching out to all the areas of the host hippocampus from the injection site at later time points. This was reflected on electrophysiological parameters, as neurons with mature membrane properties, resting membrane potential and fast, high amplitude action potentials could only be observed at six months after transplantation. The presented study shows for the first time long term survival of the transplanted human iN cells into the hippocampus, were they acquire mature neuronal properties. Further improvements are needed to achieve higher survival and integration rate of the transplanted cells, although this proof of principle study shows the potential for the iN cells as a candidate for a cell replacement therapy in epilepsy.

T-1189

SEQUENTIALLY ACTING SOX PROTEINS IN NEURAL LINEAGE SELECTION

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Neural stem cells (NSCs) generate all major cell types of the nervous system, but how neuronal, astroglial and oligodendroglial lineages are specified at the molecular level is not known. While Sox2 regulates NSC-expressed genes, it also pre-binds genes that first are activated as Sox2 is down-regulated and the binding is replaced by Sox11 in differentiating neurons. To examine if Sox2 pre-bound genes in NSCs also include genes expressed in astroglial and oligodendroglial lineages we have performed single cell RNA-seq experiments on neurons, astrocytes and oligodendrocytes to understand the unique expression profile for each cell type. Correlation of the gene expression profiles for oligodendrocytes and astrocytes and the Sox2 pre-bound genes indeed revealed glial genes bound by Sox2 in NSCs. During glial differentiation another group of Sox proteins are expressed, SoxE (Sox8, -9 and -10). Interestingly, Sox10 ChIP-Seq data from oligodendrocytes showed a significant overlap with the pre-bound glial genes and, furthermore, the corresponding enhancers were activated by SoxE proteins in a cell-based reporter system. Similar to the situation in differentiating neurons, these glial genes are activated when SoxE proteins replaces the binding of Sox2. Together, this suggests that sequentially acting Sox transcription factors preselect transcriptional programs to coordinate neuronal and glial differentiation.

T-1190

BIOMIMETIC INTERRELATION BETWEEN COLLAGEN SCAFFOLD SEEDED WITH HUMAN IPS-DERIVED NEURAL STEM CELLS AND ORGANOTYPIC DORSAL ROOT GANGLION AND SPINAL CORD SLICES

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In this report we investigate the relationship between the microenvironment of a collagen scaffold (CS) seeded with hiPSC at different stages of neural commitment, and their interactions with organotypic slices from early post natal rat spinal cord and dissociated DRG neurons. CS were prepared from porcine collagen dispersion using a lyophilization technique to obtain a 3D porous scaffold. The CS were cross-linked using carbodiimide to ensure appropriate mechanical and thermal properties. Human iPSC were harvested at different developmental stages: undifferentiated cells, neurally committed as well as differentiated into glial and neuronal lineages. The iPSC under neural-differentiating protocol revealed all typical changes from pluripotent to neuronal and glial phenotypes. Markers of pluripotency: Oct, Sox2, Nanog were gradually lost while neural markers such as nestin, neurogenin, ß-III tubulin, GFAP and Pax6 were acquired, as revealed by RT-PCR and immunocytochemical analyses. These cells formed 3D aggregates on the surface of simple 2D Matrigel substrates, creating spheroids and obtaineing mature neuronal phenotypic markers: MAP-2, DC, Synapsin, and TH, as well as glial markers such as PDGFRa, GFAP and GalC . The undifferentiated cells seeded on the chemically modified CS revealed limited viability and did not differentiate spontaneously. In contrast, iPSC-derived NSC and more differentiated neural progenitors survived and adhered in a well-defined flattened manner. After a few days of co-culture on the 3D scaffolds, these cells expressed neuronal (nestin, ß-III tub, MAP-2, DC) and glial (A2B5, NG2, PDGFRa, GFAP) markersFinally the CS seeded with hiPS-

derived neural stem cells at different developmental stages were co-cultured with suspension of DRG neurons and with organotypic spinal cord slices. The cells revealed developmental stage dependent mutual responses while being tested for cell migration and axonal outgrowth. These two systems can serve as tools for investigating the axonal outgrowth into the scaffolds from CNS or PNS neurons and possible migration/ cell processes outgrowth from the scaffolds to the organotypic tissue. Supported by WRC EIT+ (project BioMed, POIG.01.01.02-02-003/08) financed from the European Regional Development Fund (OPIE, 1.1.2) and statutory funds to MMRC

T-1191

SMEK1/2 IS A NUCLEAR CHAPERONE FOR CLEAVED WNT RECEPTOR RYK, A NOVEL NON-CANONICAL WNT SIGNALING PATHWAY REGULATING NEURAL CELL FATE DETERMINATION

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Wnt signaling is known to play an important role in neurogenesis through regulating the self-renewal and differentiation of neural progenitor cells. However, the molecular mechanism is still remained elusive due to the intricacy of Wnt signaling. The receptor-like tyrosine kinase (Ryk), a Wnt receptor, has been reported to be involved in various developmental processes and biological functions in different organisms, but the detail molecular mechanism is still unclear due to the special characteristic of its inactive kinase domain. Previously, we observed that Ryk is cleaved during neural differentiation and its intracellular domain (ICD) part is released from the cell membrane and translocated into the cell nucleus. However, how Ryk-ICD moves to the nucleus and how it functions in the nucleus to regulate neural differentiation is unclear. Here, we identified Smek1/2 as Ryk-ICD partners, which regulate its nuclear localization. Genome-wide Smek binding analysis revealed that Smek binds to the regulatory region of master transcription regulators of neuron differentiation, and regulates gene expression. Moreover, the binding is dependent on Ryk. In Smek1/2 double mutant mice, we observed a significant defect of GABAergic neuronal differentiation and cortical neurogenesis. In light of these findings, we discovered a novel non-canonical Wnt signaling, Smek proteins are the nuclear chaperone for Ryk-ICD, controlling the neural differentiation of the developing mouse brain.

T-1192

SONIC HEDGEHOG SIGNALING BALANCES QUIESCENT VERSUS ACTIVATED NEURAL STEM CELLS IN THE ADULT SUBVENTRICULAR ZONE

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The Sonic Hedgehog (Shh) signaling pathway is active in the adult rodent subventricular zone (SVZ) of the lateral ventricle where it has been proposed to regulate the maintenance of neural stem and precursor cells. However, the role of Shh signaling pathway on the distinct NSCs (quiescent vs activated) and SVZ populations has not been explored. Using a fluorescence-activated cell sorting (FACS) technique associated with accurate genetic mice models, we prove for the first time that Shh role is specific to NSCs and not their immediate progeny. We explored the cell cycle of NSCs by time lapse videomicroscopy with transgenic Fluorescence Ubiquitination Cell Cycle Indicator (FUCCI), and proved that the activation of Shh signaling in vitro shortens both G1 and S-G2/M phase specifically in activated NSCs but not in transit-amplifying cells or immature neuroblasts. As a consequence, the genetic conditional activation of Shh signaling in vivo increases the pool of quiescent and activated NSCs at short term. Strikingly, at long term, quiescent NSCs population continues to increase while the deregulation of actively proliferating NSCs cell cycle leads to a progressive exhaustion of their pool and a decrease of neurogenesis. Together, our results demonstrate the tight control of quiescent vs activated NSCs balance by Shh signaling and its crucial role in the maintenance of the production of new neurons throughout life.

T-1193

TRIM28 CONTROLS TRANSCRIPTION OF ENDOGENOUS RETROVIRUSES IN NEURAL PROGENITOR CELLS

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Transcriptional co-repressor TRIM28 (Kap1 or TIF1β) is known to regulate endogenous retroviruses in embryonic stem cells but not in somatic tissues. In this study we propose neural progenitor cells (NPCs) as an exception to this rule. By using a Nestin-Cre - loxP approach we generated embryos with a CNS-specific TRIM28 deletion. Culturing NPCs from embryonic TRIM28 knockout (KO) forebrain indicates that TRIM28 depletion has no influence on proliferation and self-renewal in vitro. RNA-seq and qPCR analysis showed an up-regulation of two groups of retroelements MMERVK10C and IAP1 upon TRIM28 KO. ChIP of NPCs shows enrichment for H3K9me3 at loci of ERVs and IAPs. Furthermore those regions showed a significant loss of H3K9me3 in depleted NPCs. By mapping our results against the USCS genome browser we were able to detect retroelement-derived long non-coding transcripts in TRIM28 KO NPCs. Moreover we observed transcription of nearby genes caused by ERV activation after TRIM28 depletion. When looking at DNA-methylation we detected that a certain proportion of MMERVK10C is spared from DNA-methylation during early development. Immunocytochemistry shows translation of IAP-gag protein in the cytoplasm of TRIM28 deficient NPCs. TRIM28-Nestin-Cre KO embryos do not show any anomalies

in development until E 13.5, but those mice die around birth. Heterozygous TRIM28 KO doesn´t result in lethality but leads to significant behavioural impairment compared to wild type littermates when performing Open Field and Elevated Plus Maze behaviour tests. We are currently performing ChIP-seq experiments on our TRIM28 KO NPCs to investigate the role of TRIM28 in regulating ERVs on a genome wide level. Altogether our data demonstrates that TRIM28 dynamically regulates transcription of endogenous retroviruses in NPCs.

T-1194

MULTIPOTENT GLIA LIKE STEM CELLS IN ADULT ADRENAL MEDULLA

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The neural crest derived adrenal medulla is closely related to the sympathetic nervous system; however, unlike neural tissue, it is characterized by high plasticity and its ability to adapt to highly demanding situations, such a stress; this suggests the involvement of stem cells. In this study we used a transgenic nestin-GFP mouse model to define a population of nestin-expressing multipotent glia-like progenitor cells in the adult adrenal medulla. For lineage tracing and to study the progenitors' role in the adrenal medulla, an inducible mouse model, Nestin-CreER:Rosa26YFP, was used. These glia-like cells share features of adrenomedullary sustentacular cells, the glia population within the adrenal medulla, and they constitute a multipotent population able to differentiate into chromaffin cells and neurons. The adrenal gland is the key organ in the response to stress crucially involved in the maintenance of homeostasis. Our results from stress experiments in vivo show the activation and differentiation of these progenitors into new chromaffin cells. In summary, we describe a population of glia-like multipotent stem cells that is involved in adrenal tissue adaptation. Our data also suggest a role of stem and progenitor cells in the adaptation of neuroendocrine tissue function in general.

T-1195

TESTING ASTROCYTIC CONTRIBUTIONS IN FAMILIAL ALZHEIMER'S DISEASE USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Successful development of effective therapeutics for Alzheimer's disease (AD) requires a deep understanding of the mechanisms of disease pathogenesis. This has been made difficult by the inability of animal models to fully recapitulate AD's molecular and cellular phenotypes, but recent human induced pluripotent stem cell (hiPSC)-derived models have shown it is possible to recapitulate the complex genetic diversity of a patient and more completely model AD pathology. While most work has focused on neuronal AD phenotypes, there is mounting evidence that damage to neighboring nonneuronal support cells factor significantly to neuronal dysfunction. Astrocytes, the most abundant non-neuronal cell type in the brain, have previously been implicated in AD in the clearance of the amyloid-β peptide and also in neuroinflammation. In order to examine all mechanisms of AD pathogenesis, we must expand our model to include both hiPSC-derived neurons and glia. Thus, using an in vitro differentiation protocol to generate astrocytes from hiPSC, we will test the hypothesis that AD astrocytes exhibit intrinsic functional defects that might contribute to neurodegeneration by modulation of neuronal phenotypes. We have generated functional human iPSC-derived astrocytes and are currently testing astrocytic contributions to neuronal dysfunction in familial Alzheimer's disease. This hiPSC-derived astrocytic-neuronal model could bring new insight to whether non-neuronal cells like astrocytes modulate AD neuronal phenotypes.

T-1196

INDUCTION AND STABILIZATION OF NOVEL EARLY NEURAL PROGENITOR CELLS FROM HUMAN FETAL BRAIN TISSUE

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In the recent past, major progress in cellular reprogramming for modeling complex neurodegenerative and neuropsychiatric diseases has been achieved. The generation of induced pluripotent stem cells (iPSCs) and their subsequent differentiation into neural progenitor cells (NPCs) as well as the direct conversion of somatic cells into NPCs emerged into a promising strategy to derive patient-specific cells for biomedical applications. However, up to date it remains unclear to which extent those reprogrammed and/or differentiated NPCs represent the physiological state. Primary NPCs derived from fetal brain tissue might serve as a bona fide model and standard population for comparative validation studies. Moreover, it could elucidate mechanisms of early human neural development. So far, studies demonstrate the derivation of either rosette-like or radial glia-like cells from primary tissue. We hypothesized that the modulation of crucial signaling pathways of the early neural development is instrumental in stabilization of early progenitors. Thus we assessed the potential of small molecules to enable the stabilization of early NPCs from isolated human fetal brain tissue. Indeed, we identified conditions that allow robust formation of neuroepithelial colonies displaying a homogeneous morphology, which could be isolated and monoclonally expanded for more than 25 passages. Further characterization by immunofluorescent stainings and quantitative PCR show a characteristic neural stem cell profile including SOX1, PAX6, Nestin and SOX2. Spontaneous differentiation analysis shows a strong neurogenic potential yielding in a high percentage of TUJ1-positive neurons. Interestingly, rare neurons stain positive for the PNS-marker Peripherin. By application of directed differentiation protocols we increased the percentage of central neuronal subtypes as well as peripheral neurons and GFAP-positive cells representing the glial lineage. In summary, we here present a study showing the derivation of a novel human fetal-derived early NPC population by defined media conditions. These cells could not only serve as a comparative cell population for differentiation and conversion studies, but might also provide a cell source for biomedical applications such as cell replacement therapy and tissue engineering.

T-1197

OPTIMIZATION OF NEURAL STEM CELL SCALE-UP FOR CLINICAL APPLICATIONS

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Stem cell-based therapies for tumors and neurodegenerative diseases depend on efficient delivery of stem cells to the areas of damage. Neural stem cells (NSCs) are inherently patho-tropic, and able to migrate to sites of tumor and damage in the central nervous system (CNS). Therefore, patho-tropic NSCs can be exploited for cell replacement, regeneration and therapeutic delivery strategies. NSCs are currently being evaluated in clinical trials for stroke, multiple sclerosis, Parkinson's and other CNS diseases. In addition, our studies have shown that genetically modified NSCs can deliver anti-cancer agents selectively to invasive brain tumor sites, and are currently in phase I first in human studies for recurrent glioma patients. However, a major bottleneck to the clinical translation of these cell-based therapies is the manufacturing and production of cell banks in sufficient quantities for clinical trials. It is highly desirable to generate large GMP grade cell banks in a way that is cost-effective, reproducible and safe. This is especially important when moving toward larger phase II trials, or when giving multiple stem cell doses. To meet this need, we have developed a method of expanding our adherent human NSCs using the Quantum Cell Expansion System. Following expansion in the Quantum system, the NSCs were characterized for viability, genetic stability, identity, and growth kinetics in comparison with NSC expansion in conventional cell culture flasks. We have optimized reproducible expansion of NSCs in the Quantum system, starting from initial seeding of 4×10^7 cells to production of a total of 2.9×10^9 cells within 7-9 days. Importantly, this was done in a closed, automated system - minimizing chances for human error. Our results showed Quantum generated NSCs were genetically and functionally normal in vitro and in vivo, equivalent to NSCs expanded via conventional cell culture methods. Major advantages of Quantum expansion include a fully closed system, complete control of gases, temperature, and flow rate. The automated system allows for reproducibility requires minimal personnel and lowers overall cell expansion costs. We plan to use this novel method for production of GMP grade NSC cell banks in our future clinical trials.

T-1198

INTRACRANIAL XENOGRAFT MODEL AS A VALIDATION SYSTEM TO EXAMINE TUMORIGENICITY OF NS/PCs FOR TRANSPLANTATION THERAPY

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Transplantation of neural stem/progenitor cells (NS/PCs) is now considered to be a promising treatment for various central nervous system disorders including spinal cord injury, Alzheimer's disease, Parkinson's disease, and brain infarction. In countries where fetal NS/PCs are not allowed to use due to ethical issues, iPS cells are especially a potential cell source of NS/PCs. To apply these cells to clinic, the risk of tumorigenicity must be eliminated. The purpose of this study is to establish an in vivo-validation system to assess tumorigenicity of transplanted NS/PCs efficiently. Human NS/PCs induced from adult dermal fibroblast-derived hiPS cells were labeled with Lenti-ffLuc (Venus fused to firefly luciferase) vector. Two kinds of xenograft models were examined in this study: intracranial and spinal xenograft models. For intracranial xenograft models, 1x10^6 cells were injected into each side of the striatum of NOG mice or NOD/SCID mice without injury. For spinal xenograft models, 5x10^5 cells were transplanted into the spinal cord of NOD/SCID mice 9 days after contusion injury. Survival of transplanted cells was evaluated using bioluminescence-imaging (BLI) system in both models. For histological analysis, mice were intracardially perfused 6 to 30 weeks after transplantation. The transplanted NS/PCs could be detected by BLI in both the intracranial and spinal xenograft models throughout this study. Histological examinations revealed that the NS/PCs proliferated at the transplantation site, differentiated into neurons predominantly, and extended neuronal process to the broad areas. In some mice of brain xenograft model, extramedullary proliferation of transplanted cells was detected. The transplanted cells survived and proliferated in both models. Compared with spinal xenograft model, intracranial xenograft model would be better to detect tumorigenicity of NS/PCs for transplantation therapy, since it allows us to evaluate a larger number of cells in each animal by the reproducible procedure. Further study exploring the character of transplanted cells around injection site is needed.

T-1199

SMALL MOLECULE-BASED DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO NEURAL PRECURSOR CELLS

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Human pluripotent stem cells (hPSCs) offer great promise for incurable neurological diseases. The conventional method for neural induction of hPSCs includes embryoid body (EB) formation. However, several problems are associated with the EB-based method for using it for clinical applications, such as the time-, labor-, and cost-intensiveness of EB-formation, which makes it difficult to scale up the protocol. To avoid the EB-based method, neural differentiation of hPSCs under adherent conditions has been tried. However, the low efficiency of neural differentiation remained a major barrier to the practical use of this method for therapeutic purposes. Herein, we report an efficient, labor-effective, scalable, and rapid method for generating neural precursor cells (NPCs) from hPSCs using a small molecule in a simple and defined medium. Furthermore, this medium does not contain animal-derived component, which make it feasible to generate xeno-free NPCs for clinical use. Taken together, this study established an efficient way to generate clinically usable xeno-free NPCs by using a small molecule in adherent culture condition. This work was supported by grants from the Stem Cell Research Program (2010-0020347) and 2012M3A9C7050130 from the MSIP, and A120254-1201-0000200 from the Ministry of Health & Welfare, Korea

T-1200

DIFFERENTIATION OF DOPAMINERGIC NEURAL CELLS FROM HUMAN ADIPOSE-DERIVED MULTILINEGE PROGENITOR CELLS

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Parkinson disease (PD) is a neurodegenerative disease that is attributed to a progressive loss of dopaminergic neurons within the substantia nigra. The current therapy for PD mostly relies on symptomatic treatments, thus it is desirable that regenerative medicine strategies will be developed for PD. Recent progress in stem cell research has raised hope for the development of stem cell therapies, which can be a valuable tool in regenerative medicine. Human adipose tissue-derived multilineage progenitor cells (hADMPCs) are multipotent stem cells that can differentiate into various types of cells, including neuronal cells. Moreover, because of their reduced risk of tumorigenesis, and their hypoimmunogenicity and immunomodulatory effects, hADMPCs are an attractive material for cell therapy and tissue engineering. In this study, we attempted to develop a dopaminergic neuronal differentiation strategy from hADMPCs. We investigated a dopaminergic neuronal differentiation potential of hADMPCs by introducing four transcription factors, Achaete-scute homolog 1(ASCL1), forkhead box protein A2 (Foxa2), LIM homeobox transcription factor 1 alpha (Lmx1a), and Nuclear receptor related 1 (Nurr1). Intriguingly, addition of basic fibroblast growth factor (bFGF) caused a formation of spheroid like cells when introducing Foxa2 or Lmx1a into hADMPCs. *Nestin expression was observed to be upregulated in* these cells, suggesting that these spheroid like cells were neuronal stem cells. Further induction of neuronal differentiation resulted in the increased expression of Tyrosine hydroxylase (TH), Neurofilament-M (NF-M), and Microtuble-associated protein 2 (MAP2) in these cells. In addition, enzyme-linked immunosorbent assay revealed that dopamine secretion was significantly increased in hADMPCs introduced with Foxa2 or Lmx1a. These data suggest that the hADMPCs introduced with Foxa2 or Lmx1a have the possibility to differentiate into dopaminergic cells via neural stem-like cells. We also try to differentiate into dopaminergic cells from iPSCs derived from hADMPCs, and found that they efficiently differentiated into dopaminergic neurons. Our study thus may help developing effective therapy for Parkinson's disease.

T-1201

A XENO-FREE CHEMICALLY DEFINED CULTURE SYSTEM TO GENERATE NEURAL STEM CELLS FROM FEEDER FREE INDUCED PLURIPOTENT STEM CELLS

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Neural stem cells (NSCs) derived from human induced pluripotent stem cells (iPSCs) are expected to be a valuable cell source for cell therapy targeting central nervous system disorders such as spinal cord injury, stroke, and many other neurodegenerative diseases. For clinical applications, NSCs should be induced and maintained under good manufacturing practice (GMP)-grade conditions, which are challenging to achieve. In the present study, we established a procedure to obtain clinical-grade long-term self-renewing neuroepithelial-like stem cells (lt-NES cells) from feeder-free human iPSCs using a newly developed xeno-free neural stem cell medium. The clinical-grade lt-NES cells expressed the neural stem cell marker genes SOX1, SOX2, NESTIN, and PAX6, and flow cytometric analysis showed that over 95% of the cells were positive for both PSA-NCAM and CD133. They could be passaged for long periods while retaining normal karyotypes for a minimum of 20 passages, and they could also be cryopreserved. Additionally, they could be differentiated into neurons and astrocytes, and showed spontaneous electrophysiological activity. Therefore, the newly generated clinical-grade lt-NES cells showed properties similar to those of non-clinical grade lt-NES cells generated using the conventional neural rosette methods. In summary, this newly developed xeno-free chemical defined culture system will be useful for clinical applications of iPSC-derived NSPCs for the treatment of neurodegenerative diseases.

T-1202

IN VIVO REPROGRAMMING OF MOUSE STRIATAL REACTIVE ASTROCYTES TO NEUROBLASTS USING MIR-302/367 CLUSTER AND VALPROATE

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Reactive astrocytes have major role in formation of glial scars that occur in neurodegenerative diseases. Recent studies demonstrated that these reactive cells could be reprogrammed to induced neuron in vivo. Cluster miR-302/367 is an embryonic stem cell specific group of microRNAs which play an important role in pluripotency. Here we showed that reactive mouse astrocytes could be reprogrammed to neural cells by miR-302/367 in vivo in the presence of valproic acid as a histone deacetylase inhibitor. When human astrocytes were transducted with cluster miR-302/36 and transplanted into mouse brain, trans-differentiated into neuronal cells. We also showed that human astrocytes can convert to neuron-like cells by miR-302/367 in vitro. Following induction of astrocytes toward neurons Oct4+ and Nanog+ cells were not observed that suggests direct cell conversion. Six weeks after transduction of human astrocytes, the induced neural cells mainly showed glutamergic fate and immature electrophysiological properties. Results indicate that reactive astrocytes can be converted to neuroblasts by miR-302/367 which seems promising for conversion of glial scar cells to repairing neuroblasts in a wide range of neurological diseases.

T-1203

SINGLE CELL RNA SEQUENCING UNCOVERS CLOSE RELATIONSHIP BETWEEN DOPAMINE AND SUBTHALAMIC NUCLEUS NEURON LINEAGES

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The extensive neuronal diversity generated from neural stem cells in the mammalian CNS is an impressive feat of development, and also presents a significant challenge to dissect and understand. Ventral midbrain (vMB) dopaminergic neurons are a neuronal subtype that have been rigorously studied, strongly motivated by the interest in engineering stem cells into dopamine neurons for cell replacement in Parkinson's disease. However, classical approaches used to probe vMB neuron diversity and development are hampered by the presence of mixtures of different cell types and maturation stages in whole tissue dissections. To resolve this limitation, 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. Importantly, the analysis provided a robust genome-wide reconstruction of how neural stem cells expressing the transcription factor Lmx1a transition into postmitotic differentiating neurons. This approach also allowed rapid, comprehensive and unbiased identification of robust gene signatures represented in Lmx1a-expressing neuronal lineages. In vivo validation of one such signature has uncovered an unexpected similarity between developing dopaminergic neurons, and more rostrally developing glutamatergic neurons of the Subthalamic Nucleus. Single-cell RNA-seq thus proves an invaluable technique in successfully interrogating lineage diversity in the developing CNS.

T-1204

SEROTONERGIC REGULATION OF POSTNATAL NEUROBLAST MIGRATION

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Neuronal migration is regulated by numerous stimuli, of which calcium from different sources plays a major role. Postnatally generated SVZ (subventricular zone)-derived neuroblasts migrate long distance to reach their final destination in several forebrain regions. As they all bear the calcium permeable serotonin receptor 3A (5HT3A), we performed in vivo experiments in mice to test the function of the receptor in neurogenesis. 5HT3A receptor deletion in neuroblasts decreased speed of migration, impaired directionality of migration and abolished spontaneous calcium waves/spikes. Mutant receptor expression evidenced that receptor gating of calcium is the cause for altered migration. We identified a signaling cascade that is triggered by the 5HT3A receptor and propagates calcium signaling from the membrane to the nucleus. Finally, we detected serotonergic axons aligned with migratory paths in both mouse and monkey brain, thus highlighting a conserved evolutionary trait of the serotonergic system in mammalian brain development.

T-1205

EFFICIENT GENERATION OF FAITHFULLY REPROGRAMMED INDUCED OLIGODENDROCYTE PROGENITOR CELLS

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The derivation of induced oligodendrocyte progenitor cells (iOPCs) from fibroblasts may enable in vitro disease modeling and cell-based therapy development. However, the reported reprogramming process is inefficient, and the resultant cells exhibit limited expansion capacity and low differentiation efficiency, hampering their potential clinical usage. Here we show that donor cell characteristics and optimal factor combination help overcome these limitations, resulting in the generation of faithfully reprogrammed iOPCs. Pericytes with endogenous PDGFRα expression, high plasticity, and high replicative potential yield iOPCs with up to 90% efficiency. Ectopic expression of *Olig2* and *Sox10* is sufficient to elicit the direct conversion of pericytes into iOPCs. The generated iOPCs are stably expandable in vitro (> 30 passages), while retaining their self-renewal and differentiation capacity. These cells efficiently differentiate into MBP$^+$ oligodendrocytes (> 48% efficiency) that could ensheath multiple axons when co-cultured with dorsal root ganglion neurons and shiverer brain slices.

T-1206

ALTERATIONS IN METABOLOMIC PROFILE OF LRRK2 MUTANT IPSC-DERIVED DOPAMINERGIC NEURONS

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Combining the technologies of induced pluripotent stem cells (iPCS) and untargeted metabolomics may provide a powerful tool for discovery of novel biomarkers and help better understand the mechanisms of neurodegenerative diseases, e.g. Parkinson´s disease (PD). Mutations in LRRK2 gene are the most common genetic cause of PD. Thus, we compared global metabolite profiles of iPSC-derived dopaminergic (DA) neurons between patients with G2019S LRRK2 mutation and non-related control subjects. Cell samples were extracted with acetonitrile and analyzed with liquid chromatography combined to high accuracy mass spectrometry (LC-qTOF-MS). The results from LC-qTOF-MS were treated with univariate and multivariate methods to identify statistically significant metabolites between two different study groups. The most important finding was the putative identification of LysoPC(20:2), which is a lysophospholipid consisting of one chain of eicosadienoic acid. This compound could be a possible biomarker for PD because it was detected only in PD samples. The LysoPC(20:2) [11Z, 14Z] was characterized earlier as a potential biomarker in the pathogenesis of PD. Secondly, the level of N-acetylhistidine was reduced in PD samples. This might be an indication of altered amino acid N-acetylation due to formation of α-synuclein oligomers, the primary components of Lewy bodies in PD. In addition, various cardiolipins (CLs) were significantly different in PD samples as compared to control samples. Altered composition and content of these CLs have been associated with mitochondrial dysfunction in several physiopathological conditions. Another finding was that the amount of creatine was significantly higher in PD samples. Previously, creatine had shown efficacy as a neuroprotective agent delaying the progression of PD. The results also suggested that there

were aberrations in the purine pathway in PD samples due to an increased amount of xanthylic acid. To conclude, our data shows a great potential of untargeted metabolomics in stem cell research. However, due to small number of replicates, additional analyses are needed to ensure the findings and validate the identification of these putative new biomarkers in PD.

T-1207

THE ABLATION OF GEMININ IN THE ADULT NEURAL STEM CELLS ALTERS THEIR SELF-RENEWAL AND FATE COMMITMENT DECISIONS

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Neurogenesis continues throughout adulthood in two restricted germinal layers: the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subependymal zone (SEZ) in the walls of the lateral ventricles. The SEZ harbors slow dividing astrocytic neural progenitors, also known as Type-B1 cells, which give rise to actively proliferating transit-amplifying cells, named Type-C cells. Type C cells predominately generate neuroblasts (Type A cells) that migrate into the olfactory bulbs (OBs), and differentiate into distinct interneurons. A small proportion of the type B1 cells progeny is committed to the oligodendrocytic lineage and migrates mainly to the corpus callosum. The mechanisms underlying the regulation of neural stem cell (NSCs) self-renewal and maintenance of their multipotency are still not completely understood. We have previously shown that early cortical progenitors in the absence of Geminin show altered cell cycle kinetics and a preference for self-renewing divisions. Conversely, Geminin overexpression resulted in cell cycle exit and increased neuronal differentiation of embryonic cortical progenitor cells. In order to investigate the in vivo role of Geminin in the adult NSCs, we have generated mice lacking Geminin expression specifically in these cells. Our findings suggest that deletion of Geminin increases the number of type B1 cells and leads to a reduced production of type C cells. Moreover, our results show that Geminin depleted NSCs display an altered differentiation profile, as they favour the gliogenic fate at the expense of the neuronal fate when they migrate away from the SEZ. Summarizing, our study suggests that Geminin is a novel player in the self-renewal and fate commitment decisions of the adult NSCs.

T-1208

MINIMALLY INVASIVE MAGNETIC RESONANCE IMAGING-GUIDED TRANSPLANTATION OF MAGNETIC NANOPARTICLE LABELED NEURAL STEM CELLS INTO THE PORCINE SPINAL CORD

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Stem cell-based therapeutics are under evaluation for a range of diseases of the spinal cord, including Amyotrophic Lateral Sclerosis, traumatic Spinal Cord Injury, and Multiple Sclerosis. Delivery of cellular therapeutics to the spinal cord is achieved through either systemic administration or direct intraparenchymal injection following surgical exposure. Direct injection reliably delivers the most cells to the target site. An MR-compatible direct injection system was developed to reduce the need for invasive surgical procedures and to improve accuracy with MRI visualization. The purpose of this study was to demonstrate proof-of-principle for minimally invasive transplantation of stem cells into the spinal cord of a live pig under MRI guidance. The pig (Göttingen minipig, n = 2) was positioned in a 3T Siemens full-body MRI scanner under general anesthesia. An MR-compatible injection platform attached to the ClearPoint SmartFrame (MRI Interventions, Inc.) was fixed to the thoracolumbar spine with titanium percutaneous pedicle screws. Pre-entry MR images of the system and the spinal cord were acquired. The ventral horn of the spinal cord was chosen as the target and a trajectory through the soft tissue was calculated using the ClearPoint software. A ceramic cannula was inserted through a 1 cm skin incision to the surface of the spinal cord. Repeat imaging was performed to confirm the correct trajectory. The injection needle was passed through the cannula and into the ventral horn of the spinal cord. A single injection 2.5e5 human neural progenitor cells labeled with ferumoxytol magnetic nanoparticles were infused into the spinal cord. After needle removal, the cell graft was observed in the ventral horn of the spinal cord as a hypointense focus on T2*-weighted MRI. No post-operative morbidity was observed and both animals returned to baseline motor and sensory function within 24 hours. The spinal cords were harvested after the procedure and histochemical staining for Iron revealed the cell graft located on target in the ventral horn, confirming the signal observed on MRI. This study supports the proof-of-principle for minimally invasive transplantation of stem cells into the spinal cord of a large animal under the guidance of MRI. Assessing safety and feasibility of this approach requires further study.

T-1209

GLUCAGON LIKE PEPTIDE 1 IMPROVE NEURAL STEM CELL PROLIFERATION IN AGING BRAIN

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Glucagon-like peptide-1 (GLP-1), which is secreted from the gastrointestinal, performs as a critical hormone in stimulating insulin secretion, has been found expressing in the brain. The protective efforts of GLP-1 in central nervous system (CNS) have been highlighted in the recent three years. Growing reports from both basic research laboratory and clinical trial had shown that GLP-1 receptor (GLP-1R) agonists have been suggested to be effective in reducing aging-associated pathologies, particularly in enhancing learning and memory functionality. In our study, we found that followed by 8 weeks administration of GLP-1R long acting agonist - Exendin-4, improvement of learning ability could be detected by Water Maze on aging mice. 5-bromo-2-deoxyuridine (BrdU) was administrated before 4 weeks OR at the same day of sacrifice for endogenous MSC labels. Data showed that, GLP1R agonist could improve proliferation of endogenous neural stem cell in SVZ area; these neural stem cells could migrate to the CA1 and DG area of hippocampus. Our data suggested that GLP-1 may contribute to the cognitive improvement of aging mice.

T-1210

INDUCED NEURONAL (IN) CELL REPROGRAMMING: INSIGHT INTO MYT1L TRANSCRIPTION FACTOR FUNCTION

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Forced expression of transcription factors is sufficient to directly reprogram distant cell types into another. Recently, our lab has shown that expression of the neuronal transcription factors Brn2, Ascl1, and Myt1l (BAM) converts fibroblasts to induced neuronal (iN) cells. The ability to generate human iN cells in a culture dish is now successfully being used for disease modeling and holds great promise in regenerative medicine. In addition, the iN cell reprogramming paradigm represents a new platform to interrogate structural and functional properties of neuronal transcription factors. One of the three key reprogramming factors, Myt1l, is a CCHHC-type zinc finger transcript factor that has been identified as one of the earliest specific markers of postmitotic neurons, indicating a role in pan-neuronal identity. Recent work found mutations in Myt1l to be associated with neurodevelopmental disorders such as schizophrenia and in brain tumors, suggesting a critical role in terminal neuronal differentiation. In this study we used our iN reprogramming technology and performed a truncation and mutagenesis screen to pinpoint the essential molecular domains of Myt1l that trigger iN cell formation and neuronal maturation. We identified a minimal Myt1l fragment that generates neurons with the same efficiency and maturity as wild type Myt1l. In addition, we employed ChIP-seq and RNA-seq to dissect the roles of Myt1l on gene expression during induced neurogenesis. Strikingly, we found that the dominant function of Myt1l during reprogramming is transcriptional repression, which was linked to interaction and chromatin recruitment of the SIN3/HDAC co-repressor. Finally, we identified the genetic networks downstream of Myt1l and showed that transcriptional repression of the Notch pathway allows Myt1l to overcome Notch inhibition during induced neurogenesis. Thus, neuronal fate induction by proneural bHLH factors is subsequently stabilized by Myt1l-mediated inhibition of Notch signaling in differentiating neurons. In summary, this work provides insight into the molecular function of Myt1l during induced and normal neurogenesis. Moreover our study highlights the usefulness of iN cell technology for detailed structure function studies of disease relevant factors in mammalian cells.

T-1211

HUMAN NEURAL STEM CELLS EXPRESSING IGF-1: A NOVEL CELLULAR THERAPY FOR ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most prevalent age-related neurodegenerative disorder. Patients exhibit progressive cognitive decline and there is currently no effective treatment for the disease. Cellular therapies represent an emerging approach to treat neurodegeneration, and our laboratory has experience translating a preclinical cellular therapy to the clinic. We recently completed Phases I and II of an FDA-approved clinical trial validating safety and feasibility of intraspinal transplantation of human neural stem cells (NSC) in amyotrophic lateral sclerosis (ALS) patients. Supporting preclinical data demonstrated that NSCs integrate to form synapses with the host, improve inflammation and reduce disease associated pathologies. Our long term objective is to develop a similar cellular therapy for AD using HK532-IGF-1, a novel human NSC line modified to express the neuroprotective factor insulin-like growth factor-1 (IGF-1). HK532-IGF-1 cells or vehicle (sham) were administered bilaterally to the peri-hippocampal area of male transgenic APP/PS1 mice. All animals received immunosuppression until the study end point. Animals were tested on two hippocampal-dependent behavioral tasks at different time points: novel object recognition (NOR) and Morris Water Maze (MWM). AD pathology was assessed using standard immunohistochemistry protocols. Transplantation of HK532-IGF-1 preserved short term memory 4 weeks post intervention in the NOR test. In the MWM, vehicle injected AD mice demonstrated significant impairments relative to wild-type controls. In contrast, HK532-IGF-1 mice performed similarly to wild-type animals, as indicated by significantly shorter latencies during training and increased time spent in target quadrant during the probe test. Tissue analysis revealed a significant reduction in β-amyloid plaques in both cortex and hippocampus of animals that received stem cell injections relative to controls. These data indicate that peri-hippocampal HK532-IGF-1 cell transplantation can impact learning and memory deficits as well as disease-associated pathology in AD. Together, these findings support further large scale preclinical studies to progress this stem cell therapy to a disease modifying intervention for AD patients.

T-1212

PHENOTYPIC SCREENING OF HUMAN STEM CELL DERIVED OLIGODENDROCYTE PROGENITORS FOR THE DISCOVERY OF REGENERATIVE DRUGS

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The demyelinating disease multiple sclerosis (MS) affects around 2.5 million people worldwide. In around 70% of the MS patients, oligodendrocyte precursor cells (OPCs) are recruited to damaged lesions but fail to differentiate into myelin producing oligodendrocytes. Identifying drugs that enhance the differentiation and maturation of the resident OPC population to myelin producing oligodendrocytes could have important therapeutic implications. We have identified a number of in vitro efficient protocols capable of generating PDGFRa+ OPCs from human neural stem cells in monolayer. These protocols were indentified via the use of CombiCult™, Plasticell's bead-based combinatorial cell culture system, which allowed us to screen 6912 combinations of unique cell

culture media. CombiCult™ derived OPCs were then characterised for their differentiation potential, protein marker expression, mRNA expression and hence suitability for screening. The OPCs were then used in a phenotypic screening to identify compounds which generate myelin positive oligodendrocytes.

T-1213

DIFFERENTIAL EXPRESSION OF THE SYNDECAN FAMILY MEMBERS DISTINGUISHES QUIESCENT FROM ACTIVATED NEURAL STEM CELLS IN THE ADULT MOUSE SUBVENTRICULAR ZONE

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The production of new neurons persists during adulthood and is ensured by neural stem cells (NSCs) in neurogenic niches such as the subventricular zone (SVZ). Quiescent NSCs are considered the reservoir for adult neurogenesis. Understanding the mechanisms regulating the balance between quiescence and proliferation of NSCs is critical for the development of new therapeutic approaches to counteract neuronal loss during aging or in various brain pathologies. Using a LeX/EGFR/CD24 triple labeling, we isolated by flow cytometry quiescent NSCs (LeXbright), activated NSCs (LeX+EGFR+) and progenitor cells (EGFR+) from the adult mouse SVZ after a 15-day bromodeoxyuridine (BrdU) incorporation followed or not by a 15-day chase period. Interestingly, 25% of LeXbright cells incorporated BrdU and all of them retained the BrdU labeling after the chase in contrast to highly proliferative LeX+EGFR and EGFR+ cells which lost the BrdU labeling. A genome-wide microarray analysis of these label-retaining quiescent NSCs versus activated NSCs led to the identification of a quiescence signature of 255 genes. Gene ontology analysis identified enrichment of gene clusters associated with cell adhesion and cell-cell communication in quiescent NSCs. In particular, the syndecan family members were found to be differentially expressed. We are currently investigating their potential role in NSC quiescence.

T-1214

RELATIONSHIP BETWEEN NURR1 EXPRESSION AND DEVELOPING DOPAMINERGIC PORGENITOR CELLS

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Parkinson's disease (PD) is one of the neurodegenerative diseases and is characterized by the death of dopaminergic (DA) neurons in the substantia nigra. The main symptoms of motor abnormalities in PD are bradykinesia, resting tremor, rigidity, and postural instability. Current therapeutic approaches for PD such as pharmacological tools and deep brain stimulation provide symptomatic relief, but they cannot control the progression of the disease. Recently, cell therapy for PD is considered as a prominent therapy because lost neurons can be replaced by new ones. The previous studies in patients with PD after intrastriatal transplantation of human fetal mesencephalic tissue that includes many DA neurons have provided proof of principle that neuronal replacement can work in the adult brain. However, it is difficult to prepare sufficient fetal brain tissue to transplant for one PD patient. Instead of fetal tissue, embryonic stem cells (ESCs) and induced pluripotnent stem cells (iPSCs) are promising cell sources for cell-replacement therapy of PD. We developed the protocol of inducing DA progenitor cells from ESCs / iPSCs and they were able to ameliorate motor symptoms of PD model rats by transplantation into the host striatum. However, all model rats were not cured because a small number of DA neurons were induced in the host brain. Furthermore, DA neurons are fragile against various stresses and therefore it is important to protect DA neurons from the stimulation of host environment and to induce DA neurons in the host brain. To dissolve these problems, we focused on NURR1 protein. NURR1 is an orphan nuclear receptor and plays an essential role in the generation and maintenance of DA neurons in the midbrain. In our protocol, the ratio of NURR1-expressing DA progenitor cells at transplantation date was low or high depending on the cell lines. This result is thought to indicate the difference of amelioration of PD model rats. In this study, we found that activation of TGFβ signaling increased the ratio of NURR1 expression. Furthermore, when these cells were transplanted into the host brain, more DA neurons were detected compared with transplantation of NURR1-low expressing cells.

T-1215

CONTRIBUTIONS OF CHROMATIN ACCESSIBILITY AND TRANSCRIPTION FACTOR ACTIVITY TO NEURAL STEM CELL GENE EXPRESSION SPECIFICITY

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A key feature of multipotent stem cells is their ability to generate differentiated progeny of specific lineages, each with its own gene expression profile. Despite extensive knowledge of genome wide chromatin accessibility and transcription factor binding in many stem cell populations, how these essential regulators balance in order to control stem cell competence and gene expression is still unknown. To elucidate this question, here we have compared genome wide chromatin accessibility, transcription factor binding and gene expression profiles of pluripotent ES-cells to those of multipotent embryonic mouse cortex and spinal cord stem cells. While DNase1-hypersensitive sites (DHSs) in stem cells of the embryonic CNS overlapped extensively with those in ES-cells, DHSs specific to a single neural stem cell population were most often generated de novo. Sox2 is a master stem cell transcription factor common to ES-cells and all neural stem cells. Although Sox2 bound thousands of DNA regions, its binding profile was, in contrast to chromatin accessibility, unique to each cell type and reflected their gene expression profiles. In accordance, enhancers bound by Sox2 in cortical and spinal cord stem cells were found to drive gene expression in a cell type specific manner using a zebrafish reporter system. Moreover, bioinformatic and mutational analyses of Sox2 bound enhancers revealed partner transcription factors responsible for the selective enhancer activity. Finally, these results were used to compile, and functionally test, a model explaining the contributions of chromatin accessibility, Sox2 binding and partner factor expression to the formation of specific and stable gene expression in different stem cell populations of the developing nervous system.

T-1216

EFFECTS OF CHANGES IN GTF2I AND GTF2IRD1 COPY NUMBER ON THE NEURAL DEVELOPMENT IN THE MOUSE MODELS OF WILLIAMS-BEUREN SYNDROME AND 7Q11.23 DUPLICATION SYNDROME

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Williams Syndrome (WS) and 7q11.23 Duplication Syndrome (Dup7) are two rare neurodevelopment disorders caused by deletion and duplication respectively, of 25 genes on chromosome 7q11.23. Individuals with WS or Dup7 present with an array of cognitive and behavioural phenotypes, including anxiety, autism, speech and language delay, and intellectual disability, but the biological basis for these neuropsychiatric symptoms remain unknown. Structural and functional abnormalities of the cortex have been identified in both WS and Dup7, suggesting that cortical development is perturbed in these disorders. Our lab has generated mouse models with altered gene dosage of two candidate genes from the WS commonly deleted region, General Transcription Factor 2 I (Gtf2i) and GTF2I Repeat Domain containing protein 1 (Gtf2ird1). These models include mice with hemizygous deletion of Gtf2i (Gtf2i+/-) or of both genes (Gtf2i/Gtf2ird1+/-) and mice with duplication of Gtf2i (Gtf2i+/Dup). We have used these mice to determine whether altered expression of Gtf2i and/or Gtf2ird1 affects neural stem cell growths and cell-fate, and potentially contributes to the neurological features of WS. Our results showed that mice with a hemizygous deletion of Gtf2i and Gtf2ird1 had an overall reduction in the number of neuronal precursors and neurogenesis with an increase in astrogenesis, whereas mice with an additional copy of Gtf2i had an increase in the number of precursors and neurogenesis along with a decrease in astrogenesis. These early changes in neural development translated into perturbed cortical organization with changes in cortical thickness and cell packing density. These data will help us understand how the developing brain is perturbed in people with WS and Dup7 and may give insight into an answer the pathological causes and possibly help identify therapeutic interventions to treat the cognitive disorders involving autism and social dysfunction.

T-1217

IMMUNOGENICITY OF HUMAN INDUCED PLURIPOTENT STEM CELLS-DERIVED NEURAL STEM CELLS AS A CELL SOURCE OF TRANSPLANTATION THERAPY FOR SPINAL CORD INJURY

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Recent studies demonstrated the effectiveness of transplantation of human induced pluripotent stem cells-derived neural stem cells (hiPSC-NSCs) for rodent as well as non-human primate spinal cord injury. Since allogeneic transplantation will be aimed in first-in-human trial in taking advantage of iPS bank in Japan, immunological investigation of hiPSC-NSCs is crucial. The purpose of this study is to determine immunogenicity and immune response of hiPSC-NSCs in vitro. Integration-free human iPS clone (414C2) derived NSCs were used in this study. The expression of immune-related surface antigens on hiPSC-NSCs was analyzed using flow cytometry and PCR. Effects of inflammatory stimulation and passage number of hiPSC-NSCs on immunogenicity were also evaluated at the same time. Furthermore, hiPSCs were analyzed in the same way to compare with immunogenicity of hiPSC-NSCs. Mixed lymphocyte reaction (MLR) was performed to evaluate the degree of immune response in vitro. hiPSC-NSCs inactivated by irradiation were co-cultured with allogeneic lymphocyte and proliferation of lymphocyte was measured quantitatively by radioactivity counter. As a positive control, two human HLA incompatible lymphocytes were mixed. More than 80% of hiPSC-NSCs expressed HLA class I. In contrast, less than 0.5% of hiPSC-NSCs expressed HLA class II or co-stimulatory molecules which play an important role in antigen presentation. IFN-γ administration significantly increased the expression of HLA class I and CD54, but did not increase the expression of HLA class II and co-stimulatory molecules. Compared to the hiPSCs, hiPSC-NSCs showed significantly higher expression of HLA class I and CD54. In MLR, proliferation of lymphocyte co-cultured with hiPSC-NSCs was significantly suppressed compared to that co-cultured with allogeneic lymphocytes. Multiple passages less than 7 times did not affect the expression of surface antigen and the activation of lymphocyte mixed with hiPSC-NSCs. Immunological examination revealed that hiPSC-NSCs exhibited low immunogenicity, and that hiPSC-NSCs suppressed the lymphocyte proliferationin MLR, suggesting their immunomodulatory function. Multiple passages of hiPSC-NSCs less than 7 times did not influence their immunogenicity.

T-1218

PROLIFERATING NEUROSPHERE CULTURES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS BY DUAL SMAD INHIBITION - AMBIGUOUS RESULTS

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Reprogramming patient derived fibroblasts to induced pluripotent stem cells (iPSC) followed by neural induction (NI) and differentiation allows the generation of patient specific neurons. Keeping neural stem cells (NSC) under proliferative conditions, followed by terminal differentiation when needed can greatly enhance the numbers of neurons generated. An efficient protocol for NI utilizes the dual SMAD inhibition by small molecules under adherent culture conditions. However to our knowledge it has not yet been studied how successful this method can be employed for generating cultures of proliferating NSC. Our objective was therefore to establish and evaluate a protocol for deriving proliferative neural cultures from iPSC by dual SMAD inhibition. Human IPSC were induced neurally by deprivation of fibroblast growth factor (FGF) and a dual SMAD inhibition by the small molecules LDN-193189 and SB-431542 under adherent culture conditions. After 10 days cells were transferred to low-attachment culture plates, where they formed neurospheres (NSPH). The proliferating NSPH were passaged, RNA collected, transcribed to cDNA and analysed by qPCR. Also NSPH were plated down at regular intervals for immunofluorescent stainings and either analysed

directly or differentiated first by addition of BDNF (brain-derived neurotrophic factor), GDNF (glia-derived neurotrophic factor), IGF (Insulin like growth factor), dibutyl cAMP and the notch inhibitor DAPT for 30 days. The experiments showed mixed results: while some cell lines developed well proliferating NSPH, others stopped proliferating after few passages. The proliferating NSPH expressed neural precursor markers nestin, PAX6 and the neuronal marker β-III tubulin. qPCR confirmed increasing nestin expression and a shift from a rostral to caudal regional phenotype during culturing. The differentiation resulted in MAP2+ neurons with a majority of BRN2+ cells, as a marker for cortical neurons, as well as lower amounts of GABAergic and dopaminergic TH-positive neurons. Although being helpful for differentiation protocols, due to the big variability and high amount of culture failure, dual SMAD inhibition might not be the best option for NI for subsequent culturing of neural precursors and embryoid body based protocols might do better.

T-1219

TOWARDS A STEM CELL-BASED THERAPY FOR SENSORINEURAL HEARING LOSS AFTER EXPERIMENTAL PNEUMOCOCCAL MENINGITIS IN INFANT RATS

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Bacterial meningitis (BM) is the most common cause of acquired profound bilateral sensorineural hearing loss (SNHL) in childhood occurring in up to 45% 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 all forms of 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 infant rat model of BM. We show that increasing inoculum concentrations elevated the hearing thresholds in a dose-dependent way. The density of spiral ganglion neurons in the basal turn of cochleas were significantly reduced in animals infected with a medium (1 x 105 cfu/ml) or high (1 x 106 cfu/ml) inoculum concentration compared to uninfected control animals. Stem cell transplantations were performed two weeks postinfection (106 cfu/ml) in rats with bacteriologically cleared meningitis. Thereby, green fluorescence protein (GFP) expressing spiral ganglion-derived stem cells were transplanted directly into the modiolus via a retroauricular, transbullar approach. The surgical access and the transplantation was performed in 6 animals with minimal trauma to the middle and inner ear structures. The grafted stem cells survived and morphologically integrated into the modiolus with the formation of axonal structures after 1 week and partial differentiation into β-III tubulin-positive neurons 4 weeks post transplantation. 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. The functional role of transplanted cells on auditory function and hearing sensitivity will be analyzed in further experiments by using auditory brainstem responses.

T-1220

INNATE IMMUNE SYSTEM MOLECULE COMPLEMENT PROTEIN C1Q HAS A CONCENTRATION DEPENDENT EFFECT ON HUMAN NEURAL STEM CELL PROLIFERATION AND DIFFERENTIATION

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Blood-brain barrier (BBB) disruption after spinal cord injury exposes the central nervous system (CNS) to serum and blood components, including innate immune cells and proteins. Post-injury, complement proteins C1q and C3a are also synthesized by infiltrating immune cells at concentrations that are very different from that present in the serum. We have recently shown that conditioned media (CM) derived from polymorphonuclear neutrophils (PMN) and macrophages (MΦ) alters migration and differentiation of multipotent human neural stem cells (hNSC) in vitro. In parallel, blockade of complement proteins C1q and C3a during the CM exposure reversed these effects. In this study, we investigated the effect of C1q doses ranging from inflammatory cell secreted concentrations to those observed in the serum (20 ng/ml to 120 µg/ml) on hNSC in vitro. C1q exposure caused a dose-dependent effect on proliferation and differentiation without changes in survival. MΦ secreted concentrations of C1q promoted differentiation towards the oligodendroglial lineage. In contrast, serum concentrations of C1q dramatically decreased proliferation, altered tri-lineage differentiation, size and cellular morphology. Time lapse live cell imaging using mouse NSC expressing Fucci-cell cycle indicator suggest that the changes associated with serum concentrations of C1q may be due to accelerated G0/G1 arrest. Serum concentrations of C1q may affect the cellular response of NSC populations in the acute post-injury period, before BBB integrity is re-established. Conversely the effects of PMN and MΦ secreted concentrations of C1q may contribute to the regulation of cell migration and fate in the later stages post-injury. Understanding the non-traditional effects of C1q and other complement proteins on hNSC populations may provide insight into therapeutic targets for controlling the inflammatory microenvironment after CNS injury and disease.

T-1221

SYSTEM XC- PROMOTES VIABILITY OF HUMAN NEURAL STEM CELLS BY DECREASING ROS-MEDIATED APOPTOSIS

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Recent progress in neural stem cell (NSC) biology includes therapeutic regenerative strategies to replace lost neural cells, promote host tissue regeneration, and harness the inherent tropism of NSCs to deliver therapeutic agents directly to sites of CNS injury or disease. Although there is potential value of transplanted NSCs for the treatment of many CNS pathologies, very little is known about their sensitivity to the hostile microenvironment. A

stable, immortalized NSC line, HB1.F3.CD, exhibits sub-optimal survival in vivo shown by increased TUNEL staining. Since many neuropathological insults are accompanied by oxidative stress, we need to have a better understanding of NSC antioxidant defense mechanisms. Exogenous treatment with the antioxidants glutathione (GSH) or N-acetylcysteine promoted the survival of the HB1.F3.CDs under hypoxic insult and oxidative stress in vitro. However, treatment under basal conditions decreased cell viability. To determine what endogenous antioxidant mechanisms may play a role in NSC health, we examined the HB1.F3.CDs for expression of system xc-. This transporter contributes to the antioxidant capacity by importing cystine into a cell, where it is then reduced to cysteine and used to synthesize GSH. We show for the first time that the HB1.F3.CDs as well as primary NSC pools express high levels of xCT (SLC7A11), the catalytic domain of system xc-. Inhibition of system xc- accomplished pharmacologically using sulfasalazine decreased NSC viability and proliferation in a dose-dependent manner while increasing apoptosis. This was correlated with a significant increase in intracellular reactive oxygen species and DNA double-strand breaks as shown by increased γ-H2AX staining. The transporter is modulated by various insults where GSH depletion by buthionine sulfoximine, an inhibitor of γ-glutamylcysteine synthetase, increased SLC7A11 and hypoxia decreased SLC7A11 expression. Our data suggest that system xc- is important in modulating NSC redox and viability. Therefore, we stably over-expressed SLC7A11 in the HB1.F3.CD NSCs to assess whether they have increased survival under insult. Genetic manipulation of system xc- may improve survival of transplanted NSCs and thus improve clinical outcome in patients receiving NSC-mediated therapies.

T-1222

MICROCARRIER PROMOTED EXPANSION OF NEURAL PROGENITOR CELLS (NPCS) CAN SURVIVE, DIFFERENTIATE, AND INNERVATE THEIR LONG DISTANCE TARGETS WHEN TRANSPLANTED INTO MICE

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Neuronal progenitor cells (NPC) derived from human embryonic stem cell (hESC) is a good cell source in the transplantation therapy in terms of its availability and ethical acceptability. However, the traditional expansion and differentiation from hESC to NPC takes long time and the cell yield is low. In this study, we use a microcarrier (MC) system for the expansion of hESC and its subsequent differentiation into NPC. We found there are 6 folds more NPCs can be generated by using this MC based system compared with traditional 2D culture system. The hESC derived NPC was subsequently transplanted into the striatum of adult NOD-SCID (NOD.CB17-Prkdcscid/NcrCrl) mice in either single cell form or cell aggregation form. Recipient mice were perfused and the in vivo survivability, differentiation potential, maturation, and targets innervation of the transplanted cells were assessed by immunostaining. Both the transplanted single NPC and aggregated NPC showed long term survival in vivo, which are revealed by human specific NCAM and nuclear staining. Compared with single cells, the transplanted cell aggregate showed better survivability. Both the transplanted single NPC and aggregate NPCs are able to differentiate into DCX positive, Tuj1 positive and NeuN positive neurons in vivo. Furthermore, both the transplanted single cells and the cell aggregates can send long neurites to integrate into the host brain tissues. These results provided preclinical evidence for the use of MC to expand and differentiate hESC-derived NPC in cell transplantation therapy in PD.

T-1223

MODELING HUMAN CORTICAL DEVELOPMENT THROUGH THE GENERATION OF FUNCTIONAL, SELF ORGANIZING NEURONS FROM INDUCED PLURIPOTENT STEM CELLS

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Human psychiatric diseases are associated with genetic variations that presumably affect the neural development. Generating patient specific induced pluripotent stem cells (iPSCs) provides the in vitro analysis of genetic variations to verify their impact on diseases. Here, we established various differentiation strategies enabling the effective generation of neural stem cells (NSCs) and cortical neurons from human iPSCs. We efficiently induced self-renewing NSC lines from iPSCs. Protein and transcript analysis of specific markers such as SOX2, PAX6, MSI-1 and NEUROG3 indicated the induction of neural cell fates. These progenitor cells efficiently differentiated into glial (GFAP, OLIG2) and neuronal cells (TUBB3, STX, NEUN) within 8 weeks. Markers representative for regional identity were also detectable (ACSL1, FOXG1). Furthermore, immunostaining and whole-cell patch clamp recordings of mature neurons revealed the presence of different neuron subtypes such as inhibitory GABAergic and excitatory glutamatergic cortical neurons (GABA, TBR1). The differentiation model and the culture conditions were transferred to a three dimensional culture system allowing the generation of cerebral organoids. In order to mimic brain regions including the cerebral cortex, NSCs were cultured as free-floating neurospheres in bioreactors. Within 4 weeks, neurospheres differentiated into cerebral organoids. Early neural development was confirmed through the expression of specific markers such as PAX6 and SOX2. Immunostaining of as well as GABA as well as TBR-1 and NEUN demonstrated the presence of mature post mitotic neurons within the cerebral organoids. These results confirmed the efficient neural differentiation of human iPSCs using both culture systems. The generation of cortical neurons from patient-specific iPSCs enables further investigations of neuronal dysfunctions associated with specific genetic variations.

T-1224

STROKE

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Stroke is a leading cause of neurological disability and mortality in adults. Despite its prevalence, there are no medical therapies for alleviating the chronic functional impairments following stroke. Human fetal neuralstem cell transplants have been shown to

produce some recovery in animal models of stroke, but ethical consideration and a short supply of fetal tissue limits this approach. Human parthenogenetic stem cells offer a good alternative because they are derived from unfertilized oocytes without destroying viable human embryos and can be used to generate an unlimited supply of neural stem cells for transplantation. We have previously reported that human parthenogenetic stem cell derived neural stem cells(hpNSCs) were safe and provided functional improvements in Parkinson disease animal models. Here we present the results of a proof of concept study testing the safety and therapeutic potential of hpNSCs in a mouse model of stroke with middle cerebral artery occlusion followed by reperfusion (MCAO/R). Twenty-four hours post-MCAO/R, control vehicle (PBS) or hpNSCs were transplanted into the ipsilesional hippocampus. To assess motor recovery, we performed the beam walk and rotarod behavioral test on each mouse for 3 days consecutively before MCAO/R(as training) and 2 to 31 days post-injury. The results of our study demonstrate that intra hippocampal administration of hpNSCs is safe and provides significant motor improvement. Overall, these results represent a first but significant step toward the therapeutic application of hpNSCs as a safe and effective cell therapy for stroke.

T-1225

IDENTIFICATION AND CHARACTERIZATION OF SECONDARY NEURAL TUBE-DERIVED EMBRYONIC NEURAL STEM CELLS IN VITRO

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Secondary neurulation is an embryonic progress that gives rise to the secondary neural tube, the precursor of the lower spinal cord region. The secondary neural tube is derived from aggregated Sox2-expressing neural cells at the dorsal region of the tail bud, which eventually forms rosette or tube-like structures to give rise to neural tissues in the tail bud. We addressed whether the embryonic tail contains neural stem cells (NSCs), namely secondary NSCs (sNSCs), with the potential for self-renewal in vitro. Using in vitro neurosphere assays, neurospheres readily formed at the rosette and neural-tube levels, but less frequently at the tail bud tip level. Further, we identified that sNSC-generated neurospheres were significantly smaller in size compared to cortical neurospheres. Interestingly, various cell cycle analyses revealed that this difference was not due to a reduction in the proliferation rate of NSCs, but rather to the neuronal commitment of sNSCs, as sNSC-derived neurospheres contain more committed neuronal progenitor cells, even in the presence of EGF and bFGF. These results suggest that the higher tendency for sNSCs to spontaneously differentiate into progenitor cells may explain the limited expansion of the secondary neural tube during embryonic development.

T-1226

SUPPRESSOR OF FUSED (SUFU) REGULATES HINDBRAIN NEURAL PROGENITOR PROLIFERATION

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Sonic hedgehog (Shh)/Gli pathway plays essential function in the patterning and proliferation of mammalian neural tube. Suppressor of fused (Sufu) was characterized as a negative modulator of Shh signaling. In the spinal cord dorsoventral (DV) patterning, Sufu plays important roles in the activation of Shh signaling via Gli transcription factors. However, the functions of Sufu in hindbrain patterning and proliferation remain elusive. To address these questions, Sufu was conditionally ablated in mouse hindbrain rhombomere (r) 4. Deletion of Sufu caused enlargement of r4, predominantly enlarged in dorsal r4. BrdU pulse labeling revealed more profound increased proliferation of dorsal Pax6/Sox2 positive progenitor domain than the ventral Nkx2.2/Sox2 positive progenitor domain, coupled by markedly reduced cell cycle exit, suggesting differential control of cell cycle progression along the dorsoventral axis of r4. Examination of DV patterning revealed dramatic dorsal expansion of pMN and p2 progenitor domains in mutant r4. However, the FoxA2 positive floor plate, and the dorsal p0 domain were not severely affected, suggesting a novel domain specific regulation of neural progenitor pools by Sufu. Interestingly, we observed spatial up-regulation of active β-catenin, Gli1 and Gli2 transcription factors that coincides with the region exhibiting highly proliferative cells. Furthermore, concomitant deletion of Gli2 in the Sufu mutant largely rescued the patterning and proliferation phenotypes of Sufu single mutant. Besides, deletion of β-catenin alleviates proliferation phenotype of Sufu mutant. Our study demonstrates novel functions of Sufu to repress both Gli2 and β-catenin in the hindbrain to ensure tightly controlled neural progenitor cell cycle progression.

T-1227

IN VITRO MONITORING OF HUMAN IPS CELL-DERIVED NEURON GROWTH WITH A MICROFABRICATED DEVICE

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Neuronal cells derived from human induced pluripotent stem cells (iPSCs) have great potential for drug discovery and transplantation. Fulfilling the goal requires further investigations of maturation processes of neuronal functions in iPSC-derived neurons. However, little is known about the time course and related factors of functional maturation, due to a lack of an effective method. We aim to evaluate the process of functional maturation from their electrical activity at both sub-cellular and network levels. In this study, we recorded electrical activity of iPSC-derived neurons with two recording systems which include a novel microfabricated device and a conventional system. The microfabricated device is composed of a microelectrode array (MEA) and microtunnels made of polydimethylsiloxane. The MEA has 64 micro-electrodes on the culture substrate, which is capable of long-term and multi-site recording. Microtunnels are able to guide axons on recording electrodes. The device allows us to record propagating action potentials along axons and to evaluate activity dependent changes in axonal conduction velocity. Meanwhile, the other system is based on the conventional MEA. Neural activity are recorded from 64 electrodes simultaneously with the MEA, which allows us to evaluate activity patterns of neuronal networks. First, human iPSCs were induced to differentiate into neural stem and progenitor cells in the dorsal regions of cerebral cortex by SB431542 and dorsomorphin. Then, these cells were seeded and allowed to differentiate into glutamatergic neurons and glial cells on the recording devices. Neural

activity was recorded every ten days and developmental changes were evaluated. Finally, we discussed the developmental changes of iPSC-derived neurons at sub-cellular and network levels from recorded activity.

T-1228

DELETION OF GEMININ DURING THE EARLY STAGES OF CORTICOGENESIS IN MICE LEADS TO A PHENOTYPE SIMILAR TO MICROCEPHALY

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Telencephalon is generated during embryonic development by proliferating neuroepithelial cells in the anterior neural tube and subsequently develops into several structures including the cerebral cortex. In the developing mouse cortex, differentiated cells are generated during neurogenesis from distinct types of neural progenitor cells occupying the ventricular and subventricular zone (VZ and SVZ). Neuroepithelial cells represent the initial population of neural progenitors, from which the earliest born neurons of the cortex and other neural progenitor populations are generated. Microcephaly in humans and mice is characterized by reduction of the brain mass, mainly concerning the cortex. Abnormal cell cycle kinetics, defects in mitosis and DNA repair mechanisms as well as impaired epigenetic regulation of gene expression in neural progenitors have been implicated in the molecular pathogenesis underlying this clinical entity. Geminin is proposed to regulate the balance between self-renewal and differentiation via interactions with transcription factors and chromatin remodeling complexes. We have previously shown that loss of Geminin expression during mid-embryogenesis leads to the transient expansion of neural progenitor cells located in the VZ and SVZ. Moreover, overexpression of Geminin promotes cell cycle exit and premature neuronal differentiation. We have specifically inactivated Geminin during early events of neuroepithelial generation in the mouse cortex. Geminin deficient mice exhibit a phenotype characterized by reduced telencephalic vesicles reminiscent of microcephaly which is coincident with aberrant neuronal differentiation and increased DNA damage and apoptosis. Our data suggest that Geminin participates in mechanisms regulating survival and neuronal differentiation of neural progenitor cells during the early stages of cortical development and assign Geminin with an important role in corticogenesis.

T-1229

TRANSPLANTATION OF HUMAN EMBRYONIC STEM CELL DERIVED NEURAL PRECURSORS IN A RAT CONTUSION MODEL OF SPINAL CORD INJURY

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Neural progenitor cells (NPCs) have been tested as an alternative treatment for several neurologic diseases due to their ability of differentiation into neurons, astrocytes and oligodendrocytes. Patients suffering from spinal cord injury still have limited treatment options to restore movement. The aim of this study has been to isolate and characterize human NPCs derived from embryonic stem cells and transplant them in a spinal cord injury model. Human embryonic stem cells (ES) of the H9 cell line were used to derive NPCs in vitro. The cells were maintained undifferentiated in DMEM-F12 plus 20% KnockOut Serum Replacement + 2 β-ME + 10 ng/ml FGF2 on the top of inactivated mouse embryonic fibroblast layers. For differentiation, colonies were cultured in low attachment plates for embryoid body formation for 4 days and transferred to matrigel coated-plates for 16 days in DMEM-F12 + 1X ITS. Rosettes were manually isolated and dissociated using Tryple Express. Single cells were maintained in KnockOut DMEM + StemPro Neural Supplement + 20 ng/ml FGF2 and 20 ng/ml EGF in CellStart-coated plates. Immunophenotyping was performed by flow cytometry using fluorochrome labeled antibodies anti-CD15, -CD29, -CD44, -CD271, -nestin and -sox2. The cells were differentiated into neurons and astrocytes using standard protocols. 5x105 cells were transplanted in a rat contusion model of spinal cord injury. 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 four further weeks after transplantation. The NPCs were cultivated for at least five passages after rosette isolation. More than 90% of the cells expressed both nestin and sox2. CD29 was expressed by more than 99% of the cells while CD15 expression was variable. Less than 10% of the cells were CD44+ or CD271+ (mesenchymal/neural crest markers). The cells were able to differentiate into neurons and astrocytes (GFAP+ cells) in vitro. Open-field locomotion in the cell transplanted groups was improved when compared with the vehicle. Analysis of the fate of transplanted cells is ongoing. A relatively pure population of NPCs was efficiently derived from ES in vitro. This data indicates that NPCs are promising candidates for cell therapy for CNS diseases.

T-1230

MICRORNA-181A PROMOTES DOPAMINERGIC DIFFERENTIATION BY ENHANCING WNT ACTIVITY AND TARGETING THE NUCLEAR RECEPTOR GCNF

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Several studies have demonstrated that a functional microRNA (miRNA) system is required for the generation of mesencephalic dopamine (mesDA) neurons, which are degenerated in Parkinson's disease. Further studies in mouse ES cells indicated that miRNAs could be envisaged as tools to modulate DA neuron specification during in vitro differentiation. Here, we used a specific population of long-term self-renewing rosette-forming neuroepithelial stem cells (lt-NES cells) derived from human pluripotent stem cells to identify novel miRNAs associated with early human neuronal differentiation and subtype specification. Based on these initial experiments, we found that miR-181a enhances neuronal differentiation in general

but also specifically promotes the differentiation into TH-positive mesDA-like neurons. Intriguingly, miR-181a*, which is encoded by the same pre-miRNA, had the opposite effect and impaired the generation of TH-positive neurons. The two sister miRNAs also showed distinct expression patterns during dopaminergic differentiation indicative of an intrinsic regulatory mechanism. The positive effect of miR-181a on the dopaminergic lineage was further confirmed using human pluripotent stem cell-derived floor plate progenitor cells as well as pre-rosette neural stem cells. In both cell culture paradigms, ectopic expression of miR-181a promoted the differentiation of FOXA2-positive progenitor cells into TH-positive mesDA neurons. We further found that miR-181a targets the germ cell nuclear factor GCNF, which has been previously associated with forebrain and midbrain development and was here identified as a negative regulator of dopaminergic differentiation. In addition, miR-181a also enhances Wnt signaling probably by targeting several negative modulators of this pathway, which further promotes the dopaminergic lineage. Finally, we have integrated these findings into a transfection-based miRNA modulation approach that could be combined with the currently used patterning protocols to generate DA neurons. Taken together, our data reveal the impact of miRNAs on human dopaminergic differentiation and delineate a miRNA that promotes the generation of this medically highly relevant neuronal subtype.

T-1231

HIGH THROUGHPUT GENE EXPRESSION ANALYSIS OF HUMAN NEURAL DEVELOPMENT TO TEST NEURONAL DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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In recent years stem cell technology has provided an in vitro cellular model that permits the study of many human diseases. The correct differentiation of stem cells to the desired cell types is a key issue, and ideally these human pluripotent stem cell (hPSC)-derived tissues/cells should be evaluated by comparison with tissue/cells profiled from the equivalent in vivo developmental area. Gene expression profiling during human development is essential for defining stage-related changes for specific anatomical regions, and these data could subsequently be applied to in vitro differentiation protocols. Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder that mainly affects the striatal medium spiny neurons (MSNs). Several efforts have been made to generate human PSC-derived MSNs although with varying degrees of success. With this in mind our aim was to generate quantitative genetic profiles of telencephalic development, subpallium specification and striatal maturation in human fetal and adult brain by high-throughput QPCR, and then apply these profiles to evaluate the efficacy of human PSCs differentiation to MSNs. Our results showed a specific developmental profile of expression of genes involved in striatal specification in fetal and adult brain tissue. Application of this expression pattern to the different stages of our in vitro differentiation protocol that were analysed here revealed that the cells acquired a WGE-like fate and were progressing towards an adult striatal identity. This study therefore establishes the qualitative and quantitative data to evaluate telencephalic differentiation from hPSCs. Of note, this quantitative method of gene expression analysis in human tissue and its application to in vitro differentiation protocols can be utilized in a broad range of experimental paradigms.

T-1232

D SERINE CONTROLS NEURAL FATES IN THE DEVELOPING HIPPOCAMPUS

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During neural development, neurons, oligodendrocytes and astrocytes differentiate in specific temporal orders at defined domains. Neural stem/progenitor cells switch neural fates from neurons to glia at specific timings. However, how the timing is controlled remains unclear. Accumulating evidence indicate that neural stem/progenitor cells are pharmacologically and/or electrically able to response to neural activities and change their differentiating properties. Here, we provide evidence that D-serine, a regulator of NMDA receptor signaling, plays crucial roles in time specific neuro-gliogenesis in the developing hippocampus. D-serine is generated from L-serine by serine racemase (SR), and starts to act at postnatal day 7 (P7) when SR starts to express in the developing nervous system. We found that P8 SR-/- mouse derived multipotent neural progenitors were biased to generate neurons and oligodendrocytes rather than astrocytes compared with wild type (WT)/SR+/- derived progenitors. The phenotype in SR-/- progenitors was rescued by administration of D-serine, suggesting that D-serine/SR are involved in mechanisms controlling neural fate specification. 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 at P13 in WT, on the other hand, few in SR-/-. We quantitatively confirmed 70% reduction in the percentage of immature astrocytes in SR-/- by dissociation of P14 hippocampus. Thus, D-serine/SR control both neural fate specification and the timing when astrogensis starts in the developing hippocampus.

T-1233

EVALUATION OF OLIGODENDROCYTE PROGENITOR CELLS FOR PERIPHERAL NERVE REGENERATION CELL THERAPIES

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Access to highly functional remyelinating cells could greatly enhance our possibilities of treating large peripheral nerve injuries, where host Schwann cells fail to fully repopulate the graft in a timely fashion unless complete nerve autografts are used. While highly functional remyelinating Schwann cells are notoriously hard to derive from stem cell sources, methods for deriving functional oligodendrocyte precursor cells (OPCs) have made great strides. It is therefore

time to evaluate the potential of OPCs for treatment of peripheral nerve injuries. We found that OPCs, isolated by magnetic activated cell sorting from Plp-eGFP neonatal mouse brain, survived and differentiated to eGFP+ cells one month after being engrafting into the sciatic nerve of NOD SCID mice. Prior to engraftment, endogenous cells at the site were killed by repeated freeze-thawing cycles, and a transection and direct nerve anastomosis was performed proximal to the site. The presence of eGFP+ cells at the one month time point suggests that the cells were able to attain a mature oligodendrocyte-like fate, alternatively a Schwann cell-like phenotype, in agreement with previous reports form in vitro studies and in vivo induced demyelinated lesions in the CNS where astrocytes were depleted. With regenerative therapies in mind, studies are ongoing to identify OPC fate in peripheral nerve injuries and evaluate their potential to support regeneration through remyelination. We also plan to evaluate the ability of OPCs to support axon regeneration in synthetic peripheral nerve guides, posing an alternative to autografts for large injuries. If successful, this opens the door for future studies using human stem cell-derived OPCs for cell therapies in the peripheral nervous system.

T-1234

OLIGODENDROCYTES GENERATION DYNAMICS IN MULTIPLE SCLEROSIS

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Oligodendrocytes wrap layers of specialized cell membrane around nerve fibers forming myelin, which facilitate fast propagation of nerve impulses and trophic support of axons. Myelination can in theory be modified by mature oligodendrocytes generating new myelin and/or by exchanging oligodendrocytes and their myelin sheaths. In a previous study we assessed the dynamics of oligodendrocyte generation and myelination in the human brain. By analyzing the concentration of ^{14}C, derived from nuclear bomb testing during the Cold War, in genomic DNA of oligodendrocytes,we found that human white matter oligodendrocytes are remarkably stable with limited turnover whereas myelin is exchanged at a high rate. This indicates that myelin modulation in humans may be carried out by mature oligodendrocytes. There are different kinetics of oligodendrocyte generation and turnover in gray and white matter, with a longer period of oligodendrocyte generation and higher turnover rate throughout life in gray matter, suggesting the possibility of de novo myelination in the sparsely myelinated cortex. However, how these generation kinetics of the oligodendrocyte population may change under pathological conditions such as in the demyelination disease multiple sclerosis (MS) has been unknown. A hallmark of MS is the loss of oligodendrocytes and myelin, as well as axons, leading to conductions deficits and a variety of neurological symptoms. In early stages of MS, regeneration of myelin (remyelination) has been observed to occur. Studies in experimental animal models of MS support that new oligodendrocyte generation contributes to the remyelination process. However, how this appears, where and to what extent this process may occur in human MS is poorly understood. To address the dynamics of oligodendrocyte generation during the pathological course of MS, we are using the strategy of analyzing the content of ^{14}C in genomic DNA of oligodendrocytes from MS brain tissue. This will enable us to understand more of the disease's processes and potentially point to targets for new therapeutic interventions aiming at affecting the remyelination process.

REPROGRAMMING

T-1235

WHITE PRIMARY HUMAN PRE-ADIPOCYTES CAN BE INDUCED TO EXPRESS UCP-1 AND TRANSDIFFERENTIATE INTO BROWN-LIKE ADIPOCYTES IN-VITRO

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Brown adipose tissue (BAT) is dedicated to regulating body temperature in hibernating mammals and newborn humans. In the many mitochondria present in brown adipocytes accumulated fatty acids are degraded and free energy is released as heat. This process called thermogenesis works by uncoupling the mitochondrial electron transport chain from the production of ATP. Expressed in the mitochondria's inner member, uncoupling protein 1 (UCP-1) bypasses the proton import path of the ATP synthase, thus releasing the energy of the proton gradient as heat. On the other hand, white adipose tissue (WAT) in subcutaneous and visceral body compartments stores excess energy in the form of triglycerides. Excess of accumulated fat, particularly in visceral adipose tissue, can lead to a number of disorders such as dyslipidemia, fatty liver disease, insulin resistance, type II diabetes, hypertension, and cardiovascular diseases that are associated with other metabolic disturbances called metabolic syndrome. The discovery of active BAT in adult humans has raised great interest in the scientific community to expand research with BAT for new therapeutic strategies in the treatment of obesity and related disorders. Activating and expanding BAT *in-vivo* may be one such strategy. BAT can be expanded through exposure to cold, or through pharmacological agents. For researchers and drug developers an *in-vitro* system of human brown adipocytes would be of great value to gain a better understanding of the key factors involved in transdifferentiation. However, the supply of adult human BAT is limited, which has scientists looking for alternatives. Here we employ primary human white adipocytes of subcutaneous and visceral origin to demonstrate that the expression of the UCP-1 marker of brown adipocytes can be induced severalfold through the treatment with forskolin. This implies that WAT derived cells may have the potential to transdifferentiate to active brown adipocytes. Treating these cells with known pharmacological factors *in-vitro* gives rise to a brown-like phenotype, as indicated by the characteristic shape of intracellular lipid droplets. This *in-vitro* system should provide results of greater relevance than those obtained with animal-derived cells.

T-1236

SOX9 RESTRAINS CHONDROCYTE TO OSTEOBLAST LINEAGE PROGRESSION- IMPLICATIONS FOR THE PATHOGENESIS OF CAMPOMELIC DYSPLASIA

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In endochondral bone development bi-potential chondro-osteoprogenitors form mesenchymal condensates followed by a cascade of chondrocyte differentiation steps to form cartilage. Osteoblasts, originating from the perichondrium, accompany vascular invasion and lay down endochondral bone to replace cartilage. It has been generally accepted that chondrocytes and osteoblasts are separate lineages derived from a common osteo-chondro-progenitor and in the terminal step, differentiated hypertrophic chondrocytes (HCs) are thought to undergo apoptosis. But whether hypertrophy is the terminal differentiation stage of chondrocytes, with apoptosis as the ultimate fate, or marks a transition preceding osteogenesis has been debated. Using a genetic recombination lineage tracing approach in mice, we showed that HCs directly contribute to the osteoblast lineage and can become osteogenic cells in fetal and postnatal endochondral bones, persist into adulthood. Therefore, hypertrophy is not the terminal differentiation state for chondrocytes, but is part of a continuum in which HCs directly contribute to the full osteoblast lineage. SOX9 is the master transcriptional regulator of chondrogenesis and mutations in the gene cause campomelic dysplasia (CD) characterized by cleft palate, short stature, bowed limbs (campomelia), XY sex reversal. The human CD $SOX9^{Y440X}$ nonsense mutation leads to truncation of the transactivation domain and is suggested to be hypomorphic. We generated a conditional mouse equivalent of the $SOX9^{Y440X}$ mutation. All the mutants recapitulated the human CD skeletal phenotypes. But heterozygous $SOX9^{Y440X+}$ mice, display a more severe skeletal phenotype especially with respect to campomelia than in $Sox9^{-/+}$, suggestive of a dominant negative or neomorphic pathogenesis. We hypothesized that the campomelia is caused by the impact of the $SOX9^{Y440X}$mutation on the chondrocyte to osteoblast lineage transition. We found dysregulated hedgehog signaling in $Sox9^{Y440X/Y440X}$ growth plates. Lineage tracing showed an increased number of HC descendants suggesting an accelerated HC to osteoblast transition. We propose that *SOX9* normally restrains the transition of HCs to osteoblasts. The campomelia in CD is a consequence of loss of control of the lineage progression from chondrocyte to osteoblast.

T-1237

THE ROLE OF SOX2 IN CANCER AND AGING

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We recently uncovered a new role for the cell cycle regulator and tumor suppressor gene p27 controlling the transcription of pluripotency gene Sox2. Failure to exert this control results in pituitary tumor development, retinal development problems and organismal hyperplasia, typical phenotypes of p27-null mice. We showed that p27 regulates Sox2 transcription through an enhancer region (SRR2) during development and that loss of p27 results in uncomplete repression of Sox2 that can be demonstrated by 2 factor reprogramming of p27-null cells. We now show the differential effects of the retinoblastoma family of proteins on Sox2 regulation. We have tested how RB, p130 and p107 exert a different degree of control over the expression of Sox2. Again, failure to properly control the expression of Sox2 in retinoblastoma deficient mice or cells has measureable consequences. Lack of proper repression in null animals results in pituitary tumors and cells deficient for some of these tumor suppressor genes can be reprogrammed with only 2 factors. Finally, we have addressed the role of Sox2+ cells in aging by repeatedly depleting these cells from the organism.

T-1238

REPROGRAMMING FROM THE CELL SURFACE: INDUCING AND INVESTIGATING PLURIPOTENCY THROUGH COMBINATORIAL ANTIBODY SCREENING

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Development is mediated by a series of events initiated through cell surface signaling, whereas reprogramming to induced pluripotent stem cells (iPSCs) is typically achieved by factors acting directly on DNA or gene transcription in nucleus. However, the precise mechanisms underlying reprogramming to pluripotency remain largely elusive. This lack of understanding may contribute to iPSC heterogeneity and creates a reliance on reprogramming methods with the potential to alter genomic integrity. To address these limitations, we hypothesized that signaling induced at the cell surface might also be able to induce reprogramming, and possibly reduce the potential for oncogenic mutation in iPSCs. In addition, this approach may identify novel pathways that can contribute to reprogramming. To test this hypothesis, we developed an antibody screening platform to efficiently screen for cell surface regulators of pluripotency. To identify antibodies that can replace the Yamanaka transcription factors Oct4, Sox2 and Klf4, we screened a library of lentiviral vectors harboring more than 109 distinct antibody specificities targeted to extracellular domains. During screening, the use of integrating lentiviruses allows us to recover the sequence of active antibodies upon discovery of a phenotype, an iPSC colony. Subsequently, each antibody is sub-cloned and their activity is confirmed using both viral and soluble antibodies. From the initial screen we identified antibodies that replace either Oct4 or Sox2 during reprogramming. Next, we confirmed the ability of these antibodies to generate iPSCs that appropriately express pluripotency markers, differentiate into the three germ layers in vitro and give rise to chimeric offspring. Furthermore, we have identified a novel target for a Sox2-replacing antibody with previously unknown roles in reprogramming and pluripotency. Currently, we are investigating the signaling pathways and mechanisms involved in the reprogramming using antibodies.

T-1239

MIR29 IMPAIRS EARLY PHASE OF REPROGRAMMING PROCESS BY MODULATING WNT SIGNALING

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Induced pluripotent stem cells (iPSC) were first generated by retroviral transduction of transcription factors Oct4, Sox2, Klf4 and cMyc into somatic cells. IPSC are functionally and morphologically similar to embryonic stem cells. However, many of the molecular mechanisms that drive nuclear reprogramming remain yet to be discovered. Moreover, new tools for improving reprogramming have been developed over the past years (i.g. small compounds and microRNAs). In this way, we emphasize the use of synthetic miRs, to explore their potential roles in stochastic cell reprogramming. Therefore, we independently transfected pre and anti-miRs (29 and CTR) into BJ1 fibroblasts and NTera2 cells. Whole-genome transcriptomes were obtained by oligonucleotide microarray. Pathways and biological processes modulated by miR29 were identified by using DAVID tool and the data was validated by qRT-PCR. Next, in order to analyze the modulatory effect of miR29 in reprogramming process, we generated mouse Oct4-GFP (OG2) iPSC with all-in-one hOCT4-hKLF4-hSOX2 (OKS) transduction and miR29 transfection. Through forced expression of miR29 we detected a decrease in the number of Oct4-GFP+ colonies. The opposite effect was found through inhibition of miR29. This finding may be due to the inhibition of miR29 targets Dkk1 and Kremen2, which we found to be repressed by miR29 (array and qRT-PCR data). These targets are components of canonical WNT signaling, a pathway whose role in reprogramming process is still controversially described. As cMyc inhibits miR29 transcription, we analyzed the expression levels of identified targets during the first 12 days of reprogramming by OKS or OKSM, compared to controls. We could confirm that DKK1, Kremen2 and sFRP2 were also modulated by cMyc during reprogramming. We suggest that this process is a consequence of miR29 repression. Aiming at confirming the miR29 modulatory role in canonical WNT signaling, we performed luciferase assay on OG2 MEFs transduced with a TCF/LEF reporter. We found that miR29 promoted TCF/LEF activity in fibroblasts, and this mechanism was CHIR99021-independent. Our findings add to the understanding of the molecular mechanisms by which miR29 impairs early phase of reprogramming, and may help in the future development of miR-mediated reprogramming with increased efficiencies.

T-1240

LOCUS-SPECIFIC ASSESSMENT OF ENDOGENOUS RETROELEMENT CONTROL DURING REPROGRAMING TO PLURIPOTENCY

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Endogenous retroelements (EREs) account for about half of the mouse or human genome, and their potential as insertional mutagens and transcriptional perturbators is suppressed by early embryonic epigenetic silencing. Yet, recent findings demonstrate that certain classes of EREs or specific ERE loci are essential to reach and maintain the pluripotent state. We previously demonstrated that EREs are markedly upregulated during the reprogramming into iPS cells of either mouse embryonic fibroblasts, human CD34+ cells or human primary hepatocytes, with hundreds of ERE loci not associated with pluripotency aberrantly unleashed. We now carry out a systematic survey of all ERE integrants (loci) during iPS reprogramming. Consistent with previous finding, we identify several pluripotency associated EREs, mostly from the HERVH/LTR7 family, but also detect the activation of elements that constitute potential threats for genome integrity and obstacle to the reprogramming process. We also find clusters of EREs that are specific of reprogramming time-points, which likely reflect cellular sub-states on the path to pluripotency. Characterizing these states and separating pluripotency-coopted ERE loci from pluripotency-counteracting EREs will be an important step in understanding the epigenetic and transcriptional changes that occur during reprogramming as well as the general mechanisms of ERE control.

T-1241

INVOLVEMENT OF THE WNT PATHWAY DURING CHEMICAL INDUCED CONVERSION OF HUMAN AMNIOCYTES INTO A PLURIPOTENT STATE

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Pluripotency can be regained by ectopic co-expression of defined reprogramming transcriptional factors. Induced pluripotent stem cells (iPSC) provide opportunities for disease modeling, drug screening, and autologous cell-based therapies. However, technical concerns preclude the translational use of iPSC. Chemical reprogramming attempts to restore pluripotency by using a combination of small molecules alone. We previously showed that human amniotic fluid stem cells (AFSC) cultured in pluripotent conditions and exposed to the histone deacetylase inhibitor, valproic acid (VPA) can be reset into a pluripotent state. They regain the ability to differentiate into lineages of the three germ layers, and homogeneously express Oct4, Sox2, KLF4, c-MYC, as well as NANOG, PODXL, ZFP42, DNMT3B, LIN28 and SAL4 at a single cell level. Here we investigate the molecular mechanisms by which AFSC regain pluripotent functionality during chemical reprogramming. Parental AFSC co-express the mesenchymal stem cells (MSC) markers CD90, CD105, and CD73. Reprogrammed AFSC down-regulated mesenchymal markers expression (CDH2, Col1a2, Col5a2, Foxc2, GNG11, MSN, TCF4, and Vimentin), and concomitantly up-regulated the expression of epithelial markers (CDH1, IL1RN, NUDT13, RGS2, SPP1). Increased levels of CDH1 protein in reprogrammed cells confirmed this. Wnt signaling plays a biphasic role in initiation and late phase of the reprogramming process. In reprogrammed cells, endogenous Wnt signaling were activated by VPA treatment, with up-regulation of Wnt1,2,3A,7A,8A, paralleling down-regulation of the Wnt repressors, DKK1, CCND1, and Cyclin D1. However, during the early initiation stage of chemical reprogramming, Wnt signaling was initially inhibited, with an up-regulation of Snail and DKK1, and down-regulation of Wnt ligands. Moreover, we observed an initial up-regulation followed

by a down-regulation of mesenchymal genes. We are investigating the sequential stages and action of Wnt, MET signaling during VPA-induced reactivation of the endogenous pluripotency network during chemical reprogramming of human amniocytes.

T-1242

IDENTIFYING NOVEL SIGNALLING NETWORKS UNDERLYING HUMAN INDUCED PLURIPOTENT STEM CELL REPROGRAMMING

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Since their first description, induced pluripotent stem cells (iPScs) enabled a substantial expansion in stem cell research due to their application in disease modeling and potential for regenerative medicine. However, protocols remain inefficient in part due to an incomplete understanding of the mechanisms underlying the reprogramming process. The current dogma, largely elucidated using mouse cells, is that iPSc reprogramming consists of three stages: initiation, maturation and stabilisation. Initiation of reprogramming occurs in almost all cells that receive the reprogramming transgenes and involves a phenotypic mesenchymal-to-epithelial transition. The initiation stage is also characterised by increased cellular proliferation and a metabolic switch from mainly oxidative phosphorylation to mainly glycolytic energy production. The maturation stage is considered the major bottleneck within the process, resulting in very few 'stabilisation competent' cells progressing to the final stabilisation phase. To reach this stage in both mouse and human cells, pre-iPS cells must activate endogenous expression of the core circuitry of pluripotency, comprising Oct4, Sox2 and Nanog, and thus reach a state of transgene independence. Despite these insights, we still lack a comprehensive temporal understanding of the molecular mechanisms underlying iPSc reprogramming, particularly in human cells. We have developed a novel lentiviral-based transcription factor (TF) activated reporter system for real-time quantitation of TF activity during human iPSc reprogramming. The 10 reporter constructs we have generated allow signalling activity to be monitored by GFP and a secreted luciferase, thus allowing quantitative measurements to be made from the conditioned medium of living cultures. Using this method, we reveal a novel signalling axis involving NRF2, HIFα and AP-1 that is likely to underlie the metabolic shift in iPSc reprogramming. We also identify roles for novel regulators of human iPSc reprogramming including NFkB and Wnt. This technology provides a tool to enable TF quantification throughout the process of iPSc reprogramming in living cells. We anticipate these data will allow us to transiently modulate key signal transduction pathways to improve the efficiency of generating iPScs.

T-1243

TRANSCRIPTOME ANALYSIS OF THE PHASES IN HUMAN SOMATIC CELL REPROGRAMMING

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The derivation of induced pluripotent stem (iPS) cells has presented the opportunity to model human disease in vitro with patient-derived cells, as well as the potential for personalized cell therapies. Understanding how the process of reprogramming somatic cells works at the cellular and molecular level is therefore critical to harness iPS cell technology. In order to study mechanisms underlying the induction of pluripotency, we systematically tested multiple ratio combinations of reprogramming factors to establish a rapid and efficient mRNA-based strategy for reprogramming human somatic cells. With this system, we performed temporal transcriptome analysis by RNA-Seq, and characterized distinct phases based on changes in gene expression during reprogramming. Our findings reveal that the previously established phases of reprogramming - initiation, maturation and stabilization - are temporally altered in humans compared to mouse. The mesenchymal-to-epithelial transition (MET) and cell cycle progression, which are hallmarks of the initiation phase in mouse, occur after upregulation of pluripotency markers in human cells. Moreover, we identified novel classes of genes important for early reprogramming of human cells. It is our aim to define the function of these genes and their influence on cellular plasticity and major cell fate transitions.

T-1244

MYOGENIC DIFFERENTIATION FROM EMBROYONIC FIBROBLAST BY DEFINED FACTORS

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Ectopic expression of Pax3 into induced pluripotent stem cell or embryonic stem cell was used to produce myogenic progenitor cells. Here, we investigate that skeletal myogenic lineage cells can be generated from mouse embryonic fibroblasts by conversion with five transcription factors. We introduced five transcription factors (Pax3, Sox2, Klf4, c-Myc, Esrrb) into mouse embryonic fibroblasts (MEFs). Cells morphologically changed were observed around 5 weeks after transduction and expressed myogenic genes such as Myf5. Compared to myoblast cell line, the cell expressed myogenic genes such as MyoD, Myogenin and MHC (myosin heavy chain) as much as myoblast cells whereas MEFs did not. We named it induced myoblast like cell, iMLC. In myogenic differentiation media, iMLC showed the process of myogenic differentiation and formation of myotube. Subcutaneous injection of iMLC into nude mice displayed the muscle fibers formation. These results represents that five factors might convert fibroblast into myogenic lineage cells.

T-1245

EXTERNAL pH AFFECTS THE TIMING OF MOUSE SOMATIC CELL REPROGRAMMING

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Mechanisms of somatic cell reprogramming have been investigated for the last decades. Previously, we found the external pH affected to somatic cell reprogramming in chicken cells and the optimal pH range to induce the reprogramming was different from that of mouse somatic cells. We here investigated further effects of the external pH on somatic cell reprogramming in mice. First the effects of pH on reprogramming efficiency were examined. Mouse fibroblasts with Oct4-GFP as an indicator of pluripotency were infected by a retroviral vector with 4 factors *(Oct3/4, Sox2, Klf4,*

and c-Myc). Cells were then cultured in different pH conditions (pH 6.6 to 7.8). The pH of culture medium was adjusted by the concentration of sodium bicarbonate in 5% CO_2 incubator and checked pH rates in each culture every day to keep constant pH. And then the GFP positive colony number was counted at day 14 and day 21. The distribution of colony number in culture was shown a normal distribution with the peak at pH7.4. At the pH6.6 and 7.8, GFP positive colonies could not obtain. Next, the effects of pH on the timing of reprogramming were examined. During the processes for inducing reprogramming in somatic cells, Oct4-GFP-positive colonies were counted at each pH condition. Oct4-GFP colonies appeared more slowly at lower pH than higher pH, and therefore the lower pH resulted in retardation on the timing of reprogramming. This may be caused by slow proliferation of fibroblasts at the low pH. Interestingly, morphology differences of colonies were also observed at different pH ranges; a compact morphology at the lower pH and a flat at the higher pH. This may suggest external pH change affected cell characteristics and cell matrix during reprogramming. These results suggest that the external pH in culture medium affects not only somatic cell reprogramming but also during differentiation of pluripotent cells.

T-1246

REAL TIME VISUALIZATION AND KINETIC MEASUREMENT OF SOMATIC REPROGRAMMING

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Somatic reprogramming enables the generation of induced pluripotent stem cells (iPSC) from diverse genetic backgrounds. The three week long process is not yet fully understood. To gain a better understanding of the reprogramming process and the differences between different reprogramming systems, it is critical to have the ability to visualize cells as they transition into iPSCs and to track the progression of reprogramming. Current methods for monitoring reprogramming rely on the qualitative inspection of morphology or staining with stem cell-specific dyes and antibodies. Here we present different approaches for using these data for more quantitative analyses. We utilized the IncuCyte FLR and Zoom Live Cell Imaging System for the continuous monitoring of fibroblasts as they are reprogrammed using different technologies, either in feeder-dependent or feeder-free media systems. The kinetics of colony formation was measured based on confluence in phase contrast images or on fluorescence signals from staining with live Alkaline Phosphatase dye or TRA-1-60 antibody. In addition, whole-well imaging was carried out to derive a more accurate colony count based on traditional AP staining or the more stringent TRA-1-60 antibody staining. The results indicated that the collection of multiple parameters such as colony size and confluence provides semi-quantitative metrics to compare reprogramming differences across the different systems. To further measure the kinetics of reprogramming, flow analysis was performed throughout the process using antibodies against self-renewal markers and fibroblast markers. The combination of real-time visualization and flow analysis enables the quantitative study of reprogramming at different stages and provides a more accurate comparison of different reprogramming systems and methods.

T-1247

GENE EXPRESSION PATTERNS OF WNT SIGNAL PATHWAY IN PLURIPOTENT CELLS

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Activity of Wnt signaling is related by the regulation of many cellular functions, including proliferation, migration, cell fate specification, maintenance of pluripotency and induction of tumorigenicity. Cell reprogramming can induce somatic cells to ESC-like cells that was named 'induced pluripotent stem cells (iPSCs), by using direct transduction of a cocktail composed of only four pluripotent transcription factors: Oct4, Sox2, Klf4 and c-Myc. Moreover, modulation of the Wnt/beta-catenin signalling pathways strikingly enhances somatic-cell reprogramming. Although Wnt signal transduction can promotes cell reprogramming process in vitro, their molecular mechanisms in differentiation processes and maintenance of pluripotency have been not completely clear. In our study, we performed gene expression including Fzd1, Ccnd1, BtrC, Axin1, APC and Adar that these genes were defined to play functions in Wnt signal pathway network in mouse induced pluripotent stem cells (miPSCs), mouse embryonic stem cells (mESCs) and mouse bone marrow-derived mesenchymal stromal cells (mBMMSCs) with comparing mouse embryonic fibroblasts (MEF) using quantitative reveres transcription polymerase chain reaction (qRT-PCR). We found that the expression of these genes was significantly reduced in miPSCs but not mESCs which is condition of maintenance for pluripotency. Our results indicated that although Wnt signal transduction can enhance cell reprogramming in vitro, probably these processes are conversely inhibited by novel mechanisms in processes of cell reprogramming.

T-1248

MODELING THE INNER EAR IN VITRO: USING DIRECT REPROGRAMMING OF MOUSE AND HUMAN FIBROBLASTS FOR REGENERATIVE INITIATIVES OF INNER EAR CELL TYPES

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Hearing loss affects 360 million people worldwide. Sensorineural hearing loss is caused by dysfunction of hair cells or spiral ganglion neurons within the cochlea, which are responsible for mechanotransduction of sound to the brain. Hair cells are hypersensitive to common chemotherapy drugs and antibiotics, and once mammalian hair cells are lost they do not regenerate. Because of the difficulty obtaining these scarce cell types from primary sources, we are developing an in vitro system using direct reprogramming of more readily available cell types. Generating an in vitro model will allow us to study the cellular mechanisms that underlie hair cell vulnerability and their lack of regenerative capacity. We have a identified a set of transcription factors that can be used to reprogram cells towards a hair cell fate when virally transduced

into mouse embryonic fibroblasts or human iPSC-derived fibroblasts, and using fluorescent reporters. This set of TFs is able to activate genes characteristic of hair cells, such as Atoh1, parvalbumin, and Myosin6. In addition, to improve reprogramming efficiency, we have begun to experiment with various culture conditions that recapitulate the developmental environment in vivo. This approach yields several hundred cells per well of a 96 well plate that are positive for the hair cell Atoh1 transgenic reporter. Our results demonstrate that a specific set of transcription factors is sufficient for reprogramming somatic cells towards a hair cell-like fate. This model will help us to pursue regenerative initiatives for these specialized and vulnerable cells. Further development of this model will be useful for studying the cellular mechanisms that underlie hearing loss, and the identification of either protective or ototoxic compounds through high throughput screening.

T-1249

NATURAL FACTORS DERIVED FROM LACTIC ACID BACTERIA CONVERT HUMAN FIBROBLASTS TO MULTIPOTENTIAL CELLS

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Humans are in contact with components of the microflora from birth. Thus, a delicate balance exists in the symbiotic relationship between microorganisms and the human host during metabolic activities. Lactic acid bacteria (LAB) form a group of related, low-GC-content, gram-positive bacteria that are considered to offer a number of probiotic benefits to general health. While the role of LAB in gastrointestinal microecology has been the subject of extensive study, little is known about how commensal prokaryotic organisms directly influence eukaryotic cells. Recently, we demonstrate the generation of multipotential cells from adult human dermal fibroblast (HDF) cells by incorporating LAB. LAB-incorporated cell clusters are similar to embryoid bodies derived from embryonic stem cells and can differentiate into endodermal, mesodermal, and ectodermal cells in vivo and in vitro. LAB-incorporated cell clusters express a set of genes associated with multipotency, and microarray analysis indicates a remarkable increase of NANOG and a notable decrease in HOX gene expression in LAB-incorporated cells. During the cell culture, the LAB-incorporated cell clusters stop cell division and start to express early senescence markers without cell death. Next, we purified the LAB-derived factors from the homogenized LAB through several biochemical methods by observing their cell clusters forming activity. When we applied the LAB-derived factors to our culture assay system, HDF cells formed the cell clusters expressing the mutipotent markers. Thus, the LAB-derived factors have potentially wide-ranging implications for cell generation and reprogramming.

T-1250

HIGHLY EFFICIENT INDUCTION OF SKELETAL MYOCYTES BY FORWARD PROGRAMMING UNDER CHEMICALLY DEFINED CONDITIONS

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Human pluripotent stem cells (hPSCs) represent a virtually unlimited source of human cells which, in theory, can give rise to all cells of the organism. However, for certain cell types that are of considerable interest for regenerative medicine, such as skeletal myocytes, the robust differentiation protocols are still lacking. Reprogramming technologies based on overexpression of key transcription factors offer an alternative route for rapid and efficient generation of such cell types. We developed an improved method to conditionally overexpress transgenes in human pluripotent stem cells by using an optimized doxycycline-responsive inducible system. The method is simple, rapid, and very efficient (>90%). It allows tightly controlled, dose dependent, and transient overexpression of transgenes of interest in hPSCs. We applied this system to overexpress MYOD1, a key determinant of the myogenic lineage. By combining the forced expression of MYOD1 with environmental cues from developmental myogenic differentiation we derived a deterministic protocol for the highly efficient generation of skeletal myocytes from human pluripotent stem cells within five days.

T-1251

HIGHLY EFFICIENT GENERATION OF INDUCED NEURONS FROM HUMAN FIBROBLASTS THAT SURVIVE TRANSPLANTATION INTO THE ADULT RAT BRAIN

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Induced neurons (iNs) offer a valuable source of human neurons for applications of disease modeling, diagnostics, drug screening and cell replacement therapy. These cells have been successfully obtained from mouse and human fibroblasts by lentiviral infection with only three transcription factors (TFs), Ascl1, Brn2, Myt1L (ABM). Our group has shown that by combining ABM with dopaminergic (DA) fate determinants, it is possible to generate subtype-specific human iNs (hDA-iNs). Our most recent results, obtained when trying to improve our conversion protocol, show that a delay in transgenes activation leads to increased conversion efficiency. Also, that the use of a delay in transgene activation in combination with small molecules (SMs) that inhibit SMAD signaling and activate WNT signaling (SB-431542, Noggin, LDN-193189 and CHIR99021), highly improves conversion efficiency and neuronal purity. Furthermore, we transplanted the hiNs into the striatum of immunosuppressed rats, in order to determine their in vivo survival ability. The TFs ABM and the DA fate determinants were activated in vitro before transplantation, and the grafting of the cells occurred four and nine days after activation of the genes. All the groups of transplanted animals formed grafts containing similar number of neurons and innervated the host brain to the same extent. The use of SMs, identified as enhancers of in vitro conversion of iNs, did not translate to better in vivo performance of the cells, and when analyzing the grafts for potential dopaminergic neurons, we could detect TH-positive hiNs in all groups of animals, although at a low frequency. Nevertheless, our data points towards the feasibility of developing such hiNs for cell replacement therapy applications. Our studies resulted in a protocol that allows for a 10-fold improvement in conversion efficiency compared to previous reports, providing a method for highly efficient generation of iNs from human fibroblasts without the need of a

selection step. We also provide the first evidence that hiNs survive and mature after transplantation into the adult rat brain.

T-1252

CELL SURFACE MARKER EXPRESSION DEFINES LATE STAGE OF REPROGRAMMING TO PLURIPOTENCY IN HUMAN CELLS

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Our current understanding of the induction of pluripotency by defined factors indicates that this process occurs in discrete stages characterized by specific alterations in the cellular epigenome and transcriptome. Analysis of the cellular reprogramming process relies on the availability of specific molecular markers to identify and prospectively isolate defined cellular intermediates in the reprogramming process. We used several combinations of stem cell surface markers to isolate colonies emerging after transduction of human fibroblasts with reprogramming factors, and then analysed their expression of a panel of genes associated with pluripotency and early germ layer lineage specification. We found that the activation of the expression of a small panel of genes characterized a late stage in the reprogramming process. Furthermore we showed that combined live cell staining with the antibody GCTM-2 and anti-CDH3 could identify colonies of cells that had traversed this boundary. These results will facilitate study of the final phases of reprogramming of human cells.

T-1253

EPIGENETIC PREDISPOSITION TO REPROGRAMMING FATES IN SOMATIC CELLS

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Reprogramming to pluripotency is a low efficiency process at the population level. Despite notable advances to molecularly characterize key steps, several fundamental aspects remain poorly understood, including when the potential to reprogram is first established. Here, we apply live-cell imaging combined with a novel statistical approach to infer when somatic cells become fated to generate downstream pluripotent progeny. By tracing cell lineages from several divisions before factor induction through to pluripotent colony formation, we find that pre-induction sister cells acquire similar outcomes. Namely, if one daughter cell contributes to a lineage that generates induced pluripotent stem cells (iPSCs), its paired sibling will as well. This result suggests that the potential to reprogram is predetermined within a select subpopulation of cells and at least heritable over the short term. We also find that expanding cells over several divisions prior to factor induction does not increase the per-lineage likelihood of successful reprogramming, nor is reprogramming fate correlated to neighboring cell identity or cell-specific reprogramming factor levels. By perturbing the epigenetic state of somatic populations with Ezh2 inhibitors prior to factor induction, we successfully modulate the fraction of iPSC forming lineages. Our results therefore suggest that reprogramming potential may in part reflect preexisting epigenetic heterogeneity that can be tuned to alter the cellular response to factor induction.

T-1254

A REPROGRAMMABLE MOUSE LINE REPORTING THE EXPRESSION OF OKMS AND THE ACTIVATION OF ENDOGENOUS OCT4

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Mouse embryonic fibroblasts can be reprogrammed to pluripotency by the forced expression of Oct4, Klf4, cMyc and Sox2 (OKMS). When these factors are introduced by stably integrated transgenes, their expression is variable from cell to cell, due to the random genomic insertion sites and the number of integrated transgenes. This heterogeneity is the major contributor to the low efficiency of the reprogramming. We developed an efficient 2° reprogramming system equipped with fluorescent visualization of both the Yamanaka factors (mCherry) and the Oct4 pluripotency hallmark gene (GFP) expressions, allowing the live monitoring of the reprogramming process in vivo and in vitro. The piggyBac transposon containing the doxycycline (dox)-inducible promoter driving OKMS linked to mCherry reporter and transposon with a ubiquitously expressed rtTA transgene were transfected into MEFs carrying an Oct4promoter-GFP transgene, and dox-independent primary (1°) iPSCs were obtained. Four clones were selected for diploid embryo-iPSC aggregation. 2° MEFs were derived from the resulting embryos at E12.5. The 2° MEFs were incubated in dox and FACs analysis confirmed that each clone activated OKMS-mCherry. After dox induction, mCherry positive colonies formed and Oct4-GFP fluorescence was observed by day 8. To assess transgene independent pluripotency, dox was removed at day 10. 82% of the clones were dox-independent at day 18. Tail tip fibroblasts, bone marrow, peripheral blood, and neurospheres from adult chimeras derived from all iPSC clones reprogrammed, and dox-independent 2° iPSCs resulted within 20 days. Germ line transmission was obtained for 2 of the 1° iPSC lines and these founders generated 2 new reprogrammable mouse lines. When OKMS-carrying mice were fed with dox for 3 to 7 days, they developed kidney tumors starting 4 weeks after treatment. Activation of OKMS early in pregnancy disrupts development as all embryos display an abnormal morphology by E8.5. Conclusion: We established an efficient secondary reprogramming system and a new reprogrammable mouse line with double fluorescent reporter. This system will enable us to uncover specific properties and molecular events underlying the generation of pluripotent cells from the soma.

T-1255

RAPID AND EFFICIENT DIRECT CONVERSION OF HUMAN ADULT SOMATIC CELLS INTO NEURAL STEM CELLS BY HMGA2/LET-7B

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A recent study has suggested that fibroblasts can be converted into mouse-induced neural stem cells (miNSCs) through the expression

of defined factors. However, successful generation of human iNSCs (hiNSCs) has proven challenging to achieve. Here, using microRNA (miRNA) expression profile ana- lyses, we showed that let-7 microRNA has critical roles for the formation of PAX6/NESTIN-positive colonies from human adult fibroblasts and the prolif- eration and self-renewal of hiNSCs. HMGA2, a let- 7-targeting gene, enables induction of hiNSCs that displayed morphological/molecular features and in vitro/in vivo differentiation potential similar to H9-derived NSCs. Interestingly, HMGA2 facilitated the efficient conversion of senescent somatic cells or blood CD34+ cells into hiNSCs through an interac- tion with SOX2, whereas other combinations or SOX2 alone showed a limited conversion ability. Taken together, these findings suggest that HMGA2/let-7 facilitates direct reprogramming toward hiNSCs in minimal conditions and maintains hiNSC self- renewal, providing a strategy for the clinical treat- ment of neurological diseases.

T-1256

THE EFFECT OF SHEAR STRESS ON THE REPROGRAMMING OF SOMATIC CELLS INTO INDUCED PLURIPOTENT STEM CELLS

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Forced expression of the Yamanaka factors can reprogram somatic cells into induced pluripotent stem cells. Using murine doxycycline inducible system, we show that culturing reprogramming cells on an orbital shaker increases the number of Nanog positive colonies. Compared to a static environment, cells on shaker experience both oscillatory shear stress and the mixing of culture media. We adjust the experimental parameters to decouple these 2 effects and demonstrate that oscillatory shear stress plays the dominant role in enhancing reprogramming efficiency. Time course studies suggest that shear stress enhance reprogramming efficiency by enhancing the maturation of nascent colonies. This surprising finding demonstrates the role of mechanical factors in enhancing reprogramming efficiency, and elucidation of the underlying pathways will yield interesting insights into mechanotransduction and the mechanism of reprogramming.

T-1257

METHODS OF REPROGRAMMING TO iPSC ASSOCIATED WITH GENOMIC INTEGRITY AND DELINEATION OF A CHR 5q CANDIDATE REGION FOR GROWTH ADVANTAGE

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Induced pluripotent stem cells (iPSCs) have brought great promises for disease modeling and cell-based therapies. One concern related to the use of reprogrammed somatic cells is the loss of genomic integrity and chromosome stability, a hallmark for cancer and many other human disorders. We investigated the cytogenetic rearrangements in 32 human iPSC lines reprogrammed by two different viral delivery systems; 16 iPSC lines were generated by non-integrative Sendai virus (SeV) whereas 16 iPSC lines were generated using integrative lentivirus. At early passages we detected cytogenetic rearrangements in 44% (7/16) of iPSC lines generated by lentiviral integration whereas the corresponding figure was 6% (1/16) using SeV based delivery. The rearrangements were numerical and/or structural with chromosomes 5 and 12 as the most frequently involved chromosomes. We present herein the karyotypic aberrations, including a novel 5q13-q33 duplication that restricts a candidate region for growth advantage in iPSC. Our results suggest that the use of integrative lentivirus confers an increased risk for cytogenetic abnormalities at early passages compared to SeV based reprogramming. In combination, our findings expand the knowledge on acquired cytogenetic aberrations in iPSC.

T-1258

MITOFUSINS DEFICIENCY ELICITS MITOCHONDRIAL METABOLIC REPROGRAMMING TO PLURIPOTENCY

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Cell reprogramming technology, controlling cell fate transition, has opened the door to generating highly desired cell types and recapitulating in vivo developmental processes and architecture. Nevertheless, the precise molecular mechanisms underlying the reprogramming process remain to be defined. Here, we show that depletion of p53 and p21, a barrier to reprogramming, yields a high reprogramming efficiency, which is related to a distinct mitochondrial background with low expression of oxidative phosphorylation subunits and mitochondrial fusion proteins, including mitofusin 1 and 2 (Mfn1/2). Importantly, Mfn1/2 depletion reciprocally inhibits the p53-p21 pathway and promotes both the conversion of somatic cells to a pluripotent state and the maintenance of pluripotency. Mfn1/2 depletion facilitates the glycolytic metabolic transition through activation of Ras-Raf and hypoxia-inducible factor 1α (HIF1α) signaling in an early stage of reprogramming. HIF1α is required for increased glycolysis and reprogramming by Mfn1/2 depletion. Taken together, these results demonstrate that Mfn1/2 constitutes a new barrier to reprogramming, and Mfn1/2 ablation facilitates the induction of pluripotency through restructuring mitochondrial dynamics and bioenergetics.

T-1259

SYNTHETIC DNA-BINDING EPIGENETIC SWITCHES FOR CELL FATE CONTROL

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Artificial induction of pluripotency in somatic cells through transcriptional reprogramming has changed the scientific view that the fate of the specialized cells is irreversible. Chromatin modifying enzymes turn `ON` and `OFF` the transcriptional machinery at the right place and time to regulate cell fate. Since the epigenome is inherently flexible, it could be modulated through pharmacological interventions. Accordingly, several small molecules targeting the

epigenetic enzymes were shown to enhance the somatic cell reprogramming. However, these effectors alter the epigenome in a sequence independent manner. Since transcriptional regulatory network gets orchestrated with complicated and precise epigenetic modifications, artificial epigenetic modifiers with selective DNA-binding capability could be harnessed as the tool to reprogram cells or differentiate them to desired phenotype. Accordingly, as a novel chemical approach to control cell fate, we synthesized a new class of dual-functional small molecule termed SAHA-PIP containing sequence-specific pyrrole-imidazole polyamides (PIPs) and HDAC inhibitor SAHA. Evaluation of the effect of SAHA-PIPs on genome-wide gene expression in human fibroblasts divulged that SAHA-PIPs with different sequence specificity activated exclusive clusters of therapeutically important developmental genes. For instance, SAHA-PIP K enforced the transcriptional activation of germ cell genes in human fibroblasts. Likewise, SAHA-PIP X activated transcription of a series of genes that are associated with retinal development. Notably, SAHA-PIP `I` dramatically switched `ON` the core pluripotency gene circuitry. Interestingly, conjugation of PIP with a p300 activator also resulted in the activation pluripotency gene network. Recently, we tested the effect of our SAHA-PIPs on the gene expression of human pluripotent stem cells and identified certain SAHA-PIPs capable of activating cardiovascular gene markers. Unlike other small molecules currently employed to improve reprogramming efficiency, PIP conjugates can be tailored to bind predetermined DNA sequences. Therefore, strategies to expand our tunable epigenetic switches could create an epoch-making approach in cellular reprogramming as they may precisely coax a type of cells into another one.

T-1260

IN VIVO REPROGRAMMING OF STRIATAL NG2 GLIA INTO FUNCTIONAL NEURONS THAT INTEGRATE INTO LOCAL HOST CIRCUITRY

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In vivo reprogramming is an interesting alternative to conventional cell replacement therapy as it removes the need for an exogenous cell source. It aims to target a non-neuronal population of cells, resident in the brain that can be reprogrammed into neurons to replace cells lost due to disease. NG2 glia are the most abundant, proliferating cell in the CNS with a remarkable self- renewal capacity. They serve as oligodendrocyte progenitor cells (OPC:s) and receive synaptic input indicating that they are part of the neuronal network. There are also reports of NG2 cells neurogenic potential when explanted to neurogenic zones in the brain or when cultured under neurological conditions. This apparent plasticity, their synaptic connection to neurons and their self-renewal capacity makes NG2 glia cells interesting targets for in vivo reprogramming. We here show the conversion of resident NG2 cells into neurons by injecting Cre inducible AAV vectors coding for the transcription factors Ascl1, Lmx1a and Nurr1 (ALN), into the striatum of NG2-Cre transgenic mice. By injecting GFP under the control of a ubiquitously expressed promoter we show that we can selectively target the NG2 population as determined by co-expression of GFP with NG2, SOX10 and PDGFr-α. By co-injecting GFP under the neuronal specific synapsin promoter together with ALN only reprogrammed neurons express GFP and can thus be distinguished from the resident neurons. The number of converted neurons is robust and reach approximately 7000/brain already after four weeks. After twelve weeks, 50% of the new neurons express the neuronal marker NeuN, compared to 25% after four weeks, indicating increasing maturation with time. At twelve weeks the converted cells express pan-neuronal markers and lose their NG2 expression indicating the transcriptional switch from NG2 glia to neuron. The majority of the cells express GAD65/67 indicating a GABAergic phenotype and patch clamp recordings reveal that converted cells exhibit electrophysiological properties of functional neurons. Using a modified rabies virus that monosynaptically trace neurons in a retrograde manner we show that the newly formed neurons integrate locally into the pre-existing neuronal network and receives input from medium spiny striatal neurons.

T-1262

CONVERSION OF PRIMED HUMAN EMBRYONIC STEM CELLS TOWARDS A MORE NAIVE STATE OF PLURIPOTENCY

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Recently, several groups succeeded for the first time in derivation and/or conversion of naive human embryonic stem cells (hESCs), using various culture conditions. As it is known that Wnt signaling is playing a major role in maintaining the naive state of pluripotency in mice, we wanted to investigate if global stimulation of non-canonical Wnt signaling by Wnt5a could promote the conversion of primed hESCs towards a more naive state. Therefore, UG11 (XY) hESC line was cultured on mouse embryonic fibroblasts (MEFs) in KODMEM based medium with 20% KOSR, supplemented as following: with bFGF (4ng/mL) (control); or with bFGF (8ng/mL), hLIF (1000U/mL), PD0325901 (1µM), Chir99021 (3µM), and Wnt5a (200ng/mL) (experimental group). After a few passages, colony morphology changed from flat to domed-shape in the experimental group, and time-to-passaging reduced from every 5-6 days to every 2-3 days. Expression of the pluripotency markers NANOG and SOX2 and of the naive markers REX1, KLF2, KLF4, and ESRRB was examined by RT-qPCR after 4 passages as single cells using trypsin. Expression analysis revealed a significant upregulation of KLF4 ($p=0.0006$) and a slight increase in KLF2 expression ($p=0.054$), while the expression of ESRRB, SOX2 and NANOG was comparable to the primed control. The expression level of REX1 was significantly lower in the Wnt5a condition compared to the primed hESCs. Therefore, we suggest that this novel medium formulation is stimulating the conversion of hESCs towards a more naive state of pluripotency, but needs to be optimized and analyzed further in depth to see how long term single cell passaging is affecting the morphology, gene expression and chromosomal stability of these hESCs. Moreover, the number of hESC lines needs to be increased, including both XX and XY lines. These results support the current theory that multiple routes are leading towards naive-like pluripotency in human. It will be important to investigate the unique properties of these different types of hESCs, as well as their functional differentiation potential.

T-1263

EFFICIENT REPROGRAMMING OF CENTENARIANS SOMATIC CELLS, PERSONALIZED MEDICINE IN GERIATRIC MEDICINE

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Since 2006, following successful reprogramming of somatic cells to pluripotency, induced pluripotent stem (iPS) cell has become a valuable tool for regenerative medicine, disease modeling, drug discovery and basic research in biology. The need for regenerative medicine and cellular therapies is perhaps greatest in the geriatric population. To address this issue, patient-specific iPS cells and ultimately organs could be required. However, reprogramming somatic cells from geriatric patients faces additional hurdles not encountered with the younger cells normally used for such studies. We report methods for efficient reprogramming of dermal fibroblasts isolated from subjects 104-109 years of age. Standard conditions were those recommended by the manufacturer for the CytoTune reprogramming Kit that uses three Sendai virus (SeV) vectors for delivering and expressing four reprogramming factors, OCT4, SOX2, KLF4, and c-MYC. Fibroblasts from three centenarians and cells from neonatal foreskin (HFF) and young adult dermal fibroblasts (HDF) served as controls. Preliminary transduction experiments with SeV-based GFP vector showed all cells are amenable to SeV transduction, however the percentage of cells expressing GFP in centenarian fibroblasts were significantly lower than control cells. The percent of GFP positive cells 48 hours after transduction was 14-26% for HFF and 10-14% for HDF. In the same experiment (MOI=3), the results for centenarian fibroblasts are 3-7%. Culture conditions for viral exposure were altered in attempts to improve vector transduction. Maintaining the same MOI per cell, the volume of media used for exposure was reduced from 300µL to 200µL and hydrodynamic pressure was applied by centrifuging culture plates at 300 g for 15 minutes to facilitate viral integration. These combined procedures significantly increased viral transduction and consequently GFP expression 19.02 and 27.19 fold in HFF and HDF cells respectively. A full reprogramming protocol was applied to all cell types. While the standard procedure recommended by the manufacturer efficiently reprogrammed fibroblasts from young individuals, no reprogrammed cells were observed in cultures of centenarian fibroblasts. However, the use of the optimized protocol resulted in efficient reprogramming of centenarian cells.

T-1264

ABROGATION OF SENESCENCE ASSOCIATED MICRORNA 195 IN AGED SKELETAL MUSCLE CELLS FACILITATES REPROGRAMMING TO PRODUCE INDUCED PLURIPOTENT STEM CELLS

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Advanced age is one of the leading risk factors for degenerative diseases. Reprogramming of a patient's own cells to produce induced pluripotent stem cells (iPSCs) is emerging as a promising strategy for autologous cell-based therapies for combatting this problem. However, low reprogramming efficiency in cells from elderly patients is a pitfall that remains to be overcome. SIRT1 demonstrates diminished expression in aged cells and increased expression upon reprogramming. Recently, it was shown that senescence-associated microRNA (miR)-195 targets SIRT1. We investigated, based on this relationship, whether abrogation of miR-195 expression could improve reprogramming efficiency in old skeletal myoblasts (SkMs). SkMs were isolated from young (2 months) and aged (24 months) C57Bl/6 mice. We found that miR-195 expression was significantly higher in Old SkMs (O-SkMs) as compared to young SkMs (2.89±0.70 fold). In addition, expression of anti-aging factors including SIRT1 and telomerase reverse transcriptase (TERT) was down regulated in O-SkMs, and transfection of O-SkMs with lentiviral miR-195 inhibitor significantly restored expression of SIRT1 and TERT in O-SkMs. Intriguingly, quantitative fluorescent in situ hybridization (Q-FISH) analysis showed significant telomere elongation in O-SkMs transfected with anti-miR-195 (1.90 fold increase). O-SkMs were transfected with Yamanaka quartet together with miR-195 inhibitor to produce iPSCs in order to examine whether silencing miR-195 in O-SkMs can facilitate reprogramming efficiency. Interestingly, blocking miR-195 expression significant increased the reprogramming efficiency of O-SkMs as compared to scramble (2.5 fold increase). Transfection of anti-miR-195 did not alter their karyotypes or pluripotency marker expression such as OCT4, NANOG and SSEA-1, and iPSCs transfected with anti-miR-195 successfully formed embryoid bodies that spontaneously differentiated into three germ layers, indicating that deletion of miR-195 does not affect pluripotency in SkMs. Abrogation of age-induced miR-195 is a novel and promising approach for efficient iPSCs generation from old donor subjects, which has the potential to contribute to successful autologous transplantation of iPSCs in elderly patients.

IPS CELLS

T-1265

MULTICLONAL LARGE-SCALE RNASEQ ANALYSES TO DEFINE IPSC VARIABILITY, GENE REGULATORY NETWORKS AND EQTLS RELATING TO A COMPLEX HUMAN DISEASE MODEL

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Induced pluripotent stem cells (iPSCs) offer a unique opportunity

to model human diseases and serve as an unlimited cell source for regenerative medicine. However, patient based and iPSC clonal variability remain a limitation for their use. Additionally, the effect of genetic variation on the molecular pathways driving self-renewal and differentiation are still not completely understood. To model insulin resistance (IR), a serious complex human condition, we have generated (through a non-integrative Sendai virus methodology), more than 1000 iPSC lines from 200 individuals that reflect the spectrum of IR in human populations. The primary aim is to use iPSCs as a model to find critical genes that participate in the origin and development of IR. To that end, we have performed RNAseq of 328 iPSC clones (103 individuals). Differential expression analysis has identified that transcription of key molecular pathways related to energy metabolism, insulin signaling and cellular growth vary substantially across the spectrum of IR, hinting at a cell autonomous mechanism. The genetic program underlying this phenotype has been explored using co-expression network analysis of iPSCs derived from insulin resistant and insulin sensitive (IS) individuals. These analyses have identified sub-networks (modules) and "key driver" genes that may underlie the condition. Of particular interest are the modules enriched for differentially expressed genes that significantly overlap between the IR and IS co-expression networks, giving potential clues to explain the mechanisms of the IR phenotype. A secondary aim is to describe the transcriptional landscape and variability found in iPSCs. We have identified ~ 800 iPSC-specific expression quantitative trait loci (eQTLs) lending insight into regulatory networks of iPSCs. We have described groups of variable and non-variable genes in iPSCs and developed approaches based on principal component analyses of pluripotency associated markers and transcription factors to define iPSC identity and "outliers". This large-scale study has not only given us a broad view of the cellular and molecular underpinnings of insulin resistance, but also generated a resource to explore variability in iPSCs, thereby allowing the design of more efficient iPSC production and differentiation strategies.

T-1266

A RAPID FLUORESCENT IMAGE CYTOMETRY METHOD FOR VALIDATION AND MONITORING OF 2O REPROGRAMMED IPSC COLONIES

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Flow cytometry (FC) and fluorescent microscopy (FM) have been commonly used for the detection of induced Pluripotent Stem Cell (iPSC) reprogramming, which often have limitations, where flow cytometry requires disruption of adherent iPSCs by trypsinization, and fluorescent microscopy requires manual qualitative analysis that is low throughput. In the recent years, image-based cytometry systems have been utilized to perform direct whole well cell-based assays in microplates without trypsinization and with comparable sensitivity as current fluorescence detection methods. In this work, we developed an automated method for detection of iPSC colonies utilizing the combination of the Celigo S Imaging Cytometer and secondary iPSC reprogramming. This approach is based on the fluorescent identification of iPSC colonies that express the four reprogramming factors, Oct4, Sox2, Klf4 and c-Myc expressing mOrange following ires. The reprogramming progress is also monitored using fluorescent detection of the pluripotency reporter Nanog-GFP+ cells within these colonies. Results demonstrated the capability of the imaging cytometer showing the increase in Nanog-GFP+/mOrange+ iPSC colonies in respect to time and also increase in iPSC colonies when treated with shRNA X. The Celigo Imaging Cytometer allowed high throughput whole well fluorescence imaging and analysis of the iPSC colonies, which provided accurate direct measurement of colony numbers as well as improve research efficiency through automation. This method can be used to not only follow the reprogramming kinetics, but could also be used to examine the effect of extrinsic factors, thus, providing a strong tool to investigate molecular mechanisms of reprogramming.

T-1267

MURINE INDUCED PLURIPOTENT STEM CELLS DIFFERENTIATE TOWARDS ENDOTHELIAL CELLS MORE EFFICIENTLY THAN MESENCHYMAL STROMAL CELLS

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Pluripotent stem cells, namely ESCs and iPSCs, can be differentiated to all cell types. Adult stem cells have limited potential, but the claims on wide differentiation capacities of mesenchymal stromal cells (MSCs) are common. Here, we compared the endothelial properties of differentiated murine iPSCs and bone marrow-derived MSCs. iPSCs were obtained by reprogramming of fibroblasts from wild type (wt) C57Bl6/J and leptin receptor deficient (db/db) mice using lentiviral STEMMCA vector. Their pluripotency was confirmed by teratoma formation, expression of Oct3/4A, Nanog, SSEA1 and differentiation towards all three germ layers. MSC (CD45-CD31-Sca-1+CD140a+) differentiated to adipocytes and osteoblasts. Differentiation of iPSCs or MSCs towards endothelium (iPS-EC and MSC-EC, respectively) was performed according to published protocols with minor modifications. Differentiated iPS-EC showed stable CD34+Tie-2+Sca-1+CD45- phenotype with less frequent expression of c-kit, CD133 and CD146. Endothelial phenotype of iPSC-EC was stable for at least 120 days. Contrary to iPS-EC, MSC-EC did not express CD34 or Tie-2, however both showed upregulation in KDR mRNA. iPS-EC and MSC-EC were positive for Ser1177 phospho-eNOS and von Willebrand factor. Moreover, laminar shear stress (3 days at 10 dynes/cm2) upregulated nitric oxide and VEGF production in iPS-EC. In spheroid assay MSCs and MSC-EC formed sprouts, similarly to iPS-ECs. Interestingly, decreased angiogenic properties of db/db iPS-EC were seen in this assay, reflecting the impairment observed also for db/db CD34+ lung endothelial cells. Wild type iPS-EC, transduced with GFP-lentiviral vector and injected with Matrigel under the skin of WT mice facilitated within 14 days the formation of mostly GFP-negative vessels, with some GFP-positive cells. In contrast, C57Bl6/J-GFP MSC-EC in Matrigel in vivo did not contribute to the vessel formation neither promoted endogenous angiogenesis. In summary, murine iPSCs can be differentiated into stable iPSC-ECs cells, with some activities typical for endothelium and can stimulate in vivo angiogenesis. In contrast, MSC, although showing some features resembling endothelium, do not improve them after differentiation and do not show angiogenic properties in vivo.
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T-1268

DEVELOPMENT OF LARGE SCALE PRODUCTION SYSTEM OF HUMAN PLATELETS BY IPS CELL-DERIVED MEGAKARYOCYTE CELL LINE

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According to Japanese Ministry of Health, Labour and Welfare statement, the blood product supply for transfusion will have shortage by 20% of what is required in 2027, due to the decreasing of youths donating blood. In particular, HLA-identical platelets will be required because of platelet refractoriness in patient receiving multiple platelet transfusions.). Although we cannot rule out the possibility of tumorigenicity by iPSC-derived cell products, platelets and erythrocytes (red blood cells), which are anucleate cells, can be γ-irradiated prior to their use for inactivating contaminated nucleated cells. We are particularly focusing on developing a method to create transfusion artificial platelets ex vivo instead of donor-platelets. In order to obtain more than 1011 platelets from rare HLA or HPA (human platelet antigen) donor-iPS cells, our task is to develop as many platelet precursors and megakaryocytes (MKs) as possible in vitro. We have recently established megakaryocytic cell lines (imMKCLs) derived from human iPS cells to grow continuously for up to 5-6 months, which was achieved by inducible overexpression (O/E) system with sequential expression of c-MYC, BMI1, and BCL-XL to control either MK expansion or platelet yield in 6-well or 10-cm dish culture in the presence of mouse feeder cells. To further address the definite protocols including expansion of imMKCLs in a scale of more than 20L liquid culture in the absence of mouse feeder cells, we have optimized the condition of liquid culture system, and also have developed an equipment for condensation of platelets from 20L to 200 mL using hollow fiber-based filtration system suitable for real transfusion in hospitals. During the process of developing the protocols, we have found some positively influenced reagents through MK maturation and proplatelet formation. We are now studying the underlying mechanisms how these reagents promote proplatelet generation from imMKCL-derived mature MKs. Therefore, our system sheds light on novel mechanical aspects for platelet biogenesis as well as clinical application.

T-1269

MITOCHONDRIAL DYSFUNCTION AND ANTIOXIDANTS MODIFY STEM CELL FUNCTIONS

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We have previously shown that, in mtDNA Mutator mice with proofreading deficient PolG, mitochondrial mutagenesis leads to severe self-renewal defects in somatic stem cell (SSC) lineages. These defects can be rescued with antioxidant treatment, and thus we have proposed that disturbances in reactive oxygen species (ROS) -mediated signaling underlie SSC dysfunction, which is likely to led to the progeroid manifestations in these mice. We now further studied the effect of mtDNA mutations on reprogramming of somatic cells to pluripotency and on the pluripotent stem cell characteristics. Fibroblasts with mutant PolG and increased mtDNA mutagenesis, showed reduced reprogramming efficiency, which was rescued by antioxidant treatment with either N-acetyl-cysteine (NAC) or mitochondrial-targeted ubiquinone (MitoQ). Mutator iPSCs (induced pluripotent stem cells) show increased mitochondrial ROS and despite their highly glycolytic nature, they manifest severe growth and self-renewal defects. Treatment with antioxidants can attenuate the stemness defect, indicating sensitivity of stem cells to ROS-mediated signaling. This however, renders stem cells highly sensitive to antioxidants. MitoQ induced cell type specific responses and the therapeutic window was very narrow. Relatively low MitoQ concentrations hampered the stemness of iPSCs and induced toxic effects in neural stem cells both in vivo and in vitro. These results suggest that minor changes in the redox state of a cell lead to disturbed ROS-mediated signaling and this can impair specifically the highly sensitive stem cell compartment. Further, the results imply that the stem cell compartment warrants special attention, when assessing safety of new antioxidants.

T-1270

NEW TYPE OF SENDAI VIRUS VECTOR PROVIDES TRANSGENE-FREE IPS CELLS DERIVED FROM CHIMPANZEE BLOOD

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Induced pluripotent stem cells (iPSCs) are potentially valuable cell sources for disease models and future therapeutic applications; however, inefficient generation and the presence of integrated transgenes remain as problems limiting their current use. Here, we developed a new Sendai virus vector, TS12KOS, which has improved efficiency, does not integrate into the cellular DNA, and can be easily eliminated. TS12KOS have mutation on a component of SeV RNA polymerase, result in SeV transcription were reduced at temperatures above 38°C. TS12KOS carries KLF4, OCT3/4, and SOX2 in a single vector and can easily generate iPSCs from human blood cells. In The iPSCs from human blood cells using conventional vector, temperature shifts from 37°C to 38°C at passages 1 and 2 induced no virus removal. In contrast, when TS12KOS vector was used under the same conditions, 65% and 47%, respectively, of the clones were negative for viral genome. Using TS12KOS, we established iPSC lines from chimpanzee blood and used DNA array analysis to show that the global gene-expression pattern of chimpanzee iPSCs is similar to those of human embryonic stem cell and iPSC lines. These results demonstrated that our new vector is useful for generating iPSCs from the blood cells of both human

and chimpanzee. In addition, the chimpanzee iPSCs are expected to facilitate unique studies into human physiology and disease.

T-1271

E-CADHERIN-FC CHIMERA AND ALBUMIN-ASSOCIATED LIPIDS ARE ABLE TO INHIBIT EXCESS AGGREGATION AND IMPROVE GROWTH EFFICIENCY OF HUMAN INDUCED PLURIPOTENT STEM CELLS (IPSCS) IN SIMPLE SUSPENSION CULTURE

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Suspension culture is one of the most promising approaches to obtain the enough number of human induced pluripotent stem cells (hiPS cells) for clinical applications. Especially, direct suspension culture systems such as spinner flasks or culture bags are simple enough to expand easily. However, iPS cells tend to form aggregates and the control of the aggregation is challenging and significant for stable production of iPS cells. In this report, we investigated the possibility that bioactive molecules were able to prevent excess aggregation in simple suspension culture. Briefly, we suspended and cultured human iPS cells in Essential 8 or mTeSR1 medium containing various bioactive molecules (E-Cadherin-Fc, albumin, insulin etc.) in rotary-shaking culture plates. In usual suspension culture, hiPS cells formed a single clamp larger than 1 mm, whereas the aggregation of hiPS cells were limited and they formed a lot of size-controlled aggregates if some bioactive molecules were added in culture medium: E-Cadherin-Fc, KnockOut Serum Replacement (KSR), and lipid-rich albumin (AlbuMax). Especially, an aggregate diameter was able to be controlled from 75 µm to 200 µm by changing the concentration of KSR (1%-20%) or AlbuMax (0.2%-2%). According to cell counting analyses, the appropriate inhibition of aggregation (2% of KSR or 0.2% of AlbuMax) improved the growth efficiency on day 5 by three to five times. In addition, we investigated the pluripotency of aggregates and showed that size-controlled aggregates preserved their pluripotency after 5 days of suspension culture. Interestingly, usual bovine serum albumin did not show this inhibition of aggregation, thus albumin-associated lipids (which consists of KSR) have the important role for the inhibition of the excess aggregation of hiPS cells. Now we are investigating the detailed mechanism of the inhibition of aggregation by adding albumin-associated lipids and trying to scale-up (from 20 to 100 mL) suspension culture of hiPS cells with aggregation control with AlbuMax.

T-1272

RAPAMYCIN SUPPRESSES NK CELL-MEDIATED REJECTION OF HLA HAPLOTYPE HOMOZYGOUS HEMATOPOIETIC CELLS BY HAPLOIDENTICAL RECIPIENT

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Transplantation of cells derived from autologous iPSCs will be advantageous because of minimum immunogenicity. However, this strategy has problems in terms of time and cost and quality control. To solve these issues, iPSCs bank project has been launched in Japan. In this project, iPSCs are planed to be produced from donors with homozygous HLA haplotype (HLA-homo). It is expected that HLA-homo iPSC-derived cells can be transplanted to recipients who have identical haplotype in one of alleles (HLA- hetero). In homo-to-hetero transplantation, rejection by allo-reactive T cells may not occur. However, in homo-to-hetero setting, recipient NK cells may attack the graft to a certain extent, by the mechanism called hybrid resistance (HR). HR has been mainly studied in mouse, whereas in human it remains unstudied because homo-to-hetero transplantation has been contraindicated due to the risk of fatal GVHD caused by T cells in the graft. If HR takes place also in human, it would be problematic in the iPS bank project. The aim of this study is to examine whether HR occurs in human and, if it does, to seek for immunosuppressive drugs which can suppress HR. NK cells express inhibitory receptors called killer immunoglobulin-like receptors (KIRs), and HLA-C serve as major ligands for KIRs. HLA-C are divided into two groups (C1 and C2), each of them binding to different KIRs (2DL3 and 2DL1). NK cells from a HLA-hetero donor (C1 : C2) were cultured with PBMC from a HLA-homo donor (C1 : C1). We found that NK cells expressing C2 specific KIRs (2DL1) were selectively activated to produce IFN-γ. Thus, it's suggested that HR can occur in human. We then searched for immunosuppressive drugs which can suppress NK mediated HR in vivo. In the mouse model, it has already reported that F1 hybrid mice rejects parental bone marrow cells and this rejection is caused by NK cells. HR in F1 hybrid mice can be quantified by the number of colonies formed on the spleen after the transplantation of parental BM cells. Using this system, we evaluated three different immunosuppressive drugs, methylprednisolone, cyclosporine A and rapamycin. We found that only rapamycin was able to suppress HR. In conclusion, these results show that HR may occurs in human, and rapamycin would be a candidate drug that can suppress it.

T-1273

DEVELOPMENT OF NOVEL MEDIUM FOR CULTURING NAïVE IPS CELLS

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Human embryonic stem cells (ES cells) and induced stem cells (iPS cells) differ from mouse stem cells in important areas such as pluripotency and growth characteristics. Recent papers have reported the existence of human naïve stem cells that are equivalent to mouse ES/iPS cells. We have developed a new culture medium for converting conventional human iPS cells (primed stem cells) into naive human iPS cells without genetic modification. After 1-3 passages of conventional primed human iPS cells in naïve iPS cell culture medium on 4500 cells/cm2 neomycin-resistant SL10 feeder cells (ReproCELL Inc., RCHEF001), the cell colonies are converted to a dome-shape with packed round cell morphology typical of naïve cells. The average doubling time was significantly reduced from around 26 hours for primed human iPS cells down to approximately 20 hours for these converted cells. Nuclear localization of transcription factor TFE3 was shown to be enhanced in these converted cells, indicating these cells are in a naïve state. Chromosome X inactivation marker gene XIST, which is suppressed in naïve state stem cells, was also weakly expressed in the converted

cells. Finally, these converted cells express representative pluripotent marker genes OCT, NANOG, TRA1-81. This medium requires no genetic modification and provides researchers with higher growth rates than with previous medium. Moreover, this culturing system with neomycin-resistant SL10 feeder-cells can be useful for introducing genetically modified genes and single cell cloning by neomycin selection. This system can support uncovering mechanisms of naïve state conversion. This medium will enhance stem cell research as well as have applications for disease modeling, studying mechanisms of stem cell biology, drug screening and regenerative medicine.

T-1274

DEVELOPMENT OF A NOVEL METHOD FOR REMOVAL OF CELLS DEVIATED FROM THE UNDIFFERENTIATED STATE IN COLONIES OF HIPSCS USING BOTULINUM HAEMAGGLUTININ

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Along with the opportunities offered by human induced pluripotent stem cells (hiPSCs), together with the tremendous advances in maintaining their pluripotency in culture, many technological issues remain to be solved. In this study, we used the botulinum haemagglutinin (HA) and investigated their effects on the removal of deviated cells from the undifferentiated state in colonies of hiPSCs, accompanied by disrupting E-cadherin binding. After exposure of hiPSCs cultures to HA for 24 h, colonies with deviated cells occurring in central region have lost contact with each of its neighbors and shrinked and most of them tend to detach from the substrate following exposure to HA. With longer culture, the eliminated space inside colonies was filled by dividing of the neighboring cells, and formed tight compact colonies, similar to those of undifferentiated cells. In contrast to colonies with deviation, colonies containing undifferentiated cells exhibited initially exhibit loose colony morphology after exposure to HA but become more compact as the colony grows larger during culture. As a result of the disruption of E-cadherin-mediated cell-cell contacts, cells deviated from the undifferentiated state exhibited completely loss of actin cytoskeleton and focal adhesion via disruption of E-cadherin binding, resulting in their eventual apoptotic cell death. However, the undifferentiated cells in colonies exhibited temporarily disruption of their actin cytoskeleton and focal adhesion, but an initial decrease was fully developed after culturing for up to 72 h after exposure. These cells also exhibited a recovery of E-cadherin expression as those before exposure to HA and expressed marker of an undifferentiated state, Oct3/4. We found that the Rap1-mediated positive feedback regulation could lead to reestablishment of E-cadherin at the intercellular junctions, thus allowing hiPSCs to establish, consolidate, and strengthen their cellular association and colony structures after exposure to HA. This technology opens new avenues for selectively eliminating the deviated cells expressing abnormal E-cadherin in hiPSC colonies during culture and helps the rational design of culture strategy by selectively eliminating the cells deviated from the undifferentiated hiPSCs.

T-1275

EPITHELIAL CELL ADHESION MOLECULE ENHANCES REPROGRAMMING AND PLURIPOTENCY IN INDUCED PLURIPOTENT STEM CELLS

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Human embryonic stem cells (hESCs) are unique pluripotent cells capable of self-renewal and differentiation into all three germ layers. To date, more cell surface markers capable of reliably identifying hESCs are needed. The epithelial cell adhesion molecule (EpCAM) is a type I transmembrane glycoprotein expressed in several progenitor cell populations and cancers. We found EpCAM to be highly and selectively expressed by undifferentiated rather than differentiated hESCs. The protein and transcript level of EpCAM rapidly diminished as soon as hESC had differentiated. This silencing was closely and exclusively associated with the radical transformation of histone modification at the EpCAM promoter. In addition, we used chromatin immune-precipitation analysis to elucidate the intracellular domain of EpCAM (EpICD) indirectly bind to the promoters of several reprogramming genes, including Oct4 (O), Sox2 (S), Klf4 (K), c-Myc (M), Nanog (N), to help maintain the undifferentiation of hESCs. To investigate the functional roles of EpCAM in reprogramming of fibroblasts into induced pluripotent stem cells (iPSCs), we generated iPSCs with the presence of EpCAM in OSKM-mediated iPSC formation. In this study, we induced reprogramming by piggybac transposone system and we generated Piggybac vector of EpCAM. We found that the expression of EpCAM significantly increased the iPSC formation. The characteristics of iPSCs we generated were further confirmed by in vitro and in vivo assays. The phenotype, the expression levels of stemness markers, gene expression profile, and DNA demethylation state of Oct4 promoter were similar in mouse ESCs and iPSCs. Moreover, these iPSCs could differentiate into three germ layers in teratoma and contribute to germline transmission in chimera mice. The results from our study offer a novel finding that we can promote the efficiency of iPSCs with the presence of EpCAM. This study not only enhances our fundamental understanding of EpCAM in stem cells, but also provides a new perspective by delivering membrane protein to promote reprogramming.

T-1276

E-RAS IMPROVES THE EFFICIENCY OF REPROGRAMMING BY THE ACCELERATION OF CELL CYCLE THROUGH JNK PATHWAY

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We have previously shown that pluripotent stem cells can be induced from adult somatic cells using protein extract isolated from mouse embryonic stem cells (mESC). However, which components among extracts are important to induce pluripotency is not verified. Interestingly, generation of induced pluripotent stem (iPS) cells was depending on the background of ES cell lines. Proteomic analysis of two different mES cell lines shows that embryonic Ras (E-Ras)

is expressed differently in each mES cells and a high level of E-Ras in the extracts allows iPS cells production. Here, we show that E-Ras augments the reprogramming efficiency by promoting cell proliferation. We found that over-expression of E-Ras increased cell proliferation which was caused by up-regulating cell cycle related genes, cyclin D and E. E-ras over-expression shortened the duration of G1 phase and accelerated the transition to S phase of the cell cycle. Our data show that E-Ras activated SP-1 by c-Jun N-terminal kinases (JNK)-dependent phosphorylation, which caused stimulation of promoters of cyclin D and E. This accounted for the enhancing effect of E-Ras-JNK signaling on the cell cycle. These data demonstrate the role of E-Ras in expediting cell proliferation, elucidating that E-Ras can be one of the important factors in generating protein iPS cells.

T-1277

HUMAN IPSC-NPC AND IPSC-GRP THERAPIES AFTER LARGE WHITE MATTER STROKE IN MOUSE: DISTINCT PATTERNS OF MIGRATION AND OF ENDOGENOUS STEM CELL RECRUITMENT

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Subcortical white matter stroke (WMS) constitutes up to 25% of all stroke subtypes. We recently developed a new subcortical WMS model with a large infarct area that simulates the larger white matter lesions seen in moderate to advanced human white matter ischemia. This model has been adapted to immunodeficient NSG mice as a platform for transplant study. While there is no therapy for white matter stroke, cell transplantation is emerging as a viable therapy to restore neurological function. Human induced pluripotent stem cells (iPSCs) are an appealing cell source for cell transplantation to repair disrupted neuronal networks. We tested 2 stem/progenitor cell lines that may produce white matter repair. The first line is iPSC-neural precursor cells (iPSC-NPCs) that can differentiate into neurons, astrocytes and to a lesser extent oligodendrocytes. The second line is iPSC-glial restricted progenitor cells (iPSC-GRPCs) that will differentiate mostly into astrocytes. To characterize how these cell types respond to white matter stroke over time (15 days and 2 months) and how they differentiate in this white matter stroke environment, we tracked the migration of injected stem cells and histologically quantified the glial scar, astrocytic and microglial response, as well as axonal/myelin loss and repair. Both treatment groups demonstrated increased myelin integrity at two months. Both lines survived and proliferated, however the two different types of stem cells had distinct migratory patterns. The iPSC-NPCs remained clustered in the periinfarct region, while the iPSC-GRPs spread out over the whole brain. Most interestingly, we measured the response of endogenous neurogenesis and found that, while in the iPSC-NPC treatment endogenous stem activation was roughly equivalent to stroke-only treatment, surprisingly, in the iPSC-GRP treatment endogenous stem cell activation was four times higher.

T-1278

A COMPARISON OF INDUCED PLURIPOTENT STEM CELLS DERIVED FROM DIFFERENT SOURCE CELLS FROM THE SAME SUBJECT

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Since the initial derivation of human induced pluripotent stem cells (iPSC) from fibroblasts (FCLs), other source cells including blood, olfactory epithelium and dental pulp have been reprogrammed into iPSC. However, whether or not there are significant differences in the resulting iPSCs from different source cells remains unclear. Of the source cells that have been reprogrammed, blood has 2 distinct advantages -it is relatively non-invasive to obtain and specimens with extensive phenotypic and clinical subject data exist in biorepositories around the world. If iPSCs derived from blood could be demonstrated to display characteristics indistinguishable from iPSCs derived from FCLs (which can only be obtained from a skin punch biopsy) the available pool of source material for iPSC would be greatly increased. In order to examine this, we have reprogrammed both blood and FCLs from 3 subjects. Although at least 4 blood cell types have been reprogrammed in the literature, we chose to focus on $CD4^+$ T cells anderythroblasts. These 2 cell types were chosen because they are easily obtainable from small amounts of adult peripheral blood. $CD4^+$ T cells were chosen because they are able to be activated and expanded in culture making them a semi-renewable resource and erythroblasts were chosen because unlike the $CD4^+$ T cells they do not undergo any DNA rearrangement. In this study 5 independent colonies of each reprogramming (45 cell lines in total) were isolated and are undergoing characterization. We intend to judge the suitability of the source cells by comparing the pluripotency, neural differentiation potential, genomic stability, and gene expression of the resulting iPSC. Gene expression arrays and embryoid body formation will be used to investigate the pluripotent state of the iPSC, while CNV analysis will be used to examine their genetic stability. Since we are particularly interested in mental health disorders we will differentiate the cell lines into neurons and examine the percentage of different neuronal subtypes produced by each source cell type, as well as their ability to mature into functional neurons. Finding no advantage to using FCLs over blood cells as source cells for iPSC will allow investigators a broader range of source cells and increase the usefulness of the blood stored in biorepositories around the world.

T-1279

IMAGE-BASED COLONY MORPHOLOGY ANALYSIS FOR CULTURE PROTOCOL EVALUATION OF IPSCS

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Induced pluripotent stem cells (iPSCs) with their capabilities for unlimited self-renewal and pluripotency is becoming an ideal cellular resource in regenerative medicine and drug discovery. To

widely distribute iPSCs and promise their stable results for further applications, the consistency of their culture protocol is an important task, since their culture tends to require experiences and skills. However, since there are various steps and parameters in iPSC culture protocol, it has been difficult to feasibly figure out the most affective factor. To enhance the efficiency and cost of such culture protocol optimization, we here repot the use of image-based colony morphology analysis for evaluating the real-time responses of iPSC colonies. By combining image processing technology and effective informatics analysis, we show that information of morphological change of colonies with their growth rate can support the technical protocol optimization. We here applied our developed method to visualize the effect of cell-culture operation by analyzing nearly 1000 induced-PSC colonies under 4 types of culture protocols. By analyzing the correlation of immune-histochemical staining results of undifferentiation markers, we found that 8 clusters of morphological classes can be quantitatively defined to evaluate the differences of manipulation effects.

T-1280

EFFECT OF FEEDER-FREE CULTURE SYSTEM FOR QUALITY OF HUMAN IPS CELLS

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Pluripotent stem cells have been thought to be useful sources for regenerative medicine. Although embryonic stem (ES) cells have abilities to differentiate into several kinds of somatic cells and grow infinitely in vitro, there are several problems with using ES cells for clinical application. To overcome these problems, induced pluripotent stem cells (iPSCs) were generated from somatic cells. iPSCs have raised hopes for a new era of regenerative medicine because they can avoid the ethical problems and innate immune rejection. Our final goal is to apply the iPSC technology to the clinical application. We are proceeding the iPS cell stock project for realization of iPSC-mediated cell transplantation therapy. The important point of this project is generation of clean & safe iPSCs from HLA (Human Leukocyte Antigen) homozygous healthy donors. Human iPSCs are typically generated and maintained on feeder cells. Mouse feeder cells (SNL or MEF) are conventionally used for hiPSC culture. These cells are prepared with FBS-containing medium. For clinical use, feeder-free (Ff) and xeno-free (Xf) culture conditions seem to be better than the conventional ones. We succeeded to develop the Ff-culture conditions by using recombinant laminin proteins and Xf-medium for hiPSC establishment and long-term culture. We could generate human iPS clones from fibroblasts and some kinds of blood cells. The quality of iPSCs is important for clinical application. We must confirm the genomic stability and differentiation ability by several analyses. It has been reported that there are "Bad"-iPS clones, which show the resistant against differentiation into neuronal cells. These Bad-iPSCs have been generated and cultivated on mouse feeder cells. In this study, we cultivated Bad-iPSCs under feeder-free culture system and examine whether Bad-iPSCs can differentiate into neuronal cells. We found that the percentage of differentiation-resistant cells (TRA-1-60 positive cells) decrease after transferring to the feeder-free condition. This results indicated that our feeder-free culture system might selectively cultivate the "Good"-iPSCs but not "Bad"-iPSCs. We believe that this system is useful not only for producing clinical-grade hiPSCs in Cell Processing Centers for future application, but also for basic stem cell research.

T-1281

MANIPULATION OF KLF4 EXPRESSION GENERATES IPSCS PAUSED AT SUCCESSIVE STAGES OF REPROGRAMMING

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The detailed mechanism of reprogramming somatic cells into induced pluripotent stem cells (iPSCs) remains largely unknown. Partially reprogrammed iPSCs are informative and useful for understanding the mechanism of reprogramming, but it remains technically difficult to produce them in a predictable and reproducible manner because it is difficult to control the expression levels and stoichiometry of reprogramming factors, which affect the efficiency of iPSC generation and the quality of generated iPSCs. We have developed a unique gene transfer system, named SeVdp vectors, which remain persistently in the cytoplasm without integrating into the host genome and also enable expression of multiples genes with a constant stoichiometry from a single vector genome. And SeVdp vectors harboring four reprogramming factors (Oct4, Sox2, Klf4 and c-myc) reprogram mouse and human somatic cells very efficiently. We analyzed the effect of reduced expression of the four reprogramming factors using SeVdp vectors, and found that reduction of Klf4 expression reproducibly generates a homogeneous population of partially reprogrammed iPSCs. Up-regulation of Klf4 enabled these cells to resume reprogramming, indicating that they are "paused iPSCs" that are stalled on the path towards iPSCs. Moreover, the different level of Klf4 expression allowed generation of many types of paused iPSCs that have paused at distinct intermediate stages of reprogramming. Whole genome analyses suggested that low Klf4 expression is sufficient to repress somatic cell-specific genes but not for activating pluripotency-related genes. In accordance with the gene expression, Klf4 did not bind to the promoter of these pluripotency-related genes in the paused iPSCs although low but significant amount of Klf4 expressed in them. Now, we are investigating the mechanism of such a Klf4 dose-dependent regulation of pluripotency using our SeVdp-based stage-specific reprogramming system (3S reprogramming system).

T-1282

LANDSCAPE OF TRANSCRIPTIONAL AND EPIGENETIC PROFILE AND HEMATOPOIETIC DIFFERENTIATION CAPACITY OF HUMAN PLURIPOTENT STEM CELLS

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The potential of human iPS cells to differentiate into many cell types makes them a promising source for regeneration therapy,

drug screening, and pathogenetic study. However, there exists large variation among human iPSC lines in their differentiation capacity to specific lineages. This variation is frequently attributed to somatic memory, however, whether somatic memory affects the cellular behavior of human iPSCs especially in hematopoietic differentiation is still controversial. Some have reported that human iPSCs derived from hematopoietic cells have higher potential to differentiate into hematopoietic cells than iPSCs derived from other somatic tissues but another report concluded the opposite. The discrepancy may be attributable to different protocols of hematopoietic differentiation, as the former assessed differentiation potential in later phases by performing colony forming assays of hematopoietic precursor cells, which reflects hematopoietic maturation, whereas the latter report assessed differentiation efficiency in early phases, which reflects hematopoietic commitment. Therefore, in this study, we assessed the hematopoietic differentiation capacity of 35 human iPSC lines from four different parental tissues and four ESC lines by evaluating both the differentiation capacity in early phase (from pluripotent state to hematopoietic precursors, commitment capacity) and that in late phase (from hematopoietic precursors to mature blood cells, maturation capacity). We also investigated transcriptional and epigenetic profile of the PSCs, parental cells of iPSCs, and their differentiated derivatives, and analyzed the association between these molecular signatures and their differentiation capacities. These analyses illustrated that commitment capacity was highly associated with a specific gene expression pattern of PSCs, and the maturation capacity was associated with the amount of reprogramming-associated aberrant DNA methylation, rather than cell type origins of human iPSCs. Most importantly, cell type origins were not a major determinant factor for differentiation capacity in both phases, contrary to some previous reports.

T-1283

GENOMIC VARIATIONS IN IPSCS ARE MAINLY DONOR CELL-DERIVED AND KARYOTYPE ABNORMALITIES ARE FREQUENT ONLY AFTER EXTENDED CULTURE OF SOURCE CELLS AND IN IPSCS FROM ELDERLY DONORS

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Recent studies observed alarming levels of genetic abnormalities in human iPSCs potentially accompanied with malignant transformation and functional deficits. Some studies reported them to be in part derived from the primary cells. We have investigated to what extent different categories of genomic variations / abnormalities are present in iPSCs and are derived from donor cells of aged versus neonatal individuals. In a prospective study we generated a series of iPSC lines from endothelial cells, CD34+ cells and fibroblasts isolated from different sources of neonatal, adult (20-28y) and aged (38-88y) donors under controlled and comparable conditions. Karyotyping revealed aberrations in 40% of early passage (EP) iPSC clones derived from elderly patients, with many of them detectable in corresponding EP primary cells accompanied with significant telomere shortening. In contrast, the vast majority of analyzed EP iPSCs derived from EP neonatal cell sources had normal karyotypes. Prolonged passaging (P11-13) of neonatal primary cells, however, led to complex karyotypic aberrations, indicating that extended primary cell culture is the most critical factor for generation of genetically abnormal iPS clones. In contrast, array CGH revealed normal numbers of CNVs in EP primary cells and EP iPSCs from neonatal and aged cell sources. Finally, exom sequencing demonstrated similar levels of INDELs and SNPs in EP iPSC clones derived from neonatal and aged donors, with all analyzed SNPs and the vast majority of analyzed INDELS already detectable at least at low levels in the respective primary cells, obviously mainly representing normal non-disease-related genetic variations. Thus, the frequency of critical genetic abnormalities in EP iPSCs may be much lower than previously reported. Remarkably, donor's age and extended culture of primary cells appear to be the most critical factors with respect to the frequency of karyotypic abnormalities. Obviously, cellular mechanisms of DNA repair reliably prevent the occurrence of SNPs, INDELs and CNVs even in cultured cells of aged human individuals, whereas imperfect mitotic chromosome segregation and telomere shortening account for the occurrence of polyploidies and chromosomal translocations in late passage primary cells and EP iPSCs from aged patients.

T-1284

EARLY QUALIFICATION STUDIES FOR THE DEVELOPMENT OF AN EMBRYOID BODY-BASED ASSAY FOR SCREENING POTENTIAL PLURIPOTENCY POTENTIAL IN INDUCED PLURIPOTENT STEM CELL LINES IN THE EBISC IPSC BANK

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European Bank for induced pluripotent StemCells (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 early development of a routine assay for screening pluripotency potential in EBiSC cell lines, based on the generation of Embryoid bodies (EBs) as a paradigm for cell differentiation during early embryogenesis. This is intended to provide a candidate replacement model for the costly and labour intensive teratoma assay which is also under investigation in other work. An EB assay was adapted to investigate if each cell line could show evidence for their ability to generate each of the three germ layers without the use of any growth factors or compounds i.e. "spontaneous" differentiation. This will give a good indication of their potential ability to generate multiple cell types. EBs from each cell line were generated, via spin plate method, and left to spontaneously differentiate for 7 and 14 days. Here we report data on a sample of ten of the first cell lines obtained for the EBiSC project. We have found that after 14 days, significant increases (> 3 fold) in all germ layer marker genes were detected. There were also significant decreases in gene expression in stem cell markers .After 7 days, it was found that the cell lines had initiated these changes in gene expression, but not to the same extent as achieved at D14. Substantial variations in patterns of individual gene expression were observed. The EB's appear to require more time to develop into each germ layer. Although the assay appears to be

effective at indicating if a cell line can make all 3 germ layers, the reproducibility of the pattern of gene expression levels is poor. This is not unexpected as the assay analyses 'spontaneous' or undirected differentiation. However, some cell line associated trends have been observed in our current data including. We conclude that a standard EB protocol with cell analysis at 14 days may be used as a robust primary screen for the potential of iPSC. Further data is now being collected to investigate cell to cell variation and comparisons with other more rapid assays.

T-1285

EXPLOITING INDUCED PLURIPOTENCY IN HIV-1 IMMUNOTHERAPY

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HIV-1 infection progressively diminishes the number of CD4+ T cells, crippling the ability of the immune system to eradicate the virus. Therapeutic vaccines are an attractive approach to elimination of residual reservoirs of infectious virus that remain following anti-retroviral therapy. Nevertheless, the use of monocyte-derived dendritic cells (DCs) has had limited success in humans, due partly to their limited capacity to cross-present exogenous antigens from HIV-1 to naïve CD8+ T cells. Having demonstrated previously that induced pluripotent stem cells (iPSCs) derived from human dermal fibroblasts may be differentiated in vitro into DCs, we now investigate the potential of this novel source of DCs for vaccination to HIV-1. We have characterised these cells with respect to their phenotype, immunogenicity and capacity for chemotaxis and have demonstrated that the iPSC derived DCs (iPDCs) express DC markers, are highly phagocytic and immunostimulatory. We have also shown that these iPDCs respond to microbial stimuli and display properties of cross presenting DCs, expressing CD141 and responding to XCL1 in a chemotaxis assay. We are now investigating their ability to cross-present HIV-1 antigen to CD8+ T cells, to ascertain their potential for use as a therapeutic vaccine for HIV-1.

T-1286

THE EFFECT OF REPROGRAMMING METHOD, SOURCE CELL TYPE AND LONG-TERM CELL CULTURE ON GENOMIC STABILITY OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human induced pluripotent stem cells (hiPSCs) possess a great potential for clinical application. However, previous studies revealed the genomic instability of these cells. The reprogramming process itself may contribute to the mutational load in hiPSCs and subsequent in vitro culturing is also related to genomic aberrations increase. Various methods for iPSC generation were established and the main effort has been focused on reprogramming efficiency. In our study we try to figure out how reprogramming method, source cell type and long-term cell culture influences the genomic stability of hiPSCs. In our laboratory we established hiPSC clones from different source cells (fibroblasts or CD34+ blood progenitors) by three reprogramming methods: STEMCCA lentivirus, Sendai virus or episomal vectors. The reprogramming efficiency was comparable for all three methods and both cell types. The pluripotency of our hiPSCs was verified by differentiation into all three germ layers and by teratoma assay. In order to study genomic integrity, we monitored DNA damage response (DDR). Phosphorylated form of histone H2AX (γ-H2AX) and protein 53BP1 play key role in DDR mechanism and mark DNA lesions throughout the genome. The levels of γ-H2AX and 53BP1 were determined in source cells, hiPSCs in low passage and hiPSCs in high passage. The immunofluorescence analysis revealed the differences in spontaneously occurring foci numbers among our hiPSC lines and variations were also found between low and high passages. Moreover, the samples differ in their capacity to response to ionizing radiation. Expectedly, the two proteins were extensively co-localized. We hypothesize that observed variations in DDR will correlate with the genomic aberrations, including duplications and deletions. Therefore, CGH microarray technology will be employed to detect copy number variations that may result from impaired DDR. The genomic stability is one of the major safety concerns of hiPSCs and must be addressed before transfer of this technology into clinical therapy.

T-1287

THE UTILITY OF INDUCED PLURIPOTENT STEM CELL (IPSC)-DERIVED CARDIOMYOCYTES AND HEPATOCYTES AS AN IN VITRO HUMAN MODEL SYSTEMS FOR HIGH-CONTENT SCREENING OF COMPLEX CHEMICAL SUBSTANCES

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In vitro model-based testing is part of routine safety evaluation of drugs and chemicals. Usually, screening is performed with pure compounds. In addition, chemical structure-based similarity "read across" is widely used for predictive safety assessments of non-pharmaceutical chemicals in regulatory submissions, especially in Europe. While either biological or chemical characterization of the potential human health hazard is sensible for chemically-characterized compounds, it is not applicable to assess the hazard of mixtures or complex substances (e.g., petroleum products). Thus, we suggest that safety evaluation centered on similarities in biological responses, i.e. a biological data-based read across, may represent a feasible alternative. In this work we tested a hypothesis that induced pluripotent stem cell (iPSC)-derived cardiomyocytes and hepatocytes represent a relevant in vitro model system that is applicable for high-content, multidimensional toxicity screening and biological read across of chemically complex substances. We selected 26 petroleum products from six distinct categories: SRGO (Straight Run Gas Oils), OGO (Other Gas Oils), VHGO (Vacuum & Hydrotreated Gas Oils), Bitumens, RAE (Residual Aromatic Extracts), and HFO (Heavy Fuel Oils). iPSC-derived cardiomyocytes and hepatocytes were exposed to a DMSO-based extract dilution series in logarithmical order over five logs for up to 48 hours. The cardiomyocyte-derived phenotypes included the measurement of effects on contractility, beating pattern and amplitude, as well as cell viability, morphology and mitochondria integrity measured through high-content live cell imaging. For hepatocytes we determined changes in cell viability,morphology, and mitochondria integrity by live and fixed cell imaging. Quantitative data were then used as high-dimensional "biological" data inputs for evaluation of the similarities and differences both within and across different substance categories.

Our data clearly indicate cell- and substance group-specific effects. Collectively, our work demonstrates that iPSC- derived cardiomyocytes and hepatocytes are useful in vitro human model systems for high-content screening of complex chemical substances.

T-1288

ORPHAN NUCLEAR RECEPTOR AND ZIC FAMILY TRANSCRIPTION FACTORS SYNERGISTICALLY ENHANCE SOMATIC CELL REPROGRAMMING

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Forced expression of core pluripotency genes can reprogram somatic cells into iPS cells. We have performed a screening to identify the genes which play pivotal roles in reprogramming of mouse embryonic fibroblasts, and found that orphan nuclear receptor family genes (e.g. Esrrb and Nr5a2) and Zic family genes (e.g. Zic2 and Zic3) are specifically expressed in the cells which are committed to become iPS cells. Moreover, the overexpression of these two transcription factor family genes along with Oct4, Sox2 and Klf4 (OSK) synergistically enhances the reprogramming of mouse embryonic fibroblasts (MEFs). Introduction of OSK, Esrrb and Zic3 by retroviral vectors into MEFs led to more than 50% of Oct4+ cells 12 days after the infection. By using inducible overexpression system, we also demonstrated that Zic3 and Esrrb functions at the early and middle stage of reprogramming, respectively. Our microarray analysis showed that the Esrrb and Zic3 have largely distinct target genes and that several tens of genes are specifically up-regulated only when both of the two TFs are overexpressed. To address the mechanisms of this synergy, we performed ChIP-seq analysis by using FLAG-tagged Esrrb and Zic3, and found that Zic3 recruits Esrrb to its own binding sites. Currently, we are analyzing the crucial downstream events for this synergistic enhancement of reprogramming by orphan nuclear receptors and Zic family TFs. Our preliminary data have suggested that these TFs activate cellular metabolism which is essential for naïve pluripotent stem cells.

T-1289

REDUCTION IN REMAINING UNDIFFERENTIATED HUMAN INDUCED PLURIPOTENT STEM CELLS BY ANTIBODY-DRUG CONJUGATE

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Cardiomyogenically differentiated induced pluripotent stem cell (iPSC) is promising for cell transplantation therapy for cardiac disease, although residual undifferentiated cell fraction after induction of the differentiation has been shown to carry the risk of teratoma/tumour formation after cell transplantation to the heart. We herein hypothesised that an antibody-cytotoxic drug conjugate treatment targeting undifferentiated cells may be useful in reducing residual undifferentiated cell fraction in the cardiomyogenically differentiated iPSC preparation. We first compared CD antigen expression patterns between undifferentiated and differentiated cells and found that CD30, as well as TRA-1-60 and TRA-1-81, were highly expressed on undifferentiated cells. In addition, CD30-negative cell-fraction was less positive for Lin28, a known pluripotent marker, than CD-30 positive one. To eliminate the CD30-positive fraction, we applied an anti-CD30 antibody-drug conjugate (brentuximab vedotin, Adcetris, SGN-35) that has been shown to be potent for selectively eliminating CD30-positive cells and has been already approved by the FDA for lymphoma therapy. Brentuximab vedotin treatment at 50 μg/ml for 72 hours blocked proliferation of hiPSCs, leading to cell death in vitro, but did not affect differentiated cells (fibroblasts and cardiomyocytes) (hiPSCs and NHDF: 7.2-16.6% and 91.6% survival compared to untreated cells, respectively). In addition, the percentage of Annexin-V positive cells in hiPSCs (253G1) after treatment with brentuximab vedotin was higher than that of the NHDF (71.5% ± 10.9% versus 2.8% ± 0.6%). Furthermore, hiPSC-derived cardiomyocytes treated with brentuximab vedotin for 96 hours showed reduced Lin28 expression compared to untreated hiPSC-derived cardiomyocytes (0 μg/ml: 0.23%; 50 μg/ml: 0.12%, compared to untreated hiPSCs). Anti-CD30 antibody-drug conjugate treatment in vitro reduced residual undifferentiated cell fraction in cardiomyogenically differentiated cell preparation of hiPSCs, indicating usefulness of the drug treatment prior to transplantation in enhancing the safety of transplantation therapy of iPSC-derived cardiac constructs.

T-1290

THE SECRETOME OF INDUCED PLURIPOTENT STEM CELLS (iPSC) MODULATES MACROPHAGE PHENOTYPE IN THE FIBROTIC LUNG

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Macrophages are involved in the pathogenesis and progression of pulmonary fibrosis (PF). Imbalance between classically activated (M1) and alternatively activated (M2) macrophages could play role in progression of PF. Secreted mediators from the human induced pluripotent stem cells (iPS) reduce bleomycin induced fibrosis in the rat lung. We investigate the effect of iPS conditioned media (iPS-cm) on macrophages, with an aim to understand the antifibrotic mechanism of iPS-cm. Human monocytes were polarized towards M1 and M2 phenotype, and treated with iPS-cm; migration and secretory properties were tested in vitro. Bleomycin injured rats where treated on d7 either with iPSC-cm or CCD1-cm (fibroblast conditioned media) as control and FACS analysis was performed on d14 with isolated single cells from the digested lung. iPS-cm increased migration of macrophages in vitro compared to medium control. Levels of pro inflammatory and pro-fibrotic mediators were reduced and anti-fibrotic mediator were increased after in vitro treatment with iPSC-cm. In vivo, percentage of total macrophages increased in bleomycin injured lungs compared to normal control (26.2±1.4% vs 14.4 ± 1.0%). Moreover, M1 and M2 populations were both increased in bleomycin injured lung (M1: 32.3 ± 2.0% vs 5.7% ± 0.3) (M2: 3.2 ± 0.6% vs 1.1 ± 0.2%). Interestingly, iPS-cm treatment reduced total percentage of macrophage to 17.4% ± 1.2; moreover, the original macrophage phenotype was partially restored (M1: 18.0% ± 8.3%; M2: 1.3% ± 0.7%). These effects couldn't be detected using CCD1-cm, which didn't reduce fibrosis in our model. iPS-cm modulates migratory properties and alters the macrophage phenotype by reducing pro inflammatory and pro fibrotic mediators in vitro and alters macrophage percentage and phenotype in vivo in a lung injury and fibrosis model. Targeting the macrophage phenotypical switch could be a possible therapy for PF.

T-1291

DEVELOPMENT AND INDUSTRIAL APPLICATION OF LECTIN-BASED TECHNOLOGIES TO DETECT AND ELIMINATE TUMORIGENIC HUMAN PLURIPOTENT STEM CELLS FOR SAFE CELL-BASED THERAPY

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While human pluripotent stem cells such as human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) are attractive sources of cells for cell-replacement therapies, a major concern remains regarding their tumorigenic potential. Therefore, safety assessment of human pluripotent stem cell-based products in terms of tumorigenicity is critical. By comprehensive glycome analysis using high-density lectin microarray, we identified a pluripotent stem cell-specific lectin probe rBC2LCN recognizing hyperglycosylated podocalyxin as a cell surface ligand. We found that hyperglycosylated podocalyxin is secreted from human pluripotent stem cells into cell culture supernatants. We then established a sandwich assay system, named the GlycoStem test, targeting the soluble hyperglycosylated podocalyxin using rBC2LCN, which was sufficiently sensitive and quantitative to detect residual human pluripotent stem cells. In addition, we also developed a drug conjugate of rBC2LCN, called rBC2LCN-PE23, which could be expressed as a soluble form from the cytoplasm of Escherichia coli and purified to homogeneity by one-step affinity chromatography. rBC2LCN-PE23 bound to human pluripotent stem cells followed by internalization inside the cells, allowing intracellular delivery as a cargo of cytotoxic agents. rBC2LCN-PE23 eliminated human pluripotent stem cells just by the addition into cell culture media without pre-dispersion. The lectin-based technology to detect (GlycoStem test) and eliminate human pluripotent stem cells (rBC2LCN-PE23) should contribute to increase the safety of human induced pluripotent stem cell-based cell therapy.

T-1292

GENERATION OF SERTOLI CELLS DERIVED INDUCED PLURIPOTENT STEM CELLS IN PRE-PUBERTAL CAT

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Induced pluripotent stem cells (iPS) are resemble to embryonic stem cells having self-renewal ability and differentiation potential into 3 layer embryonic lineages. The iPS cells are traditionally produced by robustly gene transduction using the four transcription factors including *OCT-4, SOX-2, cMYC, KLF-4,* referred to as "OSKM". However, transduction efficiency of these transcription factors into fibroblasts remains poor. Sertoli cells are immune-privileged cells that play a central role in the formation of physically blood-testis barrier of the testis. They demonstrates low immunogenicity essentially by the production of immune-modulatory substances. Moreover, the Sertoli cells of pre-pubertal mice and pigs have been demonstrated to express pluripotent factors, including SOX-2 and NANOG. It is therefore hypothesized that these cells may hold a great promise for improving iPS generation. Furthermore, application of CHIR99021 and PD0325901 inhibitors (or 2i) has been reported to improve efficiency of iPS derivation. This study aimed to examine the possibility of iPS generation using Sertoli cells as donor cells and to compare the effect of the 2i treatment vs. control (without inhibitors) on efficiency of Sertoli-iPS derivation. Sertoli cells obtained from a 4-month old domestic cat were dissociated by enzymatic digestion. The Sertoli cells at early passage (1st - 3rd) were cultured with 1% (v/v) FBS in high glucose DMEM. Sertoli cell line (passage 21st) was proven by means of immunofluorescence (IF, vimentin) and gene expression of Sertoli related genes. Lentivirus mediated gene transfer was used to transduce a polycistronic lentivirus vector containing OSKM factors. The transfected cells were then further cultured onto inactivated mouse embryonic fibroblasts. The morphology, ALP staining, IF of POU5F1 and pluripotent gene expression were used to define the pluripotency of Sertoli-iPS cells. On day 14 after viral transduction, the iPS primary colonies were obtained from control group (0.7%) was higher than 2i-iPS (0.5%). Of 51 iPS colonies selected, a total of 19 iPS like colonies from control group were successfully passaged. They were positively stained with ALP, POU5F1 and also expressed pluripotent genes. This study demonstrated the possibility to generate the iPS cells from cat Sertoli cells.

T-1293

TARGETING THE BONE MORPHOGENIC PROTEIN PATHWAY IN CHRONIC MYELOID LEUKAEMIA

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Tyrosine kinase inhibitors (TKI) have resulted in significant improvements in survival of chronic myeloid leukaemia (CML) patients, however complete elimination of leukaemic stem cells (LSCs) is rare. LSC persistence remains a major obstacle to curing CML. Several reports indicate that the TGFbeta superfamily pathway is important for LSC survival and quiescence. We conducted extensive microarray analysis to compare expression patterns in normal haemopoietic stem cells (HSC) and progenitors with CML LSC and progenitor populations (chronic phase (CP), accelerated phase (AP) and blast crisis (BC)). The TGFbeta superfamily and downstream signalling molecules were significantly deregulated in CP, AP and BC in both LSC and primitive progenitor subpopulations. The changes observed could potentiate alter autocrine signalling, as BMP 2, 4 ($p<0.5$), and ACTIVIN A ($p=3.89E-09$) were all down-regulated, whereas BMP 7, 10 and TGFbeta ($p<0.5$) were up-regulated in CP. This was accompanied by up-regulation of BMPRI ($p<0.05$) and downstream SMADs ($p<0.005$). Interestingly, as CML progressed, the profile altered, with BC patients showing significant over-expression of ACTIVIN A and its receptors ACVR1C (ALK7). Epigenetic analysis of DNA methylation patterns indicated corresponding activation and repressive marks for several of the genes of interest. To further characterise the BMP pathway and identify potential candidate biomarkers within a larger cohort, gene expression of 42 genes in 60 newly diagnosed CP CML patient samples, enrolled on a phase III clinical trial (www.spirit-cml.org) with >12 months follow-up data on their response to TKI was performed. Analysis revealed that the pathway was highly deregulated, with no clear distinction when patients were stratified into good, intermediate and poor response to treatment. Therapeutic intervention using BMP receptor inhibitors in combination with TKI indicate that they act synergistically

with TKI to target CML cells both in the presence and absence of BMP ligand. Inhibition of the pathway resulted in decreased proliferation, significant irreversible cell cycle arrest, alteration in cell cycle gene expression profiles, increased apoptosis and reduction in haemopoietic colony formation. These observations offer a therapeutic window in CML.

T-1294

THE SCALABLE BIOREACTOR SERIES FOR PLURIPOTENT STEM CELL STIRRED SUSPENSION CULTURE

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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 at the last this conference. We tried expansion and scale up culture by using three types of single-use bioreactor. The ABLE Corporation published two types of bioreactor (working volume 30mL and 100mL) last year. We show the large scale bioreactor (working volume 500mL) at this conference. This bioreactor series including delta shape paddle impeller prevents a turbulent flow and agitates medium by laminar flow. The single cell suspension were prepared from 2D on-feeder or 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 culture period was adjusted by proliferation of cell. The obtained single cells were re-inoculated into bioreactor and stirred suspension culture was performed as scale up. The undifferentiated property of the expanded iPS cells was evaluated with the flow cytometric analysis. The three types of bioreactor using the delta shape paddle impeller enabled to create a lot of aggregates 200 to 300 micrometer diameter in the cultivation for 4 to 5 days. Furthermore the number of cells increased 5 to 10 times to the number of inoculated cells. The similar cell proliferation was observed in each bioreactor series. Collectively, we established the scalable expansion system from small to large scale for pluripotent stem cell.

T-1295

CONTROL OF ANTIOXIDATION AND GENERATION OF INDUCED PLURIPOTENT STEM CELLS BY JUN DIMERIZATION PROTEIN 2

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We report here that the Jun dimerization protein 2 (JDP2) plays a critical role as a cofactor for the transcription factors nuclear factor-erythroid 2-related factor 2 (Nrf2) and MafK in the regulation of the antioxidants and production of reactive oxygen species (ROS). JDP2 associates with Nrf2 and MafK (Nrf2-MafK) to increase the transcription of antioxidant response element-dependent genes. Oxidative-stress-inducing reagent led to an increase in the intracellular accumulation of ROS and cell proliferation in Jdp2 knock-out mouse embryonic fibroblasts. In Jdp2-Cre mice mated with reporter mice, the expression of JDP2 was restricted to granule cells in the brain cerebellum. The induced pluripotent stem cells (iPSC)-like cells were generated from DAOY medulloblastoma cell by introduction of JDP2, and the defined factor OCT4. iPSC-like cells expressed stem cell-like characteristics including alkaline phosphatase activity and some stem cell markers. However, such iPSC-like cells also proliferated rapidly, became neoplastic, and potentiated cell malignancy at a later stage in SCID mice. This study suggests that medulloblastoma cells can be reprogrammed successfully by JDP2 and OCT4 to become iPSC-like cells. These cells will be helpful for studying the generation of cancer stem cells and ROS homeostasis and anti-oxidation.

T-1316 *This poster board is located in a different topic area*

NEW TECHNOLOGIES FOR GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FROM PERIPHERAL BLOOD DERIVED MONONUCLEAR CELLS UNDER XENO-FREE AND DEFINED CONDITIONS

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Shah, Kevan, Fellner, Thomas
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Reprogramming of human somatic cells into human induced pluripotent stem cells (hiPSCs) offers a new source of cells with tremendous potentials for cell therapy, basic research, disease modeling, and drug development. In order for hiPSCs to fulfill their therapeutic potentials, it is necessary to develop cell culture technologies that allow generation of these cells under defined and xeno-free conditions. Moreover, widely accessible imaging technologies are needed to evaluate hiPSC behaviors in vitro. Current methods for generation of hiPSCs have some limitations including low efficiency, difficult reproducibility, or use of feeder or xeno components. This is particularly true for derivation of hiPSCs from peripheral blood mononuclear cells (PBMCs) as one of the most viable source of cells from tissue acquisition perspective. We have successfully developed new L7™ technologies that will allow high yield of PBMCs expansion; robust generation of hiPSCs from PBMCs using non-integrating episomal plasmids; and expansion of hiPSCs under entirely defined and xeno-free conditions. Our defined and xeno-free priming medium supported growth and survival of PBMCs before and after reprogramming. Using a novel reprogramming cocktail, we significantly improved the efficiency of hiPSCs generation under defined and xeno-free conditions. We have also developed a defined and xeno-free culture system that is comprised of medium, matrix, passaging solution, and cryopreservation solution that supported the generation of hiPSCs in a relatively short period of time (approximately 12-16 days); expansion and banking of hiPSCs while using an every-other-day feeding strategy. These hiPSCs showed no trace of exogenous DNA integration and maintained pluripotent stem cell characteristics including expression of pluripotency markers, normal karyotype, and differentiation into cells of all three germ layers. Furthermore, we

used live cell imaging with CytoSMART™ technology that provided detailed understanding of hiPSC colonies growth and conditions that can affect spontaneous differentiation, such as growth supplements depletion or metabolites buildup. These technologies can offer valuable solutions for generation of clinical grade hiPSCs from peripheral blood and studying cellular behavior in other applications.

IPS CELLS: DIRECTED DIFFERENTIATION

T-1296

SEX-LINKED DIFFERENCES IN CARDIOMYOCYTE DIFFERENTIATION REVEALED IN LARGE-SCALE ANALYSIS OF CHEMICALLY DEFINED MEDIA DIRECTED CARDIAC DIFFERENTIATION OF A COHORT OF HIPSC'S

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Cardiac tissue derived from human induced pluripotent stem cells (hIPSC's) hold many advantages over currently approved stem cell therapies motivating efforts to develop efficient methods of producing hIPSC-derived cardiomyocytes. Obstacles to producing clinical grade tissue include the need for defined conditions for the generation of cardiomyocytes from hIPSC's. The recently published chemically defined media (CDM) protocol addressed potential pathological contamination by determining which components of B27 are necessary for successful cardiomyocyte differentiation while also reducing the lot-to-lot variability found in B27. CDM media consists of recombinant human albumin and ascorbic acid, neither of which requires a non-human production source. We have produced a large repository of hIPSC's derived from patients afflicted with hypoplastic left heart syndrome (HLHS) and their immediate family members with the goal of producing clinical grade cardiomyocytes from these cells. We sought to compare the efficacy of the CDM media to B27 supplemented media in the differentiation of cardiomyocytes using a cohort of 62 hIPSC's originally reprogrammed from healthy and HLHS patient dermal fibroblasts. Cardiac fate was assessed by the presence or absence of beating, confirmed by flow cytometry of MF20 and cardiac troponin expression, and by ongoing RNA analysis. We also evaluated the effect of nitric oxide (NO) supplementation on cardiac differentiation in CDM media. NO levels were increased directly using the NO donor Spermine NONOate, indirectly by supplementing media with sildenafil, or through both methods. We found a significant difference in differentiation success between the two medias ($p=0.01$), but no difference based on NO treatment. Intriguingly, the difference between CDM and B27 supplemented media was sex-dependent. No difference was found in differentiation success between the two medias for male hIPS cell lines, but female cell lines were significantly less likely to beat in the CDM media. These results suggest that while CDM media is a chemically defined and more consistent alternative to B27, it is only a viable substitute for B27 when differentiating male hIPS cell lines.

T-1297

SIMPLE, EFFICIENT AND ROBUST METHOD FOR GENERATION OF HUMAN NEURAL PROGENITOR CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human neural progenitor cells (hNPCs) are promising sources for cell-based therapy such as treatment for spinal cord injury or stroke. To date, generation of hNPCs from human induced pluripotent stem cells (hiPSCs) has been reported by using small number of hiPSCs clones. However, now hiPSCs can be generated unlimitedly from every somatic cell source and they have broad clone variations in their differentiation propensity. Therefore, efficient and robust differentiation protocols are desired for generating hNPCs from various hiPSCs. In this study, we evaluated neural differentiation propensity of 21 hiPSCs lines, which were derived from human dermal fibroblasts (HDFs, 13 clones), cord blood cells (CB, 3 clones) and peripheral blood mononuclear cells (PBMN, 5 clones) by using total five neural induction methods; EB formation-based method (EBFM) and EB formation with double SMAD inhibitors (SB431542 and Dorsomolphin) method (dSMADi), which was further divided into four subconditions by combination with media (KSR-based or B27N2-based) and oxygen levels (5% or 20%). We found that dSMADi methods could improve neural induction efficiency regardless of clones differing from EBFM. We could generate expandable hNPCs from every hiPSCs derived from HDF, CB and PBMN. Interestingly their regional specificity was mid/hind brain type even initially forebrain. Moreover, their neural gene expression levels were very similar each other. We conclude that our dSMADi methods are efficient and robust for producing easily expandable hNPCs from every hiPSCs.

T-1298

IN VITRO DIFFERENTIATION OF HUMAN IPSC REPORTER LINES TO NEURONS FOR TRACTABLE IN VIVO ANALYSIS IN RODENTS

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The directed differentiation of human pluripotent stem cells (PSCs) to neuronal subtypes represents a valuable source for cell replacement therapies. Integral to the progression and realization of neuronal cell therapy is a detailed understanding of the in vivo properties of stem cell-derived neurons. Human embryonic stem cells (hESC) have proven their capacity to efficiently differentiate into neural progenitors in vitro, survive transplantation and functionally integrate into rodent brains. The generation of human induced PSCs (iPSCs) provides an avenue for the development of patient-specific cell therapies, overcoming ethical issues as well as immune-related hurdles associated with hESC-derived products. Here, we utilise human iPSC lines, which constitutively express fluorescent proteins, to efficiently generate neural progenitors in vitro for transplantation into rodent brains. The use of targeted reporter iPSC lines facilitates the identification of human PSC derivatives in rodent models, enabling anatomical and functional properties to be assessed.

T-1299

DEFINING REGIONAL IDENTITIES OF NEURAL STEM CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS AS A NOVEL APPROACH FOR MODELING NEUROLOGICAL DISEASES IN VITRO

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Disease-specific iPSCs are a powerful tool to study neurological diseases by reproducing their pathologies in vitro. It has been known that each neurological disease has specific lesion areas and iPSCs should be differentiated into specific neural cells in such studies. However, each protocol of neural induction into desired neural cells from iPSCs varies in its efficiency and cultivation process. Therefore, it is difficult to evaluate the phenotypes of neurological diseases with combined phenotypes (e.g. SCD, MSA, ALS+PD) quantitatively. We here aim to establish a method to generate all neuronal subtypes from human iPSCs based on the similar neurosphere method. We first tried to induce neural progenitors with specific regional identities. By the combinatory treatment of various patterning factors during neurosphere formation, we have succeeded in making neurospheres with the specific antero-posterior identities, ranging from the telencephalon to the spinal cord. Furthermore, the dorso-ventral identities of neural progenitors can be controlled at any given antero-posterior position. Using this system, we successfully induced specific neuronal subtypes, including cortical projection neurons, cortical interneurons, cerebellar Purkinje neurons, midbrain dopaminergic neurons, hindbrain serotonergic neurons, spinal cord sensory interneurons, and spinal cord motor neurons. We next confirmed the utility of our system by modeling typical neurological diseases, including ALS. We demonstrated that spinal cord motor neurons were specifically damaged among those neuronal subtypes, consistent with the pathology of ALS. In addition to typical diseases, our study will elucidate the pathogenesis of combined neurological diseases.

T-1300

DIRECTED CARDIAC DIFFERENTIATION OF CYNOMOLGUS MONKEY INDUCED PLURIPOTENT STEM CELLS FOR TISSUE ENGINEERING

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Induced pluripotent stem cell (iPSC)-derivatives possess great therapeutic potential for regenerative medicine. Following myocardial infarction, a promising treatment option to prevent heart failure is the replacement of fibrous connective tissue by *in- vitro* generated iPSC-derived cardiomyocytes and engineered tissue thereof. Indeed, the efficiency of such an approach still needs to be determined. Transplantation of cardiomyocytes or bioartificial cardiac tissue (BCT) into ischemic cynomolgus monkeys represents a promising strategy to confirm the therapeutic potential prior to translation to humans, as non-human primates closely match with humans in anatomy, physiology and complexity of the immune system. We previously reported the generation of cynomolgus iPSC (cyiPSC) and their general potential to form cardiomyocytes applying a spontaneous, serum-based differentiation protocol. However, as clinical applicable grafts require defined expansion and differentiation, we report here the successful establishment of a feeder-free cultivation process maintaining 90% Oct4^{+} cells in fully-defined medium. The cells were subsequently differentiated in a 2D system applying small molecule Wnt-signaling modulators resulting in beating areas around day 10. Since replacement therapies will require large quantities of de novo formed cardiomyocytes, we adapted a 3D protocol with optimized compound concentration and timing. We show efficient cardiac differentiation by emerging beating aggregates, immunofluorescence analysis for sarcomeric proteins and quantification by flow cytometry with cardiomyocyte yields of up to 51%. Furthermore, casting of BCT by mixing beating aggregates with extracellular matrix was feasible. Overall, we show that a fully-defined, feeder-free system can be used to cultivate, expand and differentiate cyiPSC towards cardiomyocytes. Noteworthy, we found that cyiPSC culture and differentiation requires specific adjustments compared to their human counterparts to enable robust large-scale production needed for transplantation purposes.

T-1301

GENERATION AND CHARACTERIZATION OF A HOMOGENEOUS POPULATION OF EARLY MESODERM CELLS USING STEMDIFF™ MESODERM INDUCTION MEDIUM

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Generating highly pure populations of mesodermal cell types from human pluripotent stem cells (hPSCs) has proven challenging. Successful in vitro protocols have generally required a stepwise approach that mimics development; however, this requires careful optimization of conditions for each step in order to reproduce each developmental stage. To support the first stage of differentiation into

early mesoderm, we have developed a defined, xeno-free medium that generates a homogeneous population of early mesoderm cells from hPSCs using a fast and simple protocol. Briefly, hPSCs were seeded as single cells at 10^5 cells/cm^2 in either mTeSR™1 or TeSR™-E8™ with Y-27632 and the next day the medium was replaced with STEMdiff™ Mesoderm Induction Medium (MIM) to induce differentiation. MIM was changed daily until cells were harvested and analysed by flow cytometry. The early mesoderm marker Brachyury (T) and the undifferentiated cell marker OCT4 were monitored throughout differentiation, and undifferentiated cell, early and late mesoderm markers were analysed at day 4. Results were consistent for 2 human embryonic stem cell lines (H1, H9) and 4 human induced pluripotent stem cell lines (WLS-4D1, WLS-1C, STiPS-M001, STiPS-F016) for all markers tested. Cells expanded rapidly during induction and T was highly expressed on days 1 - 4, while OCT4 was down-regulated at day 2 and largely absent by day 3 or 4. At day 4, cells were 92 ± 5% (mean ± SD; n=33) T^+ and 1 ± 2% $OCT4^+$ when differentiated from hPSCs maintained in mTeSR™1, and 96 ± 2% T^+ and 2 ± 1% $OCT4^+$ (n=6) from hPSCs maintained in TeSR™-E8™. The undifferentiated cell marker TRA-1-60 was down-regulated and cells switched cell adhesion marker expression from EpCAM to NCAM, an indication they had undergone epithelial-mesenchymal transition. Cells also lacked expression of markers of lateral plate mesoderm (KDR) and paraxial mesoderm (PDGFRα), as well as definitive endoderm (SOX17). Finally, we differentiated early mesoderm cells generated using MIM into endothelial cells (lateral plate mesoderm) and mesenchymal-like cells (paraxial mesoderm). In summary, STEMdiff™ Mesoderm Induction Medium can be used to direct hPSCs to early mesoderm with high efficiency, and these early mesoderm cells can be used as a basis from which to proceed to downstream mesodermal differentiation.

T-1302

ROBUST AND EFFECTIVE HUMAN IPSCS DIFFERENTIATION INTO HEPATOCYTE-LIKE CELLS

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Principal drug screening and cell toxicity approaches include application of hepatocytes. However, large-scale derivation of fully functional primary human hepatocytes in vitro is challenging due to their expansion limitation and highly invasive extraction technique. One of the alternative methods is the generation of hepatocytes from induced pluripotent stem cells (iPSCs), which could provide unlimited source of hepatocyte-like cells. During our study we developed a robust feeder and serum-free protocol of human iPSCs differentiation into hepatocyte-like cells. We took an advantage of well described in vivo hepatic development and previously developed protocols based on Wnt and TGFβ pathways activation by defined molecules. In our improved protocol we significantly reduced amounts of growth factors in cultivation media. These improvements resulted in the increased yield of differentiated derivatives, over three millions of hepatocyte-like cells could be harvested from 35mm dish of human iPSCs. During this robust differentiation procedure the cells progress through four stages which in total takes 30 days. Differentiated cells were characterized by various techniques comprising quantitative PCR, immunocytochemistry, albumin level measurement, and LDL uptake. Though hepatocyte-like cells still retain several fetal liver-specific features such as AFP and CYP3A7 expression, cells also demonstrated high levels of such adult hepatocytes markers as ALB and A1AT. Functionality of the cells was confirmed by albumin secretion as well as LDL uptake. Moreover, we established effective protocols of cryopreservation and passaging of the cells undergoing designated differentiation protocol at consecutive stages. These techniques will permit to reduce duration of time-consuming differentiation process and therefore could be beneficial for future clinical applications and drug safety screening.

T-1303

A STANDARDISED, HIGH-THROUGHPUT SINGLE CELL DIFFERENTIATION PROTOCOL FOR HUMAN INDUCED PLURIPOTENT STEM CELLS (IPSCS)

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A pipeline for deriving human iPSCs requires a robust assay for checking the pluripotency of the cells. Our current method of differentiating derived human iPS cell lines by seeding colonies onto a 24wp, directing them to differentiate with defined media and then staining the fixed plates for lineage markers gives variable results. This variability is due, in part, to person-person variability in the plating of colonies as well as issues with lack of growth factor and antibody penetration of the centre of large iPSC colonies. We have therefore developed a method which reduces this variability by dissociating the iPSCs into single cells before seeding for differentiation so that seeding density is defined. In this method we harvest the iPSCs, dissociate with accutase to obtain a single cell suspension, count and seed a defined number of cells per well of a 96wp. The iPSCs are directed to differentiate using defined media containing different concentrations of specific growth factors for each lineage- endoderm (endo), mesoderm (meso), neuroectoderm (neuro) and maintained as stem cells for a pluripotency test. These plates are fixed after a defined period and stained for well-known stem cell (oct4, sox2) and lineage markers (brachyury- meso, sox17-endo, sox1-neuro). These are read on a high-throughput imaging platform (cellomics) and results expressed as percent positive (for lineage markers) of total DAPI stained cells. The cells differentiated into all three germ layers as expected and show improved marker expression when compared to plating of colonies onto a 24wp. Preliminary data shows that for pluripotency plates stem cell marker expression is higher for the 96wp single cell differentiation when compared to 24wp colony differentiation. Similarly, for endo, neuro and meso, lineage marker expression is consistently higher and oct4 (stem cell marker) expression is consistently lower for the new 96wp single cell differentiation method when compared to 24wp colony differentiation. Moving to this 96wp format results in more efficient differentiation, reduced variability and higher throughput.

T-1304

THE ROLE OF LSD1 FOR EFFICIENT DIFFERENTIATION TO INSULIN-PRODUCING CELLS FROM HUMAN IPSCS

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Human pluripotent stem cells(hiPSCs) represent a potentially unlimited source of functional pancreatic endocrine lineage cells. However, problems remain, such as low trans-differentiation efficiency and poor maturity of trans-differentiated cells. The histone demethylase(LSD1) plays an important role in the regulation of hiPSC self-renewal and differentiation. We used two different LSD1 inhibitors (tranylcypromine and phenelzine sulfate) and RNAi technique to inhibit LSD1 activity, and we obtained hiPSCs showing 71.3%, 53.28%, and 31.33% of the LSD1 activity in normal hiPSCs. The cells still maintained satisfactory self-renewal capacity when LSD1 activity was at 71.3%. The growth rate of hiPSCs decreased and cells differentiated when LSD1 activity was at approximately 53.28%. The hiPSCs were mainly arrested in the G0/G1 phase and simultaneously differentiated into endodermal tissue when LSD1 activity was at 31.33%. We were surprised to find that 50% of the LSD1 activity was a balance point of the proliferation turn to differentiation in hiPSCs. Get 31.3% of the LSD1 activity with RNAi technology were induced to differentiate into insulin producing cells(IPCs) in four steps, the definitive endoderm cells and beta cell maturation markers were significantly increased. The differentiated human iPSCs obtained by this approach comprised nearly 39.3% insulin-positive cells as assayed by FCM, which released insulin/C-peptide in response to glucose stimuli in a manner comparable to that of adult human islets. Most of these insulin-producing cells co-expressed mature β cell-specifc markers such as NKX6-1 and PDX1, indicating a similar gene expression pattern to adult islet β cells in vivo. These IPCs derived from shRNA-hiPSCs were transplanted under the left kidney capsule of SCID-Beige diabetic mice. Interestingly, These IPCs derived from shRNA-hiPSCs can reduce blood glucose level in recipient mouse. LSD1 could regulate the histone modify of methylation and acetylation in promoter region of pluripotent or endodermal gene in hiPSCs to increase the differentiation efficiency of IPCs.

T-1305

DERIVATION AND DISEASE MODELING OF HUMAN PLURIPOTENT STEM CELL-DERIVED RETINAL GANGLION CELLS

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Derived from patient samples, human induced pluripotent stem cells (hiPSCs) have the potential to differentiate into any cell type of the body, providing a unique tool for cell replacement, disease modeling, and drug screening. To serve in this capacity, however, hiPSCs must be directed to properly differentiate to the cell type of interest. We have previously demonstrated the ability to differentiate hiPSCs to a retinal lineage, whereupon retinal photoreceptor and retinal pigment epithelium cells were the most abundant cell types produced. The ability to derive retinal ganglion cells (RGCs) from hiPSCs would not only serve as a novel model of human retinogenesis, but would also have profound implications for diseases such as glaucoma or other optic neuropathies. In the current study, we characterize the ability of hiPSCs to generate RGC phenotypes, including those cells expressing the RGC-specific transcription factors Brn3 and Math5. Furthermore, treatment of these cells with extrinsic factors known to influence the development of specific retinal cell types was demonstrated to affect the specification of RGCs from a more primitive retinal progenitor cell fate. More recent efforts have focused upon the establishment of lines of hiPSCs derived from patients with glaucoma, with the intent to develop an in vitro system with which to study inherent subcellular changes in glaucomatous neurodegeneration. The results of these studies allow for future use of these hiPSC-derived RGCs for studies of retinal development as well as neurodegenerative processes associated with optic neuropathies such as glaucoma.

T-1306

GENERATION OF MICROGLIA-LIKE CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Microglia are mononuclear phagocytes, the resident immune cell of the nervous system, derived very early in embryonic development. They are self-renewing, and do not appear to be replenished by circulating monocytes in the healthy brain. We have taken advantage of recent knowledge in the developmental path of microglia as compared to other macrophages and monocytes. Microglia arise from the Yolk Sac, and bypass AGM/Fetal Liver and Bone Marrow hematopoiesis. These very primitive macrophages take up residence in the neural tube before blood-brain barrier closure, and reside with little turnover in this privileged environment for the life of the organism. It stands to reason that direct differentiation from pluripotent stem cells, concurrent with exposure to appropriate growth factors, could recapitulate their development. Microglia encounter a different ligand of the CSF1-Receptor compared to other macrophages, Interleukin-34 (IL34). Pluripotent stem cells differentiated in a permissive neural medium, in the presence of high levels of IL34 and low levels of CSF1, rapidly produce microglia-like cells, bypassing other progenitor stages such as monocytes, or canonical hematopoiesis. These cells are long-lived, capable of extensive ramification, motile and phagocytic, and can be activated by various stimuli. Cells generated in our culture system are advantageously comparable to acutely isolated fetal microglia, unlike macrophages or transformed microglia cell lines kept in culture and often used as models. Acutely isolated adult microglia exhibit an environment-specific signature, likely due to their residence within the nervous system. This signature may have reducible components, such as exposure to the neural-enriched CX3CL1 ligand, TGFb or IL34 signaling. Likely, long-term interactions with different cell types are necessary. We investigate the effects of these factors, and the presence of conditioned media from neuro-glial cultures, on functional and molecular profiles of our cells. We also acutely re-isolate microglia-like cells grown for various periods of time with neurons and glia in 3D organotypic cultures, and assess whether in vitro residence under such conditions can further mimic the in vivo signature.

T-1307

SCALABLE DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN SUSPENSION CULTURE INTO HEPATOCYTE-LIKE CELLS THROUGH DIRECT WNT PATHWAY INHIBITION

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Hepatic diseases such cirrhosis, hepatitis and acute liver failure are the main causes of death due to the malfunction of the liver. Differentiation of pluripotent stem cells into hepatic-like cells represents a promising alternative for the treatment of hepatic diseases. Human induced pluripotent stem cell (hiPSC)-derived hepatocytes in cell transplantation is limited by scalability and poor functionality of the differentiated cells. We present a novel suspension-based embryoid body differentiation protocol for the scalable generation of hiPSC-derived hepatocyte-like cells that use two novel factors that inhibit the Wnt pathway. The inhibition of the Wnt pathway represents an important step in the in vivo hepatic differentiation. The embryoid bodies (EBSs) were derived using a new ROCKi-free/Spin-free technique previously developed in our lab that allowed us to produce scalable and uniform EBs in large quantity. The protocol developed was established based on the developmental process during liver organogenesis. The cells were tested for in vivo function and efficacy in reversing acute liver failure in a D-galactosamine-induced injury rat model. Through the inhibition of Wnt pathway we were able to generate fully functional hepatocyte-like cells in a scalable way. The differentiated cells showed typical functional activities of mature primary hepatocytes, suas LDL storage and uptake, IGC uptake and release, glycogen storage, and secretion in vitro of hepatic proteins such as Albumin, Alpha Fetoprotein, Urea and Fibrinogen. The differentiated cells displayed metabolism of Ammonium and the CYP450 activity. Cell transplantation in a rat model of acute liver failure showed improved survival and in vivo human albumin production. Our results suggest that suspension culture hiPSC embryoid bodies can be differentiated into hepatocyte-like cells in aggregates that can be used for in vitro studies as well as for pharmaceutical testing and cell therapy.

T-1308

MESENCHYMAL STEM CELLS DERIVED FROM IPS CELLS FROM AGED INDIVIDUALS ACQUIRE FETAL CHARACTERISTICS

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The in vitro expansion and application potential of human bone marrow derived mesenchymal stem cells (hBM-MSCs) derived from aged donors are limited by their short life span in culture and restricted differentiation potential. Recent studies have reported the derivation of hMSCs (iMSCs) from induced pluripotent stem cells (iPSCs) as a possible solution. However, little is known whether the age-associated phenotype is reverted into a younger state when iPSCs derived from hBM-MSCs of aged donors are re-differentiated into iMSCs. To obtain new insights into the potential roles of age in deriving iMSCs from iPSCs we induced pluripotency in hBM-MSCs from fetal femur (55 days post conception) and aged donors (60-70 years) and subsequently differentiated them into iMSCs. Higher levels of ROS, phosphorylated γH2AX and slower proliferation rates were detected in hBM-MSCs from aged individuals. Normal Karyotypes were detected in BM-MSCs of both age groups. Fetal hBM-MSCs could be reprogrammed more efficiently and faster compared to hBM-MSCs from aged donors using either retroviral or episomal based reprogramming. hBM-MSCs and their corresponding iMSCs both fetal and aged expressed a typical MSC surface marker pattern and multipotency. iMSCs from aged parental hBM-MSCs acquired morphologies, senescent phenotypes and transcriptomes similar to that of fetal hBM-MSCs and iMSCs. Additionally, hBM-MSCs and their corresponding (iMSCs) both fetal and aged shared similar (PDGF-AA, MCP-1, MIF, Serpin E1) secretome profiles. Furthermore iMSCs derived from fetal MSC-iPSCs show a similar immunophenotype as well as trilineage differentiation potential as the parental cells. These similarities were also observed in iMSCs derived from dermal fibroblasts and the ES line H1. In summary we have demonstrated that (a) the efficiency of inducing pluripotency in hBM-MSCs is dependent on donor age. (b) The transcriptomes of iPS cells derived from both fetal and aged BM-MSCs are more similar to that of hESCs than the parental cells. (c) iMSCs irrespective of donor age re-acquire features typical of BM-MSCs. In conclusion, derivation of iMSCs by-passes the shortfalls associated with the expansion of native MSCs and these cells have tremendous potential in regenerative medicine.

T-1309

LINEAGE COMMITMENT OF CAR-T CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

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The ability to derive T cells of defined specificity and function from pluripotent stem cells may be useful for the treatment of a range of pathologies, including cancer, infection and immune deficits. We recently reported that genetic engineering of T-cell derived iPSC (TiPSC) with Chimeric Antigen Receptors (CARs) is an efficient strategy to concomitantly harness the unlimited availability of iPSC and program the specificity and functional potential of iPSC-derived T cells. We found that human TiPS-derived CAR T cells (CARTiPSC-T) generated in culture display therapeutic potency in vivo in a lymphoma model. Surprisingly, the CARTiPSC-T cells possessed innate-like phenotype and function, most similar to γδ T cells although they expressed their endogenous αβ TCR on the surface. While innate T cells have anti-tumor activity, they lack some landmark features for therapeutic efficacy such as long-term memory and in vivo persistence, which characterize mature CD8+ and CD4+ TCRαβ T cells. Further investigation of the mechanisms underlying in

vitro T lymphoid differentiation of TiPSC is needed to better direct T cell subset differentiation and further shape the functional attributes of induced T cells. Our current efforts focus on directing the generation of cancer-targeted αβ-like T cells. As T cell lineage determination depends in part on the balance between the Notch and TCR signaling, we are investigating their respective role, as well as that of the CAR, in determining lineage commitment. We hypothesize that the pre-rearranged TCRαβ elicits a γδ-like cell fate and have therefore eliminated TCRαβ expression by targeted disruption of the TCRα constant region locus (TRAC) using the CRISPR/Cas9 system. TiPSC are a valuable system for the study of human T cell differentiation. They are further amenable to genetic engineering with TCRs or CARs, which may be useful for the generation of therapeutic "off-the-shelf", antigen-specific T lymphocytes.

T-1310

AN OPTIMAL MEDIUM SUPPLEMENTATION REGIMEN FOR INITIATION OF HEPATOCYTE DIFFERENTIATION IN HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human induced pluripotent stem (hiPS) cells are an ideal cell source for hepatocytes transplantable for patients with acute liver failure. Glucose and arginine are necessary for cells to survive. Hepatocytes have galactokinase (GALK), which metabolizes galactose for gluconeogenesis, and ornithine transcarbamylase (OTC), which converts ornithine to arginine in the urea cycle. Hepatocyte selection medium (HSM) lacks both glucose and arginine, but contains galactose and ornithine. Although human primary hepatocytes survive in HSM, all the hiPS cells die in three days. The aim of this study was to modify HSM so as to initiate hepatocyte differentiation in hiPS cells Hepatocyte differentiation initiating medium (HDI) was prepared by adding oncostatin M (10 ng/ml), hepatocyte functional proliferation inducer (10 nM), 2,2'-methylenebis (1,3-cyclohexanedione) (M50054) (100 µg/ml), 1× non-essential amino acid, 1× sodium pyruvate, nicotinamide (1.2 mg/ml), L-proline (30 ng/ml), and L-glutamine (0.3 mg/ml) to HSM. HiPS cells (201B7 cells) were cultured in HDI for 2 days. RNA was isolated, used as template for cDNA, and subjected to real-time quantitative polymerase chain reaction. Alpha-fetoprotein was upregulated. Expression of albumin was not observed. The expression of GALK2, OTC, and CYP3A4 were increased. ALDH showed weak expression. In conclusion, differentiation of 201B7 cells to hepatoblast-like cells was initiated in HDI.

T-1311

A NOVEL FEEDER-FREE CULTURE SYSTEM TO DERIVE HUMAN RETINAL PIGMENT-EPITHELIUM FROM PLURIPOTENT STEM CELLS

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The retinal pigmented epithelium (RPE) is a monolayer of pigmented cell located between the neural retina and the blood supplier, choroid. Its main contributions to the visual process are the synthesis and the recycling of the chromophore required for phototransduction, the phagocytosis of shed photoreceptor outer segments, the regulation of fluid and nutrient flow between the retina and the choroid. The neural retina activity relies on RPE functions and its deficiency give rise to several diseases, of which most of them result in visual impairments or blindness. The ability to generate hRPE for disease modelling, drug screening or transplantation would be particularly worth to answer these important challenges. Here we present an easy, reliable, xeno- and serum-free method to quickly generate hRPE from iPSCs in culture. Starting from feeder-free culture conditions we established a simple three-step protocol able to induce the typical RPE cobblestone appearance, and pigmented foci as early as 18 days after differentiation. After a first step consisting on the formation of embryonic body-like aggregates, the neuroephitelium induction follows and then the third phase commit the neural cells to RPE fate. The cells are characterized by their pigmentation, the expression of mRNAs of typical RPE markers associated with the retinoid cycle (RALBP and RPE65), chloride channels (BEST1), phagocytosis (MERTK) and specific coexpression of transcription factors (PAX6 and MITF). The presence of proteins involved in the tight junction formation (ZO-1, Occludine) were revealed by immunocytochemistry. To obtain pure populations of RPE, pigmented foci were manually transferred at day 23-25 on matrigel and cultured until confluence. In conclusion, the presented protocol provides a quick and consistent method to generate hRPE from pluripotent stem cells which will be utilised to generate RPE-like tissues from hiPSCs of affected patients with the aim to perform in-depth study of diseases mechanisms and test new treatments.

T-1312

CRYOPRESERVED DOPAMINE NEURONS DERIVED FROM HUMAN IPSC MAINTAIN MIDBRAIN LINEAGE AFTER TRANSPLANTATION IN ANIMAL MODELS OF PARKINSON'S DISEASE

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Efficient and reliable derivation of functional midbrain lineage dopamine neurons from embryonic and induced pluripotent stem cells (iPSC-mDA) was a major breakthrough for cell therapy in Parkinson's disease. However, a major technical hurdle towards clinical translation remains in generating a deliverable cell product that is easily prepared for surgical transplantation without manipulation. Therefore, cryopreservation of post-mitotic iPSC-mDA neurons that demonstrate both safety and efficacy in pre-clinical models of Parkinson's disease represents a major vertical advancement for clinical translation. We demonstrate here, efficient, large-scale generation of cryopreserved, lineage restricted iPSC-mDA neurons with characteristic gene and protein expression, electrophysiological firing patterns, and neurotransmitter metabolism in vitro. To test in vivo therapeutic potential, cryopreserved iPSC-mDA neurons were thawed and rinsed in medium, then immediately injected without manipulation or sub-culturing into the striatum of both rodent and non-human primate models of Parkinson's disease. Transplanted iPSC-mDA neurons survived and maintained

expression of post-mitotic dopaminergic lineage neuron markers with extensive fiber outgrowth and striatal innervation at short-term time-points tested thus far (up to 3-months). Furthermore, proliferation in grafted iPSC-mDA neurons was absent indicating initial safety in these IND-enabling studies. Long-term safety and efficacy studies are underway towards clinical translation of cryopreserved iPSC-mDA neuron therapy for Parkinson's disease patients.

T-1313

MRNA REPROGRAMMING FOR THE GENERATION AND DIFFERENTIATION OF HUMAN IPSCS

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One of the major bottlenecks of widely applying iPSC technologies to large scale production or cell-based assays is the lengthy process typically required to create cells of desired identity from the original source, such as patient's cells. We demonstrate here an optimized platform of using synthetic mRNA to reprogram human fibroblasts to iPSCs, whereby we achieve nearly synchronized reprogramming in 6 to 7 days without passaging or the need for feeder cells. These iPSCs are not exposed to virus or foreign DNA, and are therefore genuinely footprint-free. As a focus of our research, we use delivery of mRNAs encoding specific transcription factors to streamline differentiation protocols in several lineages, including neuronal cells, cardiomyocytes, mesenchymal stem cells (MSCs) and adipocytes. Cells are either expanded at the iPSC stage or as progenitors such as MSCs. The large progenitor populations are then allowed to proceed to more specific differentiation, such as adipocytes. We show that while it takes about 28 days for MSCs grown in alternating adipocyte differentiation medium and maintenance medium to essentially all become oil-drop-containing adipocytes, the addition of mRNA encoding PPARg, a master regulator of adipogenesis, to the same differentiation protocol reduces the time for MSCs to become mature fat cells to only 14 days. Thus we demonstrate a key attribute of mRNA-mediated master gene control of cell fate. We are now developing brown fat cells with mRNA. We also show that when we apply a similar strategy to neural differentiation, iPSCs are converted to neurons or glial cells within 2-3 days after transfection with just one neural differentiation factor. Underlining such dramatically improved efficiency is the nature of mRNA transfection getting into the cytoplasm to be quickly translated in almost all cells, avoiding rate-limiting nuclear transport or genomic integration. We have further transfected iPSCs or neural progenitor cells (NPCs) with dopamine (DA) neuron-specific transcription regulators in various combinations in order to efficiently obtain high purity DA neurons. The updated results will be presented.

T-1314

IPSC-DERIVED THYMIC EPITHELIAL PROGENITORS DIFFERENTIATION FOR DEVELOPMENT OF MICE WITH PERSONALIZED IMMUNE SYSTEMS

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Functional T lymphocytes play an important role in immune defense and in defining tolerance. The development of mature, tolerant and functional T cells depends not only on the migration of their progenitors to the thymus, but also on their interactions with the thymic microenvironment where thymic epithelial cells (TEC) "educate" the developing T cells through negative and positive selection. In personalized medical research, there is a growing need for humanized mouse models that would permit the analysis of an individual's immune response to particular human diseases. We propose to generate mice with a patient-specific, functional immune system. TEC progenitors (TEP), differentiated from patient-derived induced pluripotent stem cells (iPSC) in vitro, will be transplanted into NOD/SCID/γ immunodeficient mice along with human CD34+ hematopoietic stem cells. As the iPSC-derived TECs mature, functional, patient-specific T cells will be generated. By mimicking cell signals during embryonic thymus development, we sequentially activated TGFβ, WNT, retinoic acid and BMP signaling pathways to successfully differentiate pluripotent stem cells lines (human embryonic stem cells CA1 and human iPSC 110) into definitive endoderm, 3rd pharyngeal endoderm and then TEPs. At each particular differentiation stage, the cells express unique surface markers. TEPs were characterized by their expression of the key marker FOXN1. Next, TEPs will be co-transplanted with CD34+ hematopoietic stem cells reconstitution, which will contribute to the development of the mice with a personalized immune system containing fully functional, human thymus-educated T cells. These engineered mice, as real "avatars", will accelerate the understanding of the interplay between an individual's unique immune system and a particular disease.

T-1315

MESENCHYMAL STEM CELLS CONTRIBUTE TO HEPATIC MATURATION OF INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem (iPS) cells are human somatic cells that have been reprogrammed to a pluripotent state. To date, several different methods have been reported to assess hepatocyte-like cells from iPS cells. However, those hepatic cells are not yet applicable for clinical use due to their immature function compared to primary hepatocytes. Previously we have reported that Mesenchymal stem cells (MSCs) could inhibit apoptosis of hepatic cells and improve hepatic regeneration in case of acute liver injury both in vitro and vivo. Therefore, we expected that MSCs have a potential of positive contribution to maturation phase of hepatic cells. Here we demonstrate the effect of MSCs on the maturation of hepatic progenitor cells derived from human iPS cells. MSCs were isolated from human bone marrow and cultured to 70-80% confluence. MSCs conditioned medium (MSC-CM) was collected 48 hours after cultured in hepatic maturation medium. Human iPS-derived hepatic progenitor cells were then cultured for 6 days with MSC-CM. Cell viability and urea synthesis were analyzed. The expression levels of pluripotent, endoderm, and hepatocyte specific genes after 6 days of culture were evaluated compared to the cells cultured in general maturation medium. The number of viable cell was higher in MSC-

CM by MTT assay and morphological evaluation. The expression of NANOG was decreased in the cells cultured by MSC-CM, while the expressions of endoderm marker, such as FOXA2 and CXCR4, early hepatic marker, such as AFP, and hepatocyte specific marker, such as FGG, TAT, HNF1A, ALB were increased. Interestingly, urea synthesis of the cells was enhanced by MSC-CM, which was revealed as the upregulation of ornithine transcarbamylase expression, which regulates urea cycle. The enhanced expressions of Cytochrome P450 including CYP7A1, 1A2, 2A6, 2E1 were also observed in the cells cultured by MSC-CM. Secreted molecules from MSCs could promote proliferation of the iPS-derived hepatocytes and enhance their hepatic function. MSC-CM may be used as a novel efficient strategy for hepatic maturation.

T-1316 *see abstract in iPS CELLS after T-1295*

IPS CELLS: DISEASE MODELING

T-1317

PATIENT SPECIFIC INDUCED PLURIPOTENT STEM CELLS TO STUDY MEVALONATE KINASE DEFICIENCY

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Induced pluripotent stem cells (iPSC) offer the possibility to study disease in the genetic context of the patient. Moreover, iPSC provide an unlimited source of patient specific cells which are sometimes only limited in their availability from a (pediatric) patient. We have generated iPSC from mevalonate kinase deficiency (MKD) patientsMevalonate kinase deficiency is an auto-inflammatory disease caused by a mutation in the cholesterol synthesis cascade. Since monocytes and macrophages are involved in secretion of inflammatory cytokines, we differentiated the patient-induced pluripotent stem cells to patient specific monocytes and macrophages as characterized by morphology, the expression of CD45, CD14, CD11b, CD16, CD163 and the ability to differentiate into functional macrophages.Healthy PBMCs produce elevated levels of the pro-inflammatory cytokine Il1beta after stimulation with LPS and simvastatin, which mimics MKD. The iPSC derived monocytes (iMONO) mimic the PBMCs as they also show elevated levels of IL1beta after simvastatin and LPS stimulation. Moreover, the MKD-iMONO show elevated levels of secreted Il1beta after stimulation with LPS in the absence of simvastatin thereby mimicking MKD patient derived PBMCs. Initial characterization showed that the induction of secreted Il1beta in MKD-iMono can be rescued by addition of geranyl-geranyl pyrophosphate (GGPP), an intermediate in the cholesterol synthesis cascade, to the culture medium. The MKD-iMONO are currently further biochemically characterized. To repair the disease causing mutation we designed a CRISPR-Cas9 targeting construct and designed an oligo for homologous recombination mediated repair. Initial experiments suggest a double stranded break induction in both alleles of approximately 20%. This method is currently being optimized. The repaired, non-repaired and control iPSC derived monocytes will be compared for their functionality. Taken together, derivation of patient specific iPSC, the differentiation into monocytes/macrophages to study their functionality and the correction of the disease causing mutation will contribute to better understanding of the disease and will serve as models for further investigations and allow the finding of new therapeutics.

T-1318

SUCCESSFUL PROTEOLIPOSOME-BASED THERAPY FOR CHRONIC GRANULOMATOUS DISEASE USING HUMAN MACROPHAGES DIFFERENTIATED FROM PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

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Chronic Granulomatous Disease (CGD) is an inherited orphan disorder caused by mutations in genes encoding the NADPH-oxidase subunits leading to the absence of reactive oxygen species (ROS) production by phagocytes. The most frequent form is the X-linked CGD (X-CGD) due to mutations in CYBB leading to the absence of cytochrome b558 (cytb), the membrane redox element of the enzyme. CGD patients suffering from recurrent and life-threatening infections are classically protected all their life by antibacterial and antifungal prophylaxis but this treatment is not devoid of side effects, e.g. renal failure. Our objective was to generate macrophages from X-CGD patient specific iPSCs in order to possess a relevant cellular model to develop novel and safe therapeutics as protein therapy. X-CGD iPSCs reprogrammed from CGD patient's fibroblasts and fully characterized for their pluripotency, self-renewal and genetic integrity, were differentiated into CD34+ progenitors. Then macrophages were produced at will from these progenitors by differentiation. They were phenotypically and functionally identical to their human counterparts. In addition they reproduced the genetic and functional defect of CGD phagocytes: the absence of both subunits of cytb i.e. NOX2 and p22phox related to the absence of ROS production. Using an optimized E. coli cell-free expression system, NOX2 and p22phox were synthetized and integrated into specific lipids to form proteoliposomes (PLs) able to fuse with plasma membranes. The presence of NOX2 and p22phox in PLs was verified by Western blot and hemes integration was confirmed by the differential spectrum of cytb. Then we demonstrated that PL containing NOX2/p22phox were able to generate ROS in a cell-free-system assay activated by arachidonic acid in presence of NADPH and recombinant proteins p47phox, p67phox and Rac. As shown by confocal microscopy and flow cytometry, these PLs were able to (i) "vectorialize" NOX2/p22phox directly to the membrane of X-CGD iPSC-derived macrophages and (ii) restore the NADPH oxidase activity as demonstrated by the reduction of Nitro-Blue-Tetrazolium. In conclusion we demonstrated the proof-of-principle of the proteoliposome-based therapy for CGD which is a new promising technology for the delivery of functional proteins to the membrane of targeted cells.

T-1319

MOLECULAR AND CELLULAR ANALYSIS DURING IN VITRO GABAERGIC DIFFERENTIATION OF IPSC GENERATED FROM HUNTINTON'S DISEASE PATIENT

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Huntington's disease (HD) is a neurodegenerative disorder caused by an expansion of CAG tri-nucleotide repeats that results in neuronal dysfunction and death. Neuronal death occurs in many brain regions, while degeneration of DARPP-32+ GABAergic neurons in the striatum underlies motor dysfunction in HD. It was recently reported that the alterations of epigenetic enzymes lead to transcriptional deregulation of neuronal genes, which was linked to HD pathogenesis. Here, we report the molecular and cellular properties of GABAergic neurons derived from HD iPSCs during in vitro differentiation of HD iPSC into GABAergic neurons. We used RT-PCR and immunocytochemistry assay to analyze the expression patterns of GABAergic markers. We found different expression levels of various GABAergic markers, such as GABA, GAD65/67, and DARPP-32, between normal and disease hiPSCs. In addition, we found that the HD iPSC-derived GABAergic neurons showed HD cellular symptoms: decrease of DARPP32 expression, loss of synaptophysin and increase of caspase-3. When we analyzed the expression patterns of various epigenetic markers, the HD iPSC-derived GABAergic neurons showed distinct alterations in expression of several epigenetic methyltransferases. Taken together, these results suggest that our HD iPSC-derived GABAergic neurons could be provided as useful HD cellular models for the pathological mechanisms of HD. This work was supported by grants from the Ministry of Science, ICT and Future Planning (2012M3A9C6050131, and 20100023160) and grants from the Ministry of Health and Welfare (A120392120100003OO) of the Korea government

T-1320

DYSREGULATION OSTEOGENIC DIFFERENTIATION IN CISD2-DEFICIENT MURINE INDUCED PLURIPOTENT STEM CELLS

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Patients with the Wolfram syndrome 2 (WFS2), accompany with severe mitochondria disregulation, are suffered by neurological and urinary tract disorder. The mitochondria membrane protein Cisd2, previously characterized as causative gene for WFS2, plays a critical role in maintaining the integrity of mitochondria structure. Patient specific induced pluripotent stem cells (iPSCs) exhibit the features of mitochondria disregulation, which is resulted from the loss in mitochondria potential and mass. Compared to wild type, Cisd2$^{-/-}$ iPSCs show broken mitochondria structure and lower proliferation rate. We validate whether the mitochondria disregulation would restricted the establishment of pluripotency. Notably, the result of transmission electric microscope reveals that incomplete structure of mitochondria membrane is observed in Cisd2$^{-/-}$ iPSCs. Using bioinformatics analysis, we conclude that fundamental roles of Cisd2 are to regulate iPSCs differentiation and cell proliferation. Our results show that Cisd2 regulate early development genes and maintains osteogenic differentiation by repressing the pluripotency. In addition, we characterize that Cisd2 participate the expression of mitochondria complex I subunits. Collectively, our results identify Cisd2 specific targets in mitochondria complex, and show that abnormal structure of mitochondria exists in Cisd2$^{-/-}$ iPSCs, resulting in lower differentiation efficiency and proliferation rate. Moreover, the mutation of Cisd2 gene may interference the capability of multiple-lineage differentiation especially in osteogenic differentiation.

T-1321

ELUCIDATING PROGRESSIVE SUPRANUCLEAR PALSY GENOTYPE-PHENOTYPE RELATIONSHIPS USING HUMAN ISOGENIC IPSCS

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Progressive supranuclear palsy (PSP) is a primary tauopathy, which is caused by the insoluble accumulation of hyperphosphorylated forms of the microtubule associated protein tau (*MAPT* gene). Recent studies suggest that the single nucleotide polymorphism (SNP) at rs242557 confers risk of the H1c sub-haplotype that is characteristic of PSP. The rs242557 SNP resides in a highly conserved repressor domain of the *MAPT* promoter suggesting that it may confer risk to PSP through instable regulation of *MAPT* expression. While previous studies have noted rs242557 allele-specific differences in the transcriptional repression, no causative relationship has been established between rs242557 alleles and tauopathy in human neurons. To address this question, and to control for variance associated with genetic heterogeneity that we have observed when comparing patient-derived human induced pluripotent stem cell (iPSC) lines, we engineered an isogenic allelic series at the rs242557 locus of human iPSCs from a common donor, whose diploid genome has been fully sequenced in high resolution. One challenge of this project was the necessity to target RNA-guided nuclease activity to a single nucleotide in a non-coding region, and to perform homology-directed repair without disruption of the surrounding sequence. Using CRISPR-based genome editing, we targeted the rs242557 locus to generate a series of iPSC lines carrying the risk-associated rs242557A/A, non-risk rs242557G/G, and heterozygous rs242447A/G alleles in an otherwise identical genetic background. We plan to interrogate human iPSC-derived neurons from these lines for *MAPT* expression, aggregation, and viability. Ultimately, the goal of this project is to support future biomarker and drug discovery efforts for PSP and related tauopathies for which rs242557 is a known risk factor. Additionally, these studies provide insight into the challenges of genetic engineering at a non-coding SNP locus.

T-1322

A HIGHLY STANDARDIZED HUMAN MODEL OF SPG4 UNCOVERS RAPID PHENOTYPES IN AUTHENTIC PATIENT NEURONS

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Hereditary spastic paraplegia (HSP) is characterized by progressive spasticity in the lower limbs caused by axonal degeneration of corticospinal motoneurons. Spastic paraplegia 4 (SPG4) makes up 40% of all HSP cases and is the most frequent, autosomal dominant subtype. Affected patients carry mutations in the SPAST gene encoding the microtubule-severing enzyme spastin. So far, no curative treatment for HSP is available, and drug discovery screens are hampered by the lack of suitable model systems. While SPG4-associated phenotypic alterations have been described in iPSC-derived neurons, development of these in vitro phenotypes typically requires several weeks of in vitro differentiation, which limits their exploitation for high-throughput assays. We became interested in developing a SPG4 model enabling rapid phenotypic analyses within a few days. IPSCs from three patients carrying heterozygous SPAST nonsense mutations were differentiated into highly enriched neuronal cortical cultures comprising >80% glutamatergic neurons expressing the layer V/VI markers CTIP2 and TBR1. We found that axonal swellings, a hallmark of the HSP pathology, can be reliably detected already 5 days after plating of SPG4 iPSC-derived cortical neuronal progenitors. Swellings were 1-7 µm in diameter and stained positive for the axonal markers tau1, acetylated tubulin and neurofilament. In an in vitro regeneration assay SPG4 neurons exhibited a 40% reduction in overall neurite length compared to controls already 24 hours after plating. At that time point we also observed enlarged growth cones suggestive of cytoskeletal imbalance. We transferred the regeneration assay to an automated 96-well-setup and achieved low experimental variability and good separation between populations with a z-factor of 0.5 within 24 hours. We expect these fast phenotypic assays to accelerate the study of pathomechanisms underlying HSP and to promote the identification of therapeutic compounds counteracting HSP-associated neuronal degeneration.

T-1323

DERIVATION OF NAIVETROPIC INDUCED PLURIPOTENT STEM CELLS FOR PARKINSON'S DISEASE MODELING

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Parkinson's disease (PD), which is characterized by the degeneration of nigral dopaminergic (DA) neurons, can be caused by monogenic mutations in genes such as parkin. The lack of PD phenotype in parkin knockout mice and the identification of defective dopaminergic neurotransmission in midbrain DA neurons derived from induced pluripotent stem cells (iPSC) of PD patients with parkin mutations demonstrate the utility of patient-specific iPSCs as an effective system to model the unique vulnerabilities of midbrain DA neurons in Parkinson's disease. In the present study, we converted patient-specific iPSCs from the primed state to a naivetropic state by DOX-induced expression of transgenes (Oct4, Sox2, Klf4, c-Myc and Nanog) and the use of 2iL (MEK inhibitor PD0325901, GSK3 inhibitor CHIR99021 and human LIF). These patient-specific naivetropic iPSCs were pluripotent in terms of marker expression, spontaneous differentiation in vitro and teratoma formation in vivo. They exhibited morphological, proliferative and clonogenic characteristics very similar to naive mouse embryonic stem cells (ESC). The high clonal efficiency and proliferation rate of naivetropic iPSCs enabled very efficient gene-targeting of GFP to the Pitx3 locus by Transcription Activator-Like Effector Nuclease (TALEN). The naivetropic iPSCs could be readily reverted to the primed state upon the withdrawal of DOX, 2iL and the switch to primed state hESC culture conditions. Midbrain DA neurons differentiated from the reverted iPSCs retained the original phenotypes caused by parkin mutations, attesting to the robustness of these phenotypes and the utility of patient-specific naivetropic iPSCs for modeling Parkinson's disease.

T-1324

THE HIGH-THROUGHPUT IPSC GENERATION, DIFFERENTIATION AND ANALYSIS SYSTEM FOR PATIENT-SPECIFIC IPSCS

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Modeling of neurological diseases using induced pluripotent stem cells (iPSCs) derived from the patient specimen has provided a new means of elucidating pathogenic mechanisms. However, generation and differentiation of iPSCs require numerous experimental procedure and long-term cultivation (~100 days) by current iPSC technologies. Therefore, most of the on-going studies of patient-specific iPSCs analyze only a few patients and controls. Such a limitation of the numbers of samples obliged us to focus on familial and rare diseases. Here, we have developed a high-throughput pathological analysis system for sporadic disease-specific iPSCs that consists of ~100 patients specific iPSC batch-generating system and newly established ~100 iPSC batch-differentiating system.
To establish this high-throughput iPSC generating system, we first screened 8 small molecules and these combinations that enhance T-cell derived iPSC (TiPSC) reprogramming. We found that one of the chemicals enhanced the efficiency of iPSC generation (4.3%, n=5) and these results were highly reproducible (reproducibility: 88.4%, n=60). Then, we established a novel small-scale sphere-based differentiating method that can be used for high-throughput neuronal differentiation from Sev-TiPSCs. When we applied this protocol for newly established 30 TiPSC clones, 28 TiPSC clones were able to differentiate into βIII-tubulin (+) MAP2(+) neuronal cells with highly differentiation ratio (58.4% on average). This system will facilitate us to handle ~100 sporadic patient specific-iPSCs from iPSC establishment to pathological analysis for just 45 days.

T-1325

DIFFERENTIATION OF AIRWAY EPITHELIUM CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS FOR CYSTIC FIBROSIS DISEASE MODELING AND DRUG TESTING

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Cystic fibrosis (CF) affects 1 in 3,000 live births in people in the

United States. One of the major roadblocks in CF research is the lack of human epithelial cells as animal models of CF and traditionally used cell systems do not recapitulate the human condition very well. induced pluripotent stem cells (iPSC) may be the most effective strategy to develop patient specific respiratory epithelial cells. This technology makes it possible to generate CF patient-specific iPSCs, which can be differentiated in vitro into airway epithelial cells for mechanistic studies of CF and as screening models to find new treatment. However, these studies have to be performed in a physiologically relevant model that simulates the in vivo condition. In this study, we successfully differentiated iPS cells from a CF patient toward epithelial airway progenitor cells using an efficient, consistent and step-wise differentiation method. The iPSC derived airway progenitor cells were differentiated further toward a ciliated phenotype using our unique air-liquid interface (ALI) system that relies on a rotating apparatus that mimics in vivo respiratory conditions. The dynamics of differentiation were examined by qRT-PCR, flow cytometry and immunocytochemistry. iPSC-derived airway progenitor cells had phenotypic properties similar to human airway basal cells. As determined by flow cytometry, differentiated cells were 62.2 % and 54% positive for markers of basal cells, CK5 and P63 respectively. Quantitative RT-PCR also revealed that CK5 and P63, were expressed in iPS-airway progenitor cells, with the relative levels compared to freshly isolated human basal cells. We have also shown the differentiation of human airway cells to mature ciliated cells using our unique air-liquid interface bioreactor system. Our study provides a method for generating patient-specific airway epithelial cells. In addition it demonstrates that a rotating bioreactor culture system that provides an air-liquid interface is a potent inducer of airway epithelial differentiation for iPS-airway progenitor cells, and provides a method for large-scale production of airway epithelium for CF disease modeling and additional testing of selected therapeutic drugs in more physiologically-relevant system.

T-1326

LEVERAGING POPULATION DIVERSITY IN IPSC DERIVED CELLULAR MODELS

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Leveraging the genetic and phenotypic diversity of a clinical population, ORIG3N is introducing novel approaches for evaluating disease states utilizing Induced Pluripotent Stem Cells (iPSC's). ORIG3N has developed Life Capsule™, which is a direct to people blood and induced pluripotent stem cells (iPSCs) banking service that will be one of the largest repositories for patient cells. By using the clinically diverse population of inherited and idiopathic disease patients represented in Life Capsule, we will address differences in disease phenotypes in both patient populations. To this end we will use differentiation of iPSCs as a route to produce neural and cardiac cells with specific genetic backgrounds. For example, preliminary studies demonstrated that dopaminergic neurons differentiated from control or Parkinson's Disease-iPSC's appeared indistinguishable after thirty days in culture. However, a more recent study illustrated that the PD pathology can be recapitulated in long-term cultures (>60 days) of dopaminergic neurons from monogenic and idiopathic-forms of PD. Degeneration of idiopathic-PD-derived dopaminergic neurons in culture demonstrates that the resulting phenotype is an inherent characteristic of idiopathic-PD patients and can be used to study disease pathology, drug treatment, and genetic variability in disease progression. For cardiovascular disease, we are building a cardiac panel with differentiated cardiomyocytes representing patients with cardiac diseases such as familial dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), and long QT syndrome (LQT). Utilizing IPSC's in this format will improve molecular understanding of pathological mechanisms and improve drug development. ORIG3N seeks to build on this initial proof of concept success with applications targeting additional diseases. Our goal is to identify an in vitro genetically defined, disease specific platform for assessing disease progression and phenotype using patient specific cells. This work will further the understanding of cellular regeneration and lead to transformative applications in personalized medicine.

T-1327

GLOBAL GENE EXPRESSION ANALYSIS BY RNASEQ ON NEURAL PROGENITOR CELLS OF AUTISTIC INDIVIDUALS

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The investigation of autism spectrum disorders (ASD) causes is hampered by the genetic heterogeneity of these diseases. However, it has becoming evident that, although heterogeneous, all different genetic alterations should be implicated in common molecular pathways or biological processes. In this scenario, the search for differentially expressed genes (DEGs) between ASD individuals and controls is a good alternative to examine the molecular etiology of such disorders. Moreover, with the advent of cellular reprogramming, it became possible to study neuronal cells of these patients in vitro and even their development in the course of neuronal differentiation. In this study, we conducted RNAseq expression analysis on neuronal progenitor cells (NPCs) derived from 3 different clones of induced pluripotent stem cells from 6 ASD individuals and 6 controls. The induced pluripotent stem cells were derived from stem cells of the pulp of exfoliated teeth. RNAseq was conducted using Illumina Truseq RNA sample prep kit and the libraries generated were run on an Hiseq 2500 equipment. Sequences were aligned and mapped using TopHat v2.0.11 and the reads counting matrix were generated using HTSeq. We have identified 200 differentially expressed genes (DEGs) between cases and controls using DESeq2, considering a p-value <0.05. Functional enrichment analysis revealed that a significant number of these DEGs are part of canonical pathways such as CREB signaling in neurons, androgen signaling and regulation of actin cytoskeleton. A significant number of DEGs (16 genes; p<10-10) predicted to be regulated by RICTOR have a pattern of expression consistent with an inhibition of RICTOR activity. RICTOR is a subunit of the mammalian target of rapamycin complex 2 (mTORC2), which is part of mTOR signaling, and is specifically involved in actin cytoskeleton organization and cell proliferation. Interestingly, previous studies from our group using stem cells from dental pulp of autistic individuals have already pointed to the involvement of the mentioned pathways in ASD etiology. Our present study demonstrated that in this stage of neuronal development the cells of autistic patients already present differences that can be related to the phenotype.

T-1328

EFFICIENT CRISPR/CAS9-MEDIATED ELIMINATION OF THE DISEASE-CAUSING MUTATION IN LEBER CONGENITAL AMAUROSIS HUMAN INDUCED PLURIPOTENT STEM CELLS

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Leber Congenital Amaurosis (LCA), a severe retinal dystrophy, is an early-onset childhood blindness disorder. This currently incurable autosomal recessive disease presents with severe visual impairment, rapid eye movements, and increased sensitivity to light. Disease-causing mutations have been mapped to several ciliary proteins, most commonly CEP290. We have generated integration-free hiPSCs from homozygous LCA patients carrying one of the most frequent LCA-causing CEP290 mutations (c.2991+1655A→G). This single nucleotide change in intron 26 activates a nearby 128bp cryptic exon that contains a stop codon, resulting in diminished and abnormal CEP290 protein expression. We used Cas9/CRISPR mediated genome editing to normalize splicing and protein expression by inactivating the cryptic exon. hiPSCs were transiently transfected with plasmids encoding Cas9(WT)-GFP and one or two guide RNAs followed by single-cell sorting of GFP/SSEA5-positive 7AAD-negative cells. Addition of Y27632 and conditioned medium facilitated recovery of hiPSC colonies from single-cell sorted cells. PCR-Seq analysis was used to determine the frequencies of insertions/deletions (indels) resulting from non-homologous end-joining (NHEJ) of the Cas9/gRNA-mediated double-strand breaks. Around 75% of the clones contained novel indels in at least one allele, and several independent clones showed biallelic elimination of the disease-causing mutation. Off-target activity was measured for the top off-target sites as determined by the Zhang laboratory's online tool (www.genome-engineering.org/crispr). Specifically, we looked at the five off-target loci with the highest overall scores as well as the five highest-scoring off-target sites with perfect PAM sequences. We did not observe any off-target sequence alterations, and most clones were euploid. Our results provide the first evidence for successful Cas9/CRISPR-mediated restoration of a WT-equivalent genetic state in CEP290-mutant hiPSCs. The isogenic mutant and repaired hiPSCs may be useful as tools for LCA disease modeling and drug screening. Furthermore, the high efficiency, accuracy, and lack of off-target effects suggest that Cas9/CRISPR mediated gene repair may be a promising in vivo gene therapy approach for LCA patients.

T-1329

MOLECULAR MECHANISMS REGULATING THE DEFECTS IN FRAGILE X SYNDROME NEURONS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Fragile X syndrome (FXS) is caused by the absence of the fragile X mental retardation protein (FMRP). We have previously generated FXS-induced pluripotent stem cells (iPSCs) from patients' fibroblasts. In this study, we aimed at unraveling the molecular phenotype of the disease. Our data revealed aberrant regulation of neural differentiation and axon guidance genes in FXS-derived neurons, which are regulated by the RE-1 silencing transcription factor (REST). Moreover, we found REST to be elevated in FXS-derived neurons. As FMRP is involved in the microRNA (miRNA) pathway we employed microRNA-array analyses and uncovered several miRNAs dysregulated in FXS-derived neurons. We found hsa-mir-382 to be down-regulated in FXS-derived neurons, and introduction of mimic-mir-382 into these neurons was sufficient to repress REST and up-regulate its axon guidance target genes. Our data link, FMRP and REST, through the miRNA pathway, and show a new aspect in the development of FXS.

T-1330

ENHANCED SMAD1 SIGNALING IMPAIRS EARLY DEVELOPMENTAL COMPETENCE IN CFC SYNDROME-IPSCS

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Cardio-Facio-Cuteneous (CFC) syndrome is a developmental disorder caused by constitutive activation of ERK signaling mainly due to BRAF mutations. However, little is known about the role of elevated ERK signaling in the CFC syndrome during early development. To understand pathophysiology of the CFC syndrome in vitro, induced pluripotent stem cells derived from dermal fibroblasts of a CFC syndrome patient (CFC-iPSCs) were generated. CFC-iPSCs showed several defective phenotypes, including abnormal embryoid body (EB) development, impaired neuronal differentiation, and preferential nuclear localization of β-catenin. In addition to elevated ERK signaling, interestingly, SMAD1 signaling was significantly activated in CFC-iPSCs during EB formation as compared with normal iPSCs. Most of the β-catenin was dissociated from the membrane and preferentially localized into the nucleus in CFC-EBs. Notably, inhibition of SMAD1 signaling rescued aberrant EB morphology, impaired neuronal differentiation and altered β-catenin localization. These results indicate that SMAD1 signaling may be a key pathway in manifestations of the CFC syndrome during early development.

T-1331

ACTIVE MODULATION OF NAV1.1 LEVELS IN DRAVET SYNDROME-SPECIFIC NEURONS

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Dravet syndrome (DS) is a congenital disorder diagnosed in up to

1.5% of juvenile epilepsy. The disease is predominantly caused by monoallelic loss-of-function mutations in the SCN1A gene, leading to insufficient levels of the voltage-gated sodium channel (Nav) 1.1. Indeed, coinciding with the onset of Nav1.1 expression in developing GABAergic interneurons, myoclonic seizures develop within the first 3-18 months of life. To establish an in vitro model of DS, we generated induced pluripotent stem (iPS) cells from DS-patient fibroblasts (SCN1A+/mt). Long-term self-renewing neuroepithelial stem (lt-NES) cells were derived as a stable intermediary that efficiently differentiates into GABAergic neurons with anterior hindbrain identity. After 6 weeks of differentiation, DS-specific neurons exhibit voltage-dependent in- and outward currents, action potential generation, and spontaneous synaptic activity. qPCR analysis of DS-specific neurons revealed that 50% of SCN1A mRNA originate from the mutant allele. Western blot analysis confirmed a 50% loss of Nav1.1 protein, while no truncated protein was detected. We next set out to compensate for reduced neuronal Nav1.1 levels. To this end the intracellular domain of Nav subunit β2 (β2-ICD), an endogenous regulator of SCN1A expression, was fused to GFP via a self-cleaving 2A peptide and incorporated into an inducible lentiviral construct. Subsequently, a DS lt-NES cell line was transduced with this vector and sorted for GFP+ cells to near purity. Autocatalytic construct cleavage was confirmed by Western blot analysis, and transgenic β2-ICD could be detected in cell nuclei by 3D microscopy. β2-ICD transgenic lt-NES cells maintained the expression of typical neural stem cell markers and were able to differentiate into GABAergic neurons. After doxycycline-induced transgene expression, a 4 - 8-fold increase in Nav1.1 protein levels was detected in 6-week-old neuronal cultures by Western blot analysis. In addition, biotinylation studies showed a quantitative increase of Nav1.1 α-subunit levels on the cell surface. These results indicate a regulatory activity of β2-ICD on Nav homeostasis in authentic human neurons, which might be exploited to counteract Nav1.1 deficiency in DS-specific neurons.

T-1332

IPSC-DERIVED CARDIOMYOCYTES PRODUCED FROM A DONOR CARRYING THE MYH7-R403Q EXHIBIT FEATURES OF HYPERTROPHIC CARDIOMYOPATHY IN VITRO

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Hypertrophic cardiomyopathy (HCM) is a common genetic heart condition affecting approximately 1 in 500 individuals, where the heart muscle becomes thick and blood flow is restricted. The condition is characterized by a thickening of the ventricular wall as a result of enlarged cardiac myocytes, changes in blood pressure due to restricted blood flow, and arrhythmias. The most prevalent form of familial HCM arises from a missense mutation in the gene encoding the beta-myosin heavy chain protein, resulting in a change of amino acid 403, from Arg-to-Gln (MYH7-R403Q). The study of diseases affecting cardiomyocytes has been advanced by the advent of stem cell technology which has enabled the production of stem cell-derived cardiomyocytes in sufficient quantities to facilitate large scale in vitro research. Further advances in stem cell technology enabled the production of human induced pluripotent stem (iPS) cells from any individual, apparently healthy normal as well as affected individuals, prompting production of large collections of iPS cells. Cardiomyocytes (CM) can be produced from any iPS cell in a collection and used to gain a better understanding of mechanisms involved in complex heart disease. Here we describe the study of iPS cell-derived CM from normal and MYH7-R403Q. Hypertrophy can be induced in normal human donor iPS cell-derived CM with exposure to Endothelin-1 (ET-1). HCM-induced CMs exhibit classic hallmarks of cardiac hypertrophy including up-regulation of fetal genes, cytoskeletal rearrangements, and an increase in cardiomyocyte size. We show that induced and inherited HCM in iPS cell-derived CM have common features. CMs differentiated from MYH7-R403Q iPS cells exhibit cardiac morphology, and showed autonomous contractile activity similar to the control iPS cell-derived CM. MYH7-R403Q CM and ET-1 induced HCM in normal CM have similar basal gene expression. ET-1 induction increases BNP expression in both control and MYH7-R403Q cardiomyocytes, but basal BNP levels are higher in MYH7-R403Q cardiomyocytes. These data show the progression of HCM characteristics in MYH7-R403Q cardiomyocytes and underscore the advantages of modeling cardiovascular disease with iPS cell technology.

T-1333

DYSFUNCTION OF MITOCHONDRIAL RESPIRATION CAUSES ABERRANT LACTATE TURNOVER IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED HEPATOCYTES

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Mitochondrial oxidative phosphorylation (OXPHOS) is the most important pathway to generate cellular energy through aerobic respiration and carries out by mitochondrial respiratory chain complex. Although it has been known that many metabolic disorders are caused by mutations on mitochondrial respiratory chain complex, their molecular and biochemical mechanisms in specialized cell types remain to be elucidated. Here we show that hepatic metabolism is changed by OXPHOS dysfunction in hepatocytes derived from human induced pluripotent stem cells (iPSCs) having homoplasmic m.3398T>C mutation on mitochondrial-encoded NADH dehydrogenase 1 (MTND1) gene. The patient iPSC-derived hepatocytes caused lactic acidosis in an AMP-activated protein kinase (AMPK)-dependent manner and revealed aberrant mitochondrial characteristics including their morphologies and biogenesis. In addition, glycogen storage level was significantly decreased in patient iPSC-derived hepatocytes. Patient iPSC-derived hepatocytes also showed decreased complex I activity, cellular ATP level and NAD+/NADH ratio which indicate dysfunction of OXPHOS. Intriguingly, genes related to the conversion of lactate into other metabolites such as glucose and alanine were significantly decreased in patient iPSC-derived hepatocytes. These results imply that dysfunction of OXPHOS in hepatocytes may cause metabolic shift and eventually result in severe metabolic phenotypes in the patient.

T-1334

OSTEOBLASTIC DIFFERENTIATION POTENTIAL OF INDUCED PLURIPOTENT STEM CELLS DERIVED FROM SYNOVIOCYTES OF RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS PATIENTS

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It has been shown that osteoblast maturation is compromised in the patients with rheumatoid arthritis (RA), which is an autoimmune disease with chronic inflammation and bone erosion. The aim of this study was to evaluate the osteoblastic potential of induced pluripotent stem cells (iPSCs) of RA patients. iPSCs were generated from fibroblast-like synoviocytes (FLS) of RA and osteoarthritis (OA) patients and healthy dermal fibroblasts using the 4-in-1 lentiviral vector. iPSCs were cultured in osteoblast differentiation medium (DMEM, 15% FBS, 50 µg/ml ascorbate-2-phosphate, 10 nmol/L dexamethasone, 10 mmol/L β-glycerophosphate) for 4, 8, 12, 16 or 20 days. Osteoblastic differentiation was assessed with morphology and qRT-PCR for osteoblast markers. Alizarin red staining and osteoimage bone mineralization assay were also performed to evaluate calcium deposit and mineralization of osteoblasts, respectively. RA-iPSCs, OA-iPSCs and healthy-iPSCs cultured in osteoblast differentiation medium showed osteoblast-like morphology on day 4. However, only RA-iPSCs were positively stained with alizarin red S and osteoimage staining reagents on day 4. OA-iPSCs and healthy-iPSCs were stained with alizarin red S and osteoimage staining from day 12. mRNA level of Runx2, an early osteoblast marker, robustly increased on day 4 and significant amounts of Runx2 were still detected until day 12 in RA-iPSCs. In contrast, the level of Runx2 of OA-iPSCs and healthy-iPSCs reached the highest point on day 8 and barely detected on day 12. Interestingly, mRNA level of OPN which is a late osteoblast marker was lower in RA-iPSCs than in OA-iPSCs and healthy-iPSCs. These results indicate that RA-iPSCs tend to differentiate into osteogenic lineage more easily than OA-iPSCs and healthy-iPSCs do, but osteoblast maturation is compromised in RA-iPSCs compared with OA-iPSCs and healthy-iPSCs.

T-1335

NEURONS AND GLIA CELLS OBTAINED FROM PATIENT-SPECIFIC LYMPHOBLASTS FOR THE ANALYSIS OF PSYCHIATRIC DISEASES

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Psychiatric diseases such as schizophrenia are multifactorial disorders caused by the dysfunction of neurons and glia cells. The molecular mechanisms involved are poorly understood. We recently applied genome-wide association studies (GWAS) to identify DNA variations, which are associated with onset, progression, and treatment of psychiatric disorders. Here, we describe the production of patient-specific induced pluripotent stem (iPS) cells and their neural differentiation into neurons and glia. Together, these techniques provide a potent tool for the analysis of DNA variations. Patient-specific Lymphoblasts were treated with non-viral reprogramming vectors. Efficiency of vector delivery was analyzed by flow cytometry. The mRNA and protein expression of pluripotency markers was analyzed. After induction of pluripotency factors, cells were differentiated into neurons and glia using a differentiation protocol for the generation of cortical cells. The mRNA and protein expression of neuron- and glia-associated marker genes was verified. Differentiated cells were studied using patch clamp analysis to verify their functionality. Non-viral reprogramming of Lymphoblasts was demonstrated by Alkaline Phosphatase staining and the protein expression of pluripotency markers such as OCT4, SOX2, NANOG, and SSEA4. Their expression was stable for several passages. The procedure revealed about 20% pre-iPS cells at day 3. Neural induction was shown by the protein expression of neural key regulators such as PAX6, NESTIN, and NGN3. Further Differentiation and maturation of neurons and glia cells was shown including NeuN, GFAP, and O4 protein expression. Functional studies proofed the presence of GABAergic cells suggesting their differentiation into cortical neurons. In conclusion, neural conversion of patient-specific Lymphoblasts provides a powerful tool to study psychiatric disorders.

T-1336

FORMATION OF ATAXIN-3 POSITIVE NUCLEAR INCLUSIONS IN MACHADO-JOSEPH DISEASE-SPECIFIC NEURONS BY INTRACELLULAR CALCIUM-RELEASE AND AUTOPHAGY MODULATION

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Machado-Joseph disease (MJD) or spinocerebellar ataxia type 3 is the most frequent form of inherited spinocerebellar ataxia worldwide. Expansion of a polyQ repeat increases the aggregation propensity of ataxin-3, leading to the formation of ataxin-3-positive inclusions, a hallmark of MJD. Our previous studies employing patient-specific MJD neurons showed that excitation by glutamate or NMDA and subsequent Ca2+ entry via voltage-gated Ca2+ channels activates calpain-mediated cleavage of ataxin-3, which initiates the generation of SDS-insoluble ataxin-3 microaggregates . Here we set out to explore whether and to what extent intracellular Ca2+ release contributes to this disease-initiating step. We investigated the early expressed purinergic receptor family for the induction of cytosolic calcium increase and found that ATP-stimulation of P2Y receptors enables induction of microaggregates as early as 6 days after initiation of neuronal differentiation. Microscopically visible neuronal intranuclear inclusions (NIIs) positive for ataxin-3 and ubiquitin could be detected selectively in ATP-stimulated MJD cultures. Since both glutamate and ATP-mediated aggregation rely on receptor types with largely variable spatio-temporal distribution pattern, we also explored the potential contribution of ubiquitously expressed ryanodine receptors as well as sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) for their capacity to modulate aggregate induction. Both activation of ryanodine receptors and SERCA inhibition resulted in ataxin-3 NIIs after only 8 days of differentiation with SERCA inhibition yielding >80% MJD neurons carrying ataxin-3-positive aggregates. However, these paradigms still showed quantitative fluctuations of NII induction between independent experiments. We reasoned that this variability might be due to fluctuations in proteasomal degradation and autophagy. Indeed, media conditions resulting in autophagy inhibition yielded highly efficient NII formation in MJD neurons even in the absence of additional stimuli. Our data indicate that dysregulation of intracellular calcium homeostasis and autophagy may play important roles in the process of protein aggregation in MJD neurons.

T-1337

CANCER PATIENT SPECIFIC IPSCS BASED STUDY OF A POTENTIAL ONCOGENE, SFRP2, IN LFS ASSOCIATED OSTEOSARCOMA

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In our recent study, we established Li-Fraumeni syndrome (LFS - a germline p53 mutation disease) patient specific iPSCs for modeling osteosarcoma (OS). Functional assays and global gene set enrichment analysis with LFS iPSCs derived mesenchymal stem cells (MSCs) and osteoblasts (OBs) demonstrated that tumorigenic phenotype can be recapitulated using cancer patient specific iPSCs. Taking advantages from this platform, we focused on finding therapeutically important factors that lead to LFS associated OS development. Interestingly, we found that sFRP2 (secreted frizzled related protein 2) is highly expressed in LFS iPSCs derived MSCs and its expression level increases during in vitro OB differentiation. The global transcriptome analysis also revealed that sFRP2 is a differentially expression gene in LFS derived MSCs and OBs. First, we wondered how sFRP2 is overexpressed in LFS. Western blot analysis showed that the level of total AKT and active AKT (S473) markedly increase in LFS MSCs and OBs compared to WT cells. However, sFRP2 overexpression in WT did not drive AKT activation. It suggested that activated AKT caused by p53 mutation in LFS patients leads to overexpression of sFRP2. Considering the significant expression level of sFRP2, we assumed that sFRP2 is a causative factor in p53 mutation mediated OS development. To prove this idea, we performed soft agar and *in ovo* CAM assay using inducible sFRP2 and sFRP2 knock-down MSCs and OBs. These experiments showed that sFRP2 expression is sufficient to increase the number of oncogenic spheres in vitro and the size of tumor like mass *in ovo*. In the experiments to check functional OB characteristics, we observed that sFRP2 expression delays normal osteoblast differentiation process in WT MSCs. sFRP2 expressed MSCs exert lower activity of osteogenic alkaline phosphatase and lower activity of mineralization. This study demonstrates that it is feasible to study the roles of potential oncogene in the format of modeling cancer with patient specific iPSCs.

T-1338

LOSS OF MECP2 FUNCTION RESULTS IN LIN28 MISREGULATION AND IMPAIRED GLIOGENESIS IN PATIENT-DERIVED IPSCS

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Rett Syndrome (RTT) is a pervasive developmental disorder caused by mutations in the X-linked MECP2 gene primarily affecting girls. Complete loss of MECP2 function in boys causes congenital encephalopathy, which is characterized by neurodevelopmental arrest and early lethality. In rare cases, males with MECP2 mutations have survived to term and beyond. Generation of induced pluripotent stem cell (iPSC) lines from male patients harboring a loss-of-function or missense mutations in MECP2, along with control lines from their unaffected fathers, give us an opportunity to identify the earliest cellular and molecular changes associated with loss of MECP2. We differentiated iPSC-derived neural progenitor cells (NPCs) using retinoic acid (RA) and found that astrocyte differentiation is perturbed in two different patient iPSC lines. Using a highly stringent quantitative proteomic analysis, we found that LIN28, a gene important for cell fate regulation, is upregulated in mutant NPCs. Overexpression of LIN28 in control NPCs suppressed astrocyte differentiation and impaired neuronal synapse density. These results indicate that a specific gene expression change in early development may lead to compromised glial and neuronal development in RTT.

T-1339

TRANSCRIPTIONAL AND PROTEOMIC PROFILING OF HUMAN PLURIPOTENT STEM CELL-DERIVED MOTOR NEURONS: IMPLICATIONS FOR FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, fatal neurodegenerative disease characterized by the selective loss of upper and lower motor neurons. The identification of genetic triggers (e.g. mutations in C9ORF72, SOD1, FUS, and TARDBP) has informed our biological understanding of this devastating disease, but we still do not know how mutations in these genes cause selective motor neuron degeneration. Global, large-scale molecular studies on disease-relevant cell types is an attractive option to investigate the pathology of ALS, but the inaccessibility of human motor neurons combined with our inability to expand them in culture like cancer cells remains a barrier to these types of studies. Opportunely, human pluripotent stem cells can be directed to efficiently differentiate into substantial quantities of motor neurons. Here, we combined pluripotent stem cell technologies with both RNA sequencing and mass spectrometry-based proteomics to map alterations to both mRNA and protein levels in motor neurons expressing mutant SOD1. Specifically, we introduced the severe SOD1 A4V mutation into a stem cell line that reports for GFP under the control of the motor neuron-specific promoter for HB9. This approach enabled us to study the effects of mutant SOD1 in purified populations of motor neurons using multiple metrics over time. These investigations have afforded an unprecedented glimpse at the biochemical make-up of human stem cell-derived motor neurons and how they change in culture. Moreover, our results revealed subtle yet reproducible differences in gene and protein expression between motor neurons with and without the SOD1 A4V mutation. Interestingly, several of the altered proteins regulate aspects of neuronal excitability, which our group previously described a hyper-excitability phenotype induced by mutant SOD1 in motor neurons. In conclusion, our global profiling efforts offer a greater understanding of stem cell-derived neurons and provide possible links between mutant proteins and molecular pathology. Perhaps their greatest value, however, is in identifying new therapeutic targets for intervention into the disease course of ALS.

T-1340

GENERATION AND CHARACTERIZATION OF HIPSCS GENERATED FROM SIALIDOSIS PATIENTS WITH NOVEL MUTATIONS IN NEU1 GENE

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Lysosomes are cytoplasmic compartments that contain many acid hydrolases and play critical roles in the metabolism of a wide range of macromolecules. Deficiencies in lysosomal enzyme activities cause genetic diseases, called lysosomal storage disorders (LSDs). Many mutations have been identified in the genes responsible for LSDs, and the identification of mutations is required for the accurate molecular diagnoses. Sialidosis is a family of LSDs. Mutations in the NEU1 gene that encodes lysosomal sialidase cause sialidosis. Insufficient activity of lysosomal sialidase progressively increases the accumulation of sialylated molecules, and various clinical symptoms, including mental retardation, appear. Here, we sequenced the entire coding regions of NEU1 in sialidosis patient-derived fibroblasts and found the novel mutations p.R347Q in NEU1 as well as many other mutations that have been previously reported. We generated human induced pluripotent stem cells (hiPSCs) from sialidosis patient cells with NEU1 mutation (R347Q)(NEU1-hiPSCs) and further differentiated them into neural lineage to determine the disease-specific phenotypes. We determined that sialidosis patient fibroblasts, NEU1-hiPSCs, and neural precursors differentiated from NEU1-hiPSCs containing the novel mutation showed the molecular phenotypes of the corresponding disease. Further structural analysis suggested that these novel mutation sites are highly conserved and important for enzyme activity. Thus, we demonstrated that NEU1-iPSCs and their derivatives harboring disease properties can be used as a novel and convenient humanized disease models for drug discovery and development.

T-1341

HUMAN IPSCS FOR THE STUDY OF DIFFERENTIAL DRUG RESPONSE IN HYPERTENSION

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Hypertension affects over 1 billion people worldwide. Blood pressure is regulated by a complex network of physiological pathways involving cardiac contractility, extracellular fluid volume homeostasis, and vascular tone through renal, neural or endocrine systems. The advent of induced pluripotent stem cells (iPSCs) offers an opportunity for recapitulate to some extent tissue-specific disease phenotypes. It is a useful tool for preclinical drug screening as well as optimizing medical treatment, particulary in hypertensive resistant patients. Our aim is to create a library of iPSCs from the Brazilian population for the study of differential drug response in hypertensive patients. The Brazilian Longitudinal Study of Adult Health or Estudo Longitudinal de Saúde do Adulto (ELSA)-Brasil is a large multicenter cohort study of more than 15,000 civil servants from 5 universities. The study is focused on the risk of diabetes and cardiovascular diseases in adults 35-74 years old coming from different areas of Brazil. Data from patients is collected in the form of interviews, electrocardiogram, blood sample, blood pressure, as well as other clinical, biochemical and genetic tests. Cohort participants are interviewed and examined at the clinics every 3-4 years. The questionnaires include data on age, gender, race/ethnicity and history of migration. We have built a library of 1878 cryopreserved primary mononuclear cells (MNC) from peripheral blood of ELSA participants and have established a methodology for hiPSC generation by non-integrative plasmids based on Linzhao and co-workers protocol. Among those 1878 samples, we identified a group comprising 436 individuals currently treated for hypertension. Among these, 43 (9.86%) are resistant to pharmacological treatment. We have generated 8 hiPSC lines coming from the resistant hypertensive group, one hiPSC line from the responsive group and 4 hiPSC lines from normotensive individuals. These hiPSCs will serve as a basis for the development of cell-based assays aimed at predicting response to pharmacological intervention in hypertension.

T-1342

IPS CELLS DERIVED FROM HUNTINGTON´S DISEASE PATIENTS EXHIBIT MITOCHONDRIAL DYSFUNCTION AND METABOLIC DISTURBANCES

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Huntington's disease (HD) is an autosomal dominant disease caused by an expansion of CAG repeats in the HD gene encoding for huntingtin. Several pathological mechanisms have been proposed for neurodegeneration, including mitochondrial and metabolic dysfunction and oxidative stress. An attractive model to study disease mechanisms are HD patient-specific induced pluripotent stem cells (HD-iPSC). Indeed, HD-iPSC can help to reveal the role of mitochondrial and metabolic dysfunction in early stages of HD. In this study, we show that HD-iPSCs mitochondria have more negative membrane potential and increased intracellular basal Ca2+ levels, but reduced mitochondrial Ca2+ storage capacity. Moreover, increased levels of mitochondrial superoxide anion and hydrogen peroxide production were observed in HD-iPSCs versus control iPSC (C-iPSC). Similarly, HD-iPSCs produced higher levels of reactive oxygen species following an acute exposure to hydrogen peroxide. These results are consistent with the increased acetylation and decreased activity of superoxide dismutase 2 (SOD2) in HD cells. Furthermore, HD-iPSCs showed decreased complex I+III activity and mitochondria exhibited altered round shape morphology, but no differences in fission/fusion proteins were found. Additionally, HD-iPSCs consumed less O2 and resorted less to oxidative phosphorylation to produce ATP, which is accordance with the observation that HD cells rely more on glycolysis and have lower ATP/ADP ratio. Moreover, HD-iPSCs showed increased phosphorylation of pyruvate dehydrogenase (PDH) subunit E1α at Ser 232, 293 and 300 reflecting its inactivation; concordantly, increased levels of pyruvate dehydrogenase kinase, isozyme 1 (PDK1) were found. Overall, our study suggests that mitochondrial dysfunction is evident in very early stages of HD cytopathogenesis.

T-1343

CHARACTERIZATION OF IPSC FROM MONOZYGOTIC DISCORDANT TWINS - A MODEL FOR SCHIZOPHRENIA

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Schizophrenia (SCZD) is a devastating psychiatric disorder, which affects approximately 70 million people worldwide (1% of the global population). People with the disorder demonstrate a range of symptoms, including delusions, hallucinations, difficulty speaking and organizing thoughts, memory deficits and withdrawal from friends and family. Under the best circumstances medication and therapy for SCZD can help alleviate disease symptoms, yet it is estimated that anywhere from 20% to 60% of patients remain resistant to known treatments. Understanding the cellular and molecular mechanisms that give rise to the disease is therefore a crucial step in developing new drug targets and treatments. Family and twin studies have consistently demonstrated high heritability in SCZD. Indeed, genetic research on SCZD has identified a significant number of genetic variants associated with the disorder. However, despite this apparently strong genetic component, monozygotic twin discordance for SCZD (in which only one twin suffers from the disease) is on average only around 50%, a surprising and not fully explained finding. Deep sequencing studies have shown that, opposite to the conventional view, monozygotic twins may not in fact be genetically fully identical. Copy number variations (CNVs) for example, have been identified within monozygotic twins. Beside genetic differences other possible mechanisms that might play a role in monozygotic twin discordance include alternative splicing, retrotransposition events, differential methylation and imprinting, all of which can give rise to transcriptomic differences despite identical genetic backgrounds. Here, we have utilized iPSCs to develop neurons from 3 pairs of monozygotic twins discordant for SCZD and 3 pairs of control twins, and identified genetic mutations, molecular and phenotypical differences that could potentially contribute to schizophrenia disease risk or onset. Such combinatory approach will not only help to deepen our understanding of the underlying biology of the disease, but will also contribute to future approaches and improvements in pharmacological intervention and therapy.

T-1344

FUNCTIONAL ANALYSES OF MOTOR NEURONS DIFFERENTIATED FROM ALS PATIENT-DERIVED IPSC WITH MUTATIONS IN THE FUS GENE

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For the study of functional deficiencies in neurodegenerative diseases patient-derived iPSC can be used as in-vitro disease models. As proof of principle, it has been demonstrated that motor neurons (MNs) differentiated from ALS-iPSC indeed recapitulate certain disease-specific abnormalities. The aim of the present study is to unravel pathophysiological mechanisms underlying ALS in MNs differentiated from iPSC lines carrying mutations in the FUS gene. Based on a novel approach starting from an expandable population of neural precursor cells generated from iPSC, we successfully differentiated three different ALS-iPSC lines (n=5) and lines from three healthy controls (n=5) into Tuj1/Map2/SMI32/Islet1-positive MNs without observing major differences in differentiation efficiency. Cells from both conditions showed similar basic neural properties such as steady membrane potential and Na+K+ currents under patch clamp analysis. Also, no significant differences between FUS and control lines were observed in the percentage of cells that were spontaneously active and they similarly fired single action potentials upon stimulation. However, in healthy controls we observed higher frequencies of spontaneously occurring action potentials (controls: 3.3Hz; FUS 1Hz) and postsynaptic miniature events (controls: 0.72Hz; FUS 0.27Hz), both of which are physiological parameters for spontaneous activity. Furthermore, upon stepwise depolarization a higher percentage of control-cells responded with trains of action potentials (controls: 84.3%; FUS: 50%).In line with a very recently published study our results indicate that ALS cells with mutations in the FUS gene present a hypoexcitability phenotype. AT the same time our hypoexcitable FUS mutant cells present themselves with a significantly lower Na+K+ ratio. We therefore aim to test the benefits of the FDA approved antiarrhythmic drug 4-Aminopyridine in its ability to antagonize the potassium currents which are held to be at least partly responsible for the observed hypoexcitability. How exactly this phenotype is related to the observed degeneration of MNs in ALS awaits further investigation.

T-1345

HIPSC MODEL FOR HYPERTROPHIC CARDIOMYOPATHY

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Hypertrophic cardiomyopathy (HCM) is a complex autosomal-dominant disease associated with significant genotypic and phenotypic heterogeneity. HCM is one of the most common inherited cardiovascular disorders and the leading cause of sudden cardiac death in young adults. Typically hypertrophy affects the left ventricle and the interventricular septum and may lead to arrhythmias, diastolic dysfunction and sudden death. Other hallmark features of the disease are myocyte disarray and fibrosis in the heart tissue. No specific therapy is available to prevent the onset or regression of hypertrophy. Two most predominant founder mutations for HCM in Finland are in cardiac myosin-binding protein C (cMYBPC, Q1061X) accounting for 11.4% and in α-tropomyosin (TPM1, D175N) accounting for 6.5% of the Finnish HCM cases. Mutation in each sarcomeric protein is likely to result in a distinct set of clinical symptoms. The functional consequences and the mechanisms by which mutations cause diverse phenotypes in HCM are still only partly undestood. We have derived human induced pluripotent stem cells (hiPSCs) from patients carrying Finnish founder mutations as well as from healthy controls and differentiated

them into cardiomyocytes. We have studied and compared the morphology and functionality of hiPSC-derived HCM and control cardiomyocytes by immunocytochemistry, Ca^{2+} imaging, patch clamp and video-based analysis. With our HCM hiPSC model we can demonstrate and study common and new pathophysiological mechanisms of the HCM disease in humans regarding the two Finnish founder mutations in TPM1 and cMYBPC.

T-1346

CHARACTERIZATION OF A HUMAN MACHADO-JOSEPH DISEASE NEURONAL CELL MODEL DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

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Machado Joseph Disease (MJD) also known as Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominant inherited cerebellar ataxia and a progressive, adult-onset neurodegenerative disease. MJD is caused by a CAG-repeat expansion in the ATXN3 gene on chromosome 14q24.3-q32.2, which results in an abnormally long polyglutamine tract in the ataxin-3 protein. With the development of induced pluripotent stem cell (iPS) cell technology, the study of the pathology of MJD is no longer restricted to artificial disease modeling systems such as animal models or cell lines, which present limitations as models of human neurogenetic disorders. Therefore, the aim of this work was to produce a novel human cell model of Machado-Joseph disease, which is expected to complement other disease models and bring further insight on disease mechanisms and pathology. We derived disease-specific iPS cell lines from MJD patient's fibroblasts, through the induced expression of the four transcription factors OCT3/4, SOX2, KLF4 and c-MYC using a lentiviral vector. Several clones were selected after characterization based on positive pluripotent cell surface markers, endogenous levels of pluripotency related genes and in vitro and in vivo differentiation potential. Neural induction and neuronalization of these cultures was performed using a specific set of morphogens and neurotrophins to induce hindbrain/midbrain patterning and the iPS-derived neurons were then analyzed for both terminal maturation and functional markers. We evaluated the late-onset disease phenotype in the obtained neurons by exposure to excitotoxic molecules (thapsigargin and glutamate) and oxidative stressors (H2O2) regarding the impaired toxic protein clearance, increased susceptibility to oxidative stress and Ca2+ dependent proteolysis of ATXN3 via either store-operated calcium entry or excitotoxic glutamate-induced Ca2+ influx. Our final goal is the validation of this new and unique in vitro model as a robust neurodegenerative disease modeling resource, recapitulating the molecular and cellular phenotypes typical of MJD and opening new opportunities for investigating the disease pathogenesis and for drug screening.

T-1347

GENERATION OF INDUCIBLE ALPHA-SYNUCLEINOPATHY MODEL USING HUMAN PLURIPOTENT STEM CELLS

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Alpha-synuclein, encoded by the SNCA gene, is a member of synuclein family, which also includes beta- and gamma-synuclein. It is expressed in various types of brain cells such as neurons and astrocytes. Abnormal accumulation of alpha-synuclein forms aggregates in neurons and glial cells, and is a characteristic of neurodegenerative diseases called alpha-synucleinopathies, which include Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. In addition, alpha-synuclein peptides are a major component of the amyloid plaques found in Alzheimer's disease patients. Multiplications of the normal allele as well as various point mutations of SNCA are linked to familial Parkinson's disease. The mechanism by which the abnormal expression of alpha-synuclein leads to various diseases is not well understood. Also, there is no human model in which the expression level of alpha-synuclein protein can be manipulated. Here, human induced pluripotent stem cells (iPSCs), derived from skin fibroblasts from normal donors were modified to express an alpha-synuclein-GFP fusion protein in a doxycycline-inducible manner. For this, targeted genome engineering was employed simultaneously in two safe-harbor sites in the human genome, the CLYBL (citrate lyase beta like) locus on chromosome 13 and the AAVS1 (adeno-associated virus integration site 1) locus on chromosome 19, to integrate the Tet transactivator expression unit and the TRE-SNCA-GFP expression unit, respectively. The resulting iPSC lines enable us to study the effect of changing levels of alpha-synuclein protein on cell survival and function under different conditions in various types of cells such as pluripotent cells, neural precursor cells, and neurons.

T-1348

PATIENT SPECIFIC IPS-DERIVED NEUROEPITHELIAL STEM CELLS PROVIDE A ROBUST TOOL TO MODEL ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is one of the most common neurodegenerative diseases and the cause of dementia. Dementia and AD are becoming more prevalent with an aging population, but also younger people are affected. Apart from neurodegeneration, two major hallmarks of AD are extracellular deposits of Aβ peptides, so called amyloid plaques, and intracellular accumulation of filamentous hyperphosphorylated Tau protein, neurofibrillary tangles. We have modeled Alzheimer's disease with neurons spontaneously differentiated from the patient specific iPS cell derived neuroepithelial stem (NES) cells. NES cells have highly consistent characteristics in culture, including continuous expandability in the presence of growth factors, neuronal and glial differentiation competence, as well as the capacity to generate functionally mature neurons. In

the present study NES cells from an AD patients with London mutation (APP717) as well as from Down syndrome patients and healthy control individuals were used. In addition to neuronal characterization, the differentiated cells have been evaluated with the AD specific assays e.g. the concentration of different type of amyloid beta (Aβ) peptides and the phosphorylation levels of Tau-protein. Spontaneous differentiation resulted in neuronal cell cultures from all the NES cell lines in a comparable manner. Furthermore, all the lines had increased production of Aβ-peptides during neuronal maturation. However, results reveal the increased Aβ 42/40 ratio in the AD patient cells when compared to control and the Down syndrome patient lines. In addition, a difference between phosphorylated Tau protein levels in AD patient and WT-cells was seen. As a conclusion, the data indicates that NES cells provide a robust tool to model AD. Furthermore, NES cells can be used in studying the pathophysiology and mechanisms of neurodegenerative diseases.

T-1349

GENOMIC ANCESTRY OF HUMAN IPSCS FROM A CELL LIBRARY OF THE BRAZILIAN POPULATION FOR IN VITRO CLINICAL TRIALS

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The Brazilian population is one of the most genetically heterogeneous in the world, a result of 20 generations of admixture between individuals of Amerindian, European and African descent. Drugs tested and approved in Europe or North America are sold in developing countries like Brazil, without knowing how effective or safe they are. It is widely known that many drugs show a more efficient response on certain genetic backgrounds compared to others. Therefore, these drugs will not necessarily be effective in the Brazilian population, due to genetic factors modulating the individual response to a particular drug. Thus, a collection of human pluripotent stem cells representing Brazil´s genetic diversity would be a useful tool for in vitro clinical trials of drug response in that population. Since 2008, we have established 5 lines of human embryonic stem cells from embryos donated for research in private human reproduction clinics. Analysis of their genomic ancestry shows that they are mostly of European ancestry [average: 96.6 % European (E), 1.2 % African (Af) and 2.2 % Amerindian (Am)]. In contrast, the average genomic ancestry of 1,285 participants of the Brazilian cohort study ELSA (Longitudinal Study of The Adult Health) [68% E, 21% Af and 11% Am] indicated that lines of hiPSCs derived from those individuals would be more representative of Brazil´s population. We analyzed the genomic ancestry of 4 randomly chosen lines of hiPSCs from the ELSA study, and we found that three of them are closer to the average of the ELSA cohort than any of the hESCs lines [hiPSC2 - 72% E, 25% Af and 3% Am; hiPSC3 - 78% E, 19% Af and 3% Am; hiPSC4 - 52% E, 45% Af and 3% Am]. Moreover, we also tested the local ancestry for each cell line (i.e. determined the ancestry for each physical location in the genome). This information will be useful for associating the cell lines to drug response and phenotypes to the ancestry associated to specific genes and genomic regions which play a role in metabolic pathways. Both hESC and hiPSC lines were differentiated into human hepatocyte-like cells using a highly reproducible in vitro model, which will allow to determine hepatic functionality and use for in vitro clinical trials for the Brazilian population.

T-1350

INDUCED PLURIPOTENT STEM CELL DERIVED ENDOTHELIAL CELLS AS SURROGATES FOR DRUG SCREENING IN IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION

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Pulmonary arterial hypertension (PAH) whether idiopathic (IPAH) or associated with other conditions is a potentially lethal disease in which the pulmonary arteries (PA) narrow and stiffen, progressively increasing resistance to flow and eventually leading to right-sided heart failure. Endothelial cell (EC) dysfunction, is pivotal to the development of PAH but may vary between individuals, owing to both genetic factors and environmental exposures. This makes it difficult to develop therapies that will consistently reverse the disease process. Recently, cell types of interest derived from induced pluripotent stem cells (iPSC) have been shown to recapitulate disease phenotypes in culture. Therefore, we hypothesized that IPAH patient-specific iPSC differentiated EC (iPSC-EC) will preserve the gene variants and disease phenotypes that give rise to PAEC dysfunction and would be good surrogates for drug testing. We therefore tested functional changes in iPSC-EC generated from skin fibroblasts harvested from IPAH patients at the time of lung transplant and from controls that provide unused donor lungs. This allowed for comparison of native cultured PAEC and iPSC-EC from the same individuals. Comparisons were made between iPSC-EC and PAEC from a minimum of 5 IPAH patients and 5 controls. Both iPSC-EC and PAEC from IPAH patients showed significantly reduced adhesion and survival following serum withdrawal and impaired tube formation in angiogenesis assays when compared to control iPSC-EC and PAEC respectively ($p<0.05$ for all). PAEC also showed significantly reduced expression of BMPR2 and collagen IV and unrepaired DNA, and trends in the same direction were evident in iPSC-EC. We next tested iPSC-EC as surrogates for drug screening in IPAH. Our previous studies showed that the immunosuppressant FK506 and the elastase inhibitor elafin, both improve IPAH PAEC function. We therefore examined the effects of these novel agents on angiogenesis, in iPSC-EC and PAEC from the same 4 IPAH patients. Using tube formation assays in matrigel we found that IPAH PAEC showed a hierarchy of responsiveness to FK506 and elafin, and these results were reproducible in iPSC-EC from the same patients. These results indicate that iPSC-EC can be effective disease models to screen novel drug candidates as personalized therapies for IPAH.

T-1351

INVESTIGATION OF METABOTROPIC GLUTAMATE RECEPTORS IN HUMAN PLURIPOTENT STEM CELL DERIVED DENTATE GYRUS PROX1 GRANULE CELLS AND THEIR TRANSLATIONAL RELEVANCE

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Pluripotent stem cell-based differentiation protocols have become important research tools for developmental biology and molecular medicine. The self-renewable human cell lines can be used to generate various neuronal types in vitro and to study the differentiation process. Besides the potential of generating individual cell lines from patients with neurological and psychiatric disorders, these in vitro derived neurons can also be used to investigate the electrophysiological and pharmacological properties of various receptor subtypes. Metabotropic glutamate receptors (mGlus) have been extensively studied as possible targets for the treatment of schizophrenia and other psychiatric disorders. Using established protocols we generated hippocampal dentate gyrus granule cells. The dentate gyrus protocol is based on dual SMAD inhibition, Wnt- and SHH inhibition, after generating embryoid bodies from pluripotent stem cells, which results in forebrain specific Sox2 and Nestin positive neuronal progenitor cells (NPCs). This intermediate cell type can be further differentiated into PROX1 and MAP2 expressing mature neurons. Using this protocol we compared the Wnt-antagonist effects of recombinant DKK1 and XAV939, a small molecule used in other protocols. Both Wnt antagonists are sufficient to generate PROX1 neurons. The mature neurons express mGlu2, mGlu3 and mGlu7 at subcellular compartments sites corresponding to their vivo localization. Using Ca-imaging and single cell electrophysiology we plan to characterize the pharmacology of these receptors. mGlu2 and mGlu3 have been shown to modulate hippocampal circuitry in vivo, therefore these receptor subtypes warrant further investigation. The results will be discussed in the context of IPS cell based in vitro disease modeling. This study is funded by the National Brain Research Program (NAP) of Hungary (Grant NAP-B KTIA_NAP_13-2014-0011 to JR).

T-1352

MODELLING OF NEONATAL DIABETES ASSOCIATED WITH AN ACTIVATING STAT3 MUTATION WITH PATIENT-SPECIFIC IPS CELLS

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We described a patient with neonatal diabetes associated with a hypoplastic pancreas and high beta-cell autoantibody levels already at birth. Screening of the patient's DNA revealed the missense mutation in the STAT3K392R gene. Functional characterization of this mutation showed that it is highly activating, particularly in the basal state but also after IL-6 stimulation. We hypothesize that the mutation may cause pancreatic developmental failure and autoimmunity through distinct mechanisms. In this study, we tested the hypothesis using patient-derived iPS cells. Patient iPSC were differentiated towards pancreas through a 17-day stepwise protocol leading to efficient specification of endocrine progenitors. Expression levels of pancreatic progenitor markers, such as PDX1 and NKX6.1, did not differ between STAT3K392R cells and healthy controls. Thus, overactive STAT3 did not cause a developmental block or inhibit endocrine differentiation. Instead, the insulin mRNA levels were significantly higher in STAT3K392R cells already day 13 of differentiation. Also, NEUROG3 expression was upregulated earlier in patient cells. The qPCR results were confirmed by immunocytochemistry showing more NEUROG3-positive nuclei after 13 days and markedly more INSULIN-positive area after 17 days ($p=0{,}005$). A modified protocol was developed to maintain wild-type cells as pancreatic progenitors minimizing endocrine differentiation. In these conditions, the differences with the control cells were even more remarkable, with STAT3K392R cells presenting upregulated NEUROG3 ($p<0{,}005$), NKX2.2 4-5 fold ($p<0{,}05$), and INSULIN 10-fold ($p<0.001$) levels. 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 are a valuable tool for recapitulating pancreatic developmental defects, enabling the study of monogenic diabetes mechanisms.

T-1353

ANALYSIS OF CARDIOMYOCYTES DIFFERENTIATED FROM INDUCED PLURIPOTENT STEM CELLS FROM PATIENTS WITH FAMILIAL ATRIAL/VENTRICULAR SEPTUM DEFECT

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Recent molecular analysis revealed that mutation of various cardiac transcription factor genes caused congenital heart anomalies. In these diseases, the heart often has not only morphological defects but also functional abnormalities. However, direct molecular, functional and pharmacological analysis cannot be done using human disease model. To address this issue, this study generated human induced pluripotent stem cells (iPSC) and induced cardiomyocytes from patients with familial atrial/ventricular septum defect (ASD/VSD). Peripheral blood was obtained from patients with ASD (n=2; 30-year-old male and his 4-year-old daughter) and healthy volunteers (n=2; 30-year-old female who was his wife and 29-year-old male). Molecular analysis revealed no mutations in Nkx2.5, Tbx5, GATA4 and GATA6 genes. Their iPSCs were generated using activated T-cells and Sendai virus vectors containing Oct3/4, Sox2, Klf4 and c-Myc. Cardiomyocytes, which were induced from patients and control iPSCs, were purified by culturing in glucose-free lactate-supplemented media. Global gene expression patterns of purified cardiomyocytes were analyzed using DNA chips and ingenuity pathway analysis (IPA). Upstream analysis of IPA showed that candidate genes which may be associated with cardiogenesis in cardiomyocytes derived from iPSC lines generated from ASD/VSD patients. We established human iPSCs, obtained regenerated cardiomyocytes from the patient with familial ASD/VSD, and picked candidate genes which were associated with cardiogenesis. Disease-specific iPSCs possibly elucidate the mechanisms of congenital heart disease.

T-1354

MODELING PCSK9 FUNCTIONS USING HEPATOCYTE-LIKE CELLS (HLC) DIFFERENTIATED FROM URINE SAMPLE-DERIVED HUMAN INDUCED PLURIPOTENT STEM CELLS (UHIPSC)

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hiPSC are becoming a relevant model for the study of liver metabolic diseases once differentiated into HLC, and it has been shown that they can faithfully recapitulate autosomal dominant hypercholesterolemia (ADH). PCSK9 is a critical modulator of plasmatic low-density lipoprotein cholesterol (LDLc) uptake by the liver. While PCSK9 gain of function (GOF) mutations induce ADH, loss of function (LOF) mutations lead to low levels of circulating LDLc, thus making PCSK9 a hot target for ADH pharmacological treatment strategies. However, current models to study the role of PCSK9 in ADH or unknown functions are limited. Hypothesis. Hepatic differentiation of patient-derived hiPS cells will provide an accurate and appropriate tool to model PCSK9-mediated ADH and enhance our understanding of PCSK9 functions. We used urine samples as a source of somatic cells in order to obtain hiPSC upon episomal vectors-mediated reprogramming (UhiPSC). After characterization and validation, UhiPS control, carrying the GOF S127R mutation, which leads to an intracellular form of PCSK9 with unclear functions, and LOF R104C/V114A mutations, which lead to a default of PCSK9 secretion, were differentiated into HLCCompare to control cells, HLC-S127R secreted 1.6 time (±0.8; $p<0.05$) less PCSK9, and had a dil-LDL uptake decrease of 3.5 fold (±0.17; $p<0.01$). A 24h pravastatin treatment at 10µM significantly enhanced LDLR and PCSK9 gene expression and PCSK9 secretion in both control and S127R HLC. Finally, while control HLC increased their dil-LDL uptake of a factor 1.38 (±0.49; $p<0,01$), the pravastatin treatment induced a 2.19 fold (±0.77; $p<0.01$) increase of dil-LDL uptake in HLC-S127R, which brought them to a level that was not significantly different from untreated control HLC ($p=0.29$) and was correlated to the original patient response. In an other hand, our preliminary data showed that HLC-R104C/V114A displayed a 2.36 fold increase of LDL uptake compare to control cells, which could be partially inhibited by recombinant PCSK9. Altogether, our study demonstrates that not only patient's urine samples provide an attractive source of somatic cells for reprogramming and hepatocyte differentiation but also a powerful tool to further study PCSK9 functions.

T-1355

TUBEROUS SCLEROSIS PATIENTS' NEURONAL CELLS SHOW ALTERED METABOLIC ACTIVITY AND MITOCHONDRIAL FUNCTION RELATED TO mTORC PATHWAY ACTIVATION

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Tuberous sclerosis complex (TSC) is a hereditary hamartoma syndrome caused by defects in either the TSC1 or TSC2 genes. It has been reported that over 60% of TSC patients have autism. TSC patients also have wide range of behavioral, intellectual, psychiatric, academic, neuropsychological and psycho-social difficulties. Loss of TSC1/2 activates the mTOR pathway, which participates in various biosynthetic processes, including cell growth, protein synthesis, metabolism and cell proliferation. Our research group has previously shown that neurons that lack the functional TSC1/TSC2 complex have increased ER stress and are more vulnerable to environmental damage. Furthermore, TSC- deficient rodent neurons are more vulnerable to oxidative stress and have increased reactive oxygen species (ROS) in the cells. More recently, we have demonstrated that loss of TSC1/2 function in terminally differentiated rat hippocampal neurons results in autophagy initiating signals and accumulation of autolysosomes via the AMPK pathway. Similar dysfunctions were found in cortical tuber samples from TSC patients, identifying a new role for the TSC1/TSC2 complex in the neuronal stress response. Therefore, novel approaches targeting the metabolism of the cells with mTORC1 activation may have broad clinical impact for the future. Currently, rapamycin and other mTORC1 inhibitors are intensively studied in clinical trials. However, comprehensive phenotypic characterization of the patients' neurons at the cellular level is still lacking. Here, we have studied TSC-patients' iPS cell- derived neural cell populations in vitro. We have studied cell morphology, metabolic changes, mitochondrial function, and cell responses to oxidative stress in TSC2 deficient patient specific neurons. As aberrant mTORC1 signaling likely contributes regulation of cell death, morphology, autophagy induction, and AMPK activation, understanding the mTORC1 regulation in TSC2-deficient cells during oxidative stress and ER stress may aid in the development of novel therapeutics for improving TSC related neurodevelopmental deficits in the patients.

T-1356

WISKOTT-ALDRICH SYNDROME IPS CELLS PRODUCE MEGAKARYOCYTES WITH DEFECTS IN CYTOSKELETAL REARRANGEMENT AND PROPLATELET FORMATION AND RESTORATION OF WASP RESCUES THE WAS PHENOTYPE

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Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder characterized by microthrombocytopenia, complex immunodeficiency, autoimmunity, and hematologic malignancies. WAS is caused by mutations in the gene encoding WAS protein (WASP), a regulator of actin cytoskeleton and chromatin structure in various blood cell lineages. Here we report on the successful generation of induced pluripotent stem cell (iPSC) lines from two patients with different mutations in WASP (c.55C>T and c.1507T>A). When differentiated into early CD34+ hematopoietic and megakaryocyte progenitors, the WAS-iPSC lines were indistinguishable from the wild-type iPSCs. However, all WAS-iPSC lines exhibited defects in platelet production in vitro. WAS-iPSCs produced platelets with more irregular shapes and smaller sizes. Immunofluorescence and electron micrograph showed defects in cytoskeletal rearrangement, F-actin distribution, and proplatelet formation. Correction of the WASP gene in WAS-iPSCs improved proplatelet structures and F-actin distribution as well as increased the platelet size. Our findings demonstrate the successful use of iPSCs for disease modeling and provide an important step toward development of genetically corrected cell replacement therapy.

T-1357

PITUITARY HORMONAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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The pituitary is known as the master gland of the endocrine system, reigning vital processes in the body like growth, metabolism, puberty, fertility and stress. Deficient function of the pituitary with insufficient production of one or more hormones (hypopituitarism) disturbs these physiological functions, leading to severe morbidity (e.g. dwarfism, infertility) and increased mortality risk. A cause of hypopituitarism is abnormal embryonic development of the gland, due to (a) genetic mutation(s) in essential regulatory factors like PIT1, PROP1 and HESX1. Most knowledge on hypopituitarism is obtained from genetic mouse models and not much is known about the mechanisms underlying deficient pituitary development in humans. Hence, a human in vitro pituitary model would be highly valuable. Therefore, we started to test and optimize a protocol reported to induce pituitary hormonal cells in human embryonic stem cells (hESC) culture. Treatment of H9 hESC with a BMP inhibitor (transient) and a blocker of TGFβ signaling (continuous) induced preplacodal specification, as exemplified by upregulated expression of *EYA1*, *SIX1*, *DLX3* and *PAX6.* Subsequent transitory activation of the SHH pathway and culture in N2/B27-enriched neurobasal medium stimulated the expression of *TBX19* and *POMC,* the precursor molecule of adrenocorticotropic hormone (ACTH), thus suggesting development of ACTH-expressing pituitary cells in the hESC culture. Immunofluorescence staining confirmed the substantial presence of $ACTH^{+}$ cells in the treated hESC. Currently, induced pluripotent stem cells (iPSC) are being developed from skin fibroblasts obtained from hypopituitarism patients with known genetic defects (e.g. *PIT1, PROP1, HESX1*) to create an in vitro human pituitary disease model. Together, our in vitro models will be fundamental to decipher molecular mechanisms of normal and deficient development of the pituitary in humans, and eventually to evaluate potential drugs for treatment of, and/or repair in, hypopituitarism patients.

T-1358

ANALYSIS OF LRRK2 MEDIATED PARKINSONS DISEASE PATHOGENESIS USING FUNCTIONAL PHOSPHOPROTEOMICS FROM HUMAN IPSC DERIVED NEURONS

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Parkinson's disease (PD) affects over a million Europeans and is expected to significantly increase. No effective treatments are available to prevent or delay disease pathogenesis, and better treatments are urgently needed. The mutation G2019S in the gene *LRRK2* is the most common genetic cause of PD currently known, and is thought to cause PD by increasing LRRK2 kinase activity. Consistent with this finding, inhibition of LRRK2 kinase activity protects against *LRRK2* G2019S induced pathogenesis. However, the exact targets of LRRK2 kinase activity in human neurons remain unknown. To better understand the mechanism of mutant *LRRK2*-induced PD, our group recently generated isogenic induced pluripotent stem cells (iPSCs) from patients with *LRRK2* G2019S, as well as gene-corrected wild type control iPSCs thereof. We demonstrated that iPSC-derived midbrain dopaminergic neurons (mDANs) recapitulated key aspects of PD pathogenesis including increased degeneration and increased alpha-Synuclein protein compared to isogenic controls. Inhibition of LRRK2 kinase activity with various LRRK2 kinase inhibitors protected against PD pathogenesis, suggesting that LRRK2 actively phosphorylates a disease-relevant target. To identify LRRK2 kinase targets in iPSC-derived mDANs, we used mass-spectrometry based phosphoproteomics. Interestingly, we found specific serine residues in TAU as being phosphorylated in a LRRK2-dependent manner. This is particularly interesting because TAU mutations cause Parkinsonism, and TAU pathology has been reported in some PD patients with mutant *LRRK2*. Moreover, we demonstrated previously that TAU expression is higher in human iPSC-derived neurons with *LRRK2* G2019S, compared to isogenic controls. Currently, we are using CRISPR/Cas9-mediated gene targeting to mutate these phosphosites to further assess their contribution to mutant LRRK2-mediated PD. These results suggest a novel and direct connection between two key mediators of PD pathology: TAU and LRRK2.

T-1359

UNDERSTANDING THE NEUROPHYSIOLOGY OF OBESITY CAUSED BY PC1/3 DEFICIENCY WITH HESC-DERIVED HYPOTHALAMIC NEURONS

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Hypomorphism for prohormone covertase 1/3 (PC1/3) results in a complex endocrine-metabolic phenotype that includes obesity, malabsorptive diarrhea and systemic endocrinopathies. To understand the molecular neurophysiology of the obesity in PC1/3 deficient subjects, we generated PC1/3 deficient hESC lines with CRISPR or by knocking down PC1/3 with shRNA. These cells were differentiated into hypothalamic arcuate nucleus (ARC)-like neurons using a protocol recently developed by us. The 90% reduction of proprotein convertase subtilisin/kexin type 1 (PCSK1) mRNA and the absence of mature PC1/3 protein in these neurons confirmed the PC1/3 deficiency. PC1/3 plays a critical role in processing neuropeptides and prohormones such as proopiomelanocortin (POMC), proinsulin, proglucagon in the brain, islets, intestine and elsewhere. POMC neurons in the ARC are the source of α-melanocyte-stimulating hormone (αMSH) which acts on paraventricular nucleus (PVN) neurons to suppress food intake, as well as endorphins such as β endorphin (BEP) that also affect ingestive behaviors. The percentage of POMC neurons in terminally-differentiated cells was not different between control and PC1/3-deficient lines, suggesting that lacking of PC1/3 did not affect neuronal differentiation. However, the ratios of αMSH/

POMC and BEP/POMC proteins were decreased by 25% to 67% in PC1/3 deficient hESC-derived neurons, indicating that POMC processing was impaired by the imposed PC1/3 insufficiency. Levels of mRNA expression of POMC, NHLH2 and CPE, as well as total amounts of unprocessed POMC neuropeptide, were increased 1.7 to 7 fold in the PC1/3 deficient neurons. These findings provide mechanistic insight into the molecular and functional consequences of hypothalamic PC1/deficiency, and demonstrate the potential of stem cell-derived hypothalamic-like neurons for investigation of the neurophysiology and pharmacology of body weight regulation.

T-1360

DUAL OPTICAL RECORDINGS FOR CARDIAC ACTION POTENTIALS AND CALCIUM HANDLING IN IPSC MODEL OF TIMOTHY SYNDROME FOR DRUG DEVELOPMENT

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Reprogramming of human somatic cells to pluripotency has been used to investigate disease mechanisms and to identify potential therapeutics. However, methods used for reprogramming, differentiation, and phenotyping are complicated, expensive and time-consuming. To address the limitations, we first optimized a protocol for reprogramming of human fibroblasts and keratinocytes into pluripotency using single lipofection and the episomal vectors in a 24-well plate format. This method allowed us to generate multiple lines of integration-free and feeder-free induced pluripotent stem cells (iPSCs) from seven patients with cardiac diseases and three controls. Second, we differentiated human iPSCs derived from Timothy syndrome patients into cardiomyocytes using a monolayer differentiation method. We found that Timothy syndrome cardiomyocytes showed slower, irregular contractions and abnormal calcium handling compared to controls. The results were consistent with previous reports using a retroviral method for reprogramming and using an embryoid body-based method for cardiac differentiation. Third, we developed an efficient approach for recording action potentials and calcium transients simultaneously in control and patient cardiomyocytes using genetically encoded fluorescent indicators, ArcLight and R-GECO1. The dual optical recordings enabled us to observe prolonged action potentials and abnormal calcium handling in Timothy syndrome cardiomyocytes. We confirmed that roscovitine rescued the phenotypes in Timothy syndrome cardiomyocytes and that these findings were consistent with previous studies using conventional electrophysiological recordings and calcium imaging with dyes. The approaches using our optimized methods and dual optical recordings will improve iPSC applicability for disease modeling to investigate mechanisms underlying cardiac arrhythmias and to test potential therapeutics.

T-1361

THE USE OF GENOME EDITING AND IPS DIRECTED DIFFERENTIATION SYSTEMS TO MODEL HUMAN LIVER AND PANCREATIC DISEASE IN VITRO

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The liver and pancreas are key "metabolic organs" accordingly some of the largest healthcare problems result from pancreatic and liver dysfunction. Our studies have demonstrated that functional liver and pancreatic cells can be generated on a single directed differentiation platform and both patient derived iPS cell lines and genome engineering technologies can be applied to develop disease models for metabolic disorders including A1ATD, Familial Hypercholesterolemia, and MODY diabetes. The platform utilizes defined conditions to differentiate iPS from disease patients and healthy donors to terminal cell types. In iPS liver cell generation in the first stage, iPS 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. During 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. In pancreatic cell production defined culture conditions differentiate human definitive endoderm (DE) into a near homogenous population of pancreatic endoderm cells. A significant proportion of these cells expressed key beta cell markers and exhibited glucose-responsive C-peptide production at physiologically relevant levels. Genome-editing of iPS lines to generate the disease models has been achieved using CRISPR technology which has been utilized to transfect the cells. The platform has generated iPS disease models for A1ATD, Familial Hypercholesterolemia and MODY diabetes which have been validated at both the genotypic and phenotypic levels using an array of biochemical methodologies. 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.

T-1362

MODELLING NEURAL PATHOLOGY AND DEMENTIA IN DOWN SYNDROME USING INDUCED PLURIPOTENT STEM CELLS

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Down Syndrome (DS) is the most common genetic cause of intellectual disability and is associated with an increased risk of Alzheimer's disease (AD). Triplication of a chromosome 21 (HSA21) gene, amyloid precursor protein (APP) is the main cause of this pathology. Non-DS individuals with AD present with a build-up of amyloid plaques caused by over production of β-amyloid which is produced from APP. At age 35, all DS individuals show build-up of

amyloid plaques but not all will go on to develop AD, suggesting that there are roles for other genes on HSA21 in modulating severity and age of onset of AD. The LonDowns Consortium aims to draw correlations between dementia, cognitive defects, mouse models and genetics with in vitro defects in neurons derived from DS induced pluripotent stem cells (iPSCs). For iPSCs, our two strategies are: (i) isogenic iPSC DS models, and (ii) iPSCs generated from adults and infants, at extremes of the DS spectrum for intensity of pathology. For (i), we developed an integration-free isogenic DS iPSC model by using fibroblasts of an adult with constitutional mosaicism for DS. These isogenic trisomy 21 (T21) and euploid iPSC lines reproduced several cellular pathologies seen in primary DS cells. Subsequent neuronal differentiation, showed increased β-amyloid, abnormalities in mitochondria number and size, and an increase in DNA double strand breaks indicating accelerated ageing. As for (ii), to maximize consent, hair follicles and/or blood samples are collected from participants clinically stratified for cognitive ability and dementia. So far, 120 DS adults have been recruited with 85 keratinocyte lines isolated, with 10 adults considered as extremes. Infant blood samples are at times small, so we optimised methods for generating iPSCs from small numbers of PBMCs using integration-free episomal plasmids. We compared published protocols and β-tested Lonza's new blood reprogramming kit. Using non-DS PBMCs, we could generate iPSCs using all methods, albeit with higher efficiencies using Lonza's kit. With DS, efficiencies decreased, but up to 55 TRA-1-60+ iPSC colonies were obtained using 2×105 PBMCs. iPSC lines have been established from 3 DS infants and 2 adult extremes. Future studies will involve differentiation of neuronal subtypes and evaluation of defects using methods established in our lab.

IPS CELLS: EPIGENETICS

T-1363

AUTOMATED HIGH THROUGHPUT CHIP-SEQ: A VALIDATED TOOL TO CHARACTERIZE EPIGENETIC LANDSCAPES ON INDUCED PLURIPOTENT STEM CELLS

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Induced Pluripotent Stem Cells (iPSCs) are a promising tool for regenerative medicine that still requires substantial epigenomic characterization in order to assess their full potential for clinical use. Currently, most Chromatin Immunoprecipitation (ChIP) methods lack the robustness and throughput required to compare multiple samples. Here we describe an automated high-throughput ChIP-seq pipeline to process induced pluripotent cell lines, which we have validated using a range of representative histone marks, including H3K4me3, H3K27Ac and H3K27me3. We have optimized the most critical steps in order to accommodate hundreds of cell lines with different epigenetic reprogramming backgrounds. This pipeline has allowed us to reduce the variability of the results across technical repeats when compared to the manual version of the protocol and the time required for sample preparation. This ChIP pipeline allows us to generate sequencing libraries with higher complexity and significantly lower background. We are currently extending the use of this platform to other epigenetic marks, such as transcription factors, in order to better characterize the differentiation potential of iPS cell lines from different donors. Ultimately we aim to use this platform to characterize other cells types, such as those currently used for reprograming into iPSCs (fibroblasts and Peripheral Blood Mononuclear Cells-PBMCs) as well as the cells generated following the differentiation of the iPSCs. This will allow us to obtain a full picture of the epigenetic profile of iPSCs and to understand how to predict their performance in a clinical context.

T-1364

TRANSIENT GENOME-WIDE DEMETHYLATION DURING IPSC REPROGRAMMING

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Reprogramming is a continuous process that occurs in a step-wise fashion, where remodelling of epigenetic marks, including DNA demethylation of pluripotency gene promoters, is essential. Moreover, inhibition of DNA methylation has been shown to increase the efficiency of iPSC generation from partially reprogrammed cells. Global DNA demethylation occurs in vivo in the early embryo and in primordial germ cells and in vitro during the transition of primed ESC to naive pluripotency. Here we show that global demethylation also occurs during reprogramming of MEF to iPSC. We assessed the dynamic changes of methylation and hydroxymethylation during piggyBac vector mediated reprogramming, in serum conditions, of intermediate cell populations, early and late passage iPSC and ESC by genome-wide analysis. We demonstrate that cells at early stages of reprogramming undergo global demethylation of the genome reaching CpG methylation levels below 40%, and return to levels similar to those of primed ESC in the late passage iPSC. Furthermore, hydroxymethylation increases to ESC levels in early intermediate cells and this increase precedes global demethylation. Unlike in other reprogramming systems, this transient global demethylation seems to affect all genomic features, including transposons such as IAP, which regain methylation in late passage iPSC. Finally, this transient demethylation appears to be essential for stable erasure of epigenetic memory from pluripotency gene promoters in iPSC and the dynamic patterns of pluripotency gene promoter methylation inversely correlate with transcriptional transitions throughout reprogramming. We are currently addressing the mechanisms of global demethylation during iPSC reprogramming. Global demethylation appears to be a conserved feature of all reprogramming processes whether natural or experimental presumably because it is essential for the erasure of epigenetic memory.

T-1365

DYNAMIC BALANCE BETWEEN UBIQUITINATION-DEUBIQUITINATION IS IMPORTANT FOR IPSC GENERATION

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Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) is a dramatic change of cell fate, thereby, of transcriptional program. Such dramatic reconfiguration of transcriptional programs require chromatin remodeling, which is mediated by numerous histone modifications, most notable being methylation, acetylation, and ubiquitination. Previous loss-of-function studies identified Dot1L, a histone H3 K79 methyltransferase, as an important barrier of iPSC generation. Loss of histone H3 K79 methylation enhanced iPSC formation and small molecule inhibition of Dot1L successfully replaced Klf-4 and c-Myc of the Yamanaka factors. Histone crosstalk studies have shown that histone H2B monoubiquitination is a prerequisite for Dot1L-mediated histone H3 K79 methylation. Therefore, we hypothesized that the ubiquitin pathway, mediated by E3 ubiquitin ligase RNF20/RNF40 and deubiquitinase USP44, is as equally important as Dot1L in iPSC formation. In this study, we examined the reprogramming phenotype of human fibroblasts into iPSCs upon silencing or overexpression of the components of the H2B ubiquitin pathway, RNF20, RNF40, and USP44. Silencing is performed using CRISPR-Cas9 gene editing system, and overexpression is achieved by retroviral transduction of RNF20, RNF40, or USP44 into human fibroblasts. Our preliminary results show that while knockdown of RNF20 and USP44 does not have a significant affect on somatic cell reprograming, silencing of RNF40 result in a subtle increase in İPSC colony numbers. We propose that of the two E3 ubiquitin ligases, RNF40 (BRE1B) plays a more important role in reprogramming than RNF20 (BRE1A). The results are suggestive of a delicate balance in the ubiquitin pathway and its importance in cell fate.

T-1366

JMJD3-MEDIATED HISTONE H3K27 DEMETHYLATION IS A RATE-LIMITING STEP FOR SOMATIC CELL REPROGRAMMING

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The epigenetic mechanisms underlying somatic reprogramming remain largely unknown. H3K27 methylation is an important repressive mark, whose dynamic regulation by the polycomb group protein complex and demethylases governs embryonic development, embryonic stem cell differentiation and reprogramming. Recently, two papers have reported that the H3K27 demethylases Utx and Jmjd3 regulate reprogramming through different mechanisms. Hereby, we wanted to study further the roles of Utx and Jmjd3 in reprogramming, in the presence of vitamin C (Vc), which enhances epigenetic reprogramming efficiency mainly through activating Jumonji histone demethylases and the TET DNA demethylases. Interestingly, we found that ectopic expression of Jmjd3, but not Utx or the enzyme-dead Jmjd3 mutant, greatly reduces global level of H3K27me3 and enhances reprogramming efficiency. We observed that similar to Utx, Jmjd3 deficiency--using either shRNA or knockout--inhibits reprogramming significantly in the presence of Vc. Mechanistically, Jmjd3 promotes epithelial-like gene expression during the early phase mesenchymal-to-epithelial transition and in the late phase contributes to activating the pluripotency gene network. In conclusion, our work has uncovered an important rate-limiting role of H3K27 demethylase Jmjd3 in somatic reprogramming with Vc.

CHROMATIN IN STEM CELLS

T-1367

ANALYSIS OF LONG-NONCODING RNA INTERACTION AT CHROMATIN BY CHROMATIN ISOLATION BY RNA PURIFICATION (CHIRP)

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Gene regulation plays a critical role in complex cellular processes such as development, differentiation, and cellular reprogramming. 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 discovery of the sites of interaction of chromatin-associated RNAs (e.g. lncRNAs) with genomic DNA sequences by using probe-based hybridization to target RNA molecules in chromatin. To perform ChIRP multiple biotinylated oligonucleotide probes complementary to the RNA of interest are used. To help eliminate non-specific signals two different pools of probes are used (even and odd probe sets). These probe sets are combined with chromatin and hybridized to the chromatin-associated RNA. Complexes containing biotinylated-probes bound to the chromatin-associated RNA are then isolated using streptavidin magnetic beads. DNA can then be recovered and analyzed by quantitative PCR or next generation sequencing (ChIRP-seq). Alternatively, RNA may also be isolated from an aliquot of the recovered chromatin to detect other RNA molecules that may be associated with the RNA of interest. 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. Additionally, negative and positive control probe sets, and detection primers were developed to ensure first time success. We have performed ChIRP experiments with a HeLa cell lysate and capture oligos for the NEAT1 lncRNA. Isolated DNA was subjected to NGS library construction and sequenced on an Illumina HiSeq™ instrument. Sequences were aligned to the reference genome (hg19). The peaks were called separately for odd and even reactions, and only common peaks were selected. We successfully identified several NEAT1 binding sites in the genomic DNA sequence.

T-1368

BINDING OF THE NFκB SUBUNIT RELA TO REGULATORY REGIONS OF OCT4, SOX2 AND KLF4 GENES IS ASSOCIATED WITH THEIR TRANSCRIPTIONAL REPRESSION DURING DIFFERENTIATION

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The molecular mechanisms responsible for the maintenance of human pluripotent stem cells (hPSCs) identity are not fully understood. OCT4, NANOG, SOX2, KLF4 and MYC are considered major pluripotency transcription factors (TF) in hPSCs, nevertheless, distinct pathways and downstream TFs are involved in their transcriptional regulation, ensuring the maintenance of pluripotency or differentiation. In this study we investigated the involvement of NFκB TF subunits, responsible for the downstream transcriptional regulation of the alternative canonical (RelA and NFKB1 subunits) and the non-canonical NFκB pathways (RelB and NFKB2 subunits), in the regulation of selected pluripotency factors. To this end, we used the pluripotent embryonal carcinoma cell line NTera-2 as a model, inducing cell differentiation by all trans-retinoic acid (atRA). Binding of the RelA and RelB TFs in putative regulatory regions (containing predicted NF-kB binding sites) of OCT4, SOX2, KLF4 and MYC genes were evaluated by chromatin immunoprecipitation (ChIP); the associated transcriptional regulation was evaluated by quantitative real-time PCR (qPCR). Our results showed that undifferentiated cells exhibited low expression levels of RelA and NFKB1, while cells induced to differentiate for 4 days exhibited upregulated expression of these factors. In contrast, RelB and NFKB2, and the pluripotency factors OCT4, NANOG, SOX2 and KLF4 were expressed in high levels in undifferentiated cells and were downregulated upon differentiation, whereas MYC was upregulated. ChIP revealed that RelA binds to regulatory regions of OCT4, SOX2, KLF4 and MYC only when cells are induced to differentiate, while RelB is bound in both, undifferentiated and differentiated cells. Our data suggests that the canonical NFκB pathway may be associated to differentiation, by downregulating key pluripotency genes (although inducing MYC) and that non-canonical NFκB pathway may act in the maintenance of pluripotency.

T-1369

CHROMATIN INTERACTIONS AT DEVELOPMENTAL GENES LOCI ARE REESTABLISHED BY SOMATIC CELL REPROGRAMMING

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Higher-order chromatin structures are involved in gene regulation. In pluripotent stem cells, it has been known that several developmental genes are in a poised state. Recent works have demonstrated that chromatin interactions between enhancer and promoter regions of pluripotency genes are reorganized prior to their reactivation during somatic cell reprogramming. However, little is known about the changes of chromatin structures at the developmental gene loci. Here, we report genome-wide analysis of chromatin interactions at developmental gene loci during iPSCs induction by using 4C-Seq, a method for detecting long-range chromatin interactions with a bait locus. First, by combining 4C-Seq with multiplexed PCR system, we analyzed chromatin interaction profiles both in iPSCs and their original cells at several tens of bait loci simultaneously. As a result, we found that developmental gene loci are colocalized with each other in iPSCs. In addition, 3D DNA FISH analysis showed similar chromatin interaction profiles in human iPS cells and ES cells at the single-cell level. These data suggest that reorganization of chromatin 3D structures occurred at the developmental gene loci as well as pluripotncy gene loci by somatic cell reprogramming. To investigate chromatin states that are related to the colocalization of developmental gene loci, we integrated our 4C-Seq data to public data sets on ChIP-Seq. By the bioinformatics approach, we observed that some epigenetic modification and their regulatory proteins are enriched both in developmental gene loci and their interacted loci, indicating that epigenetic regulation might play an important role in chromatin reorganization of developmental genes loci during somatic cell reprogramming. Taken together, our study provides an insight into regulatory mechanisms of developmental genes in cell fate conversion.

T-1370

EARLY DEVELOPMENTAL GENES REGULATED BY H3K27ME3 DEMETHYLASE INHIBITOR

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The removal of histone H3 trimethylation at lysine residue 27 (H3K27me3) plays a critical role in the transcriptional initiation of developmental genes. The H3K27me3-specific demethylases JMJD3 and UTX are responsible for the transcriptional initiation, whereas other genes expressed in a demethylation-independent manner. To address the role of H3K27me3 in retinoic acid (RA)-induced differentiation of the human carcinoma NCCIT cell line, we inhibited JMJD3 and UTX using the H3K27me3 demethylase inhibitor GSK-J4. Cell commitment of JMJD3/UTX-inhibited cells was delayed, and transcriptome profiling also revealed the differential expression of genes related to cell fate specification in demethylase-inactivated cells; RA metabolism and HOX family genes displayed significant expression decreases. We observed weak correlation between H3K27me3 enrichment and transcriptional repression in the control and JMJD/UTX-inhibited cells, except for a few sets of developmental genes indispensable for cell fate specification. Taken together, these results provide the H3K27me3 landscape of a differentiating cell line and suggest that both demethylase-dependent and -independent transcriptional regulation play a role in early differentiation and developmental gene expression activated by H3K27me3 demethylation.

T-1371

CANCER-LIKE EPIGENETIC CHANGES ASSOCIATED WITH GENOMIC INSTABILITY OF HUMAN PLURIPOTENT STEM CELLS

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The capacity of human pluripotent stem cells (hPSC) to proliferate without known limit and differentiate into all the cell types found in adult body makes them promising tool for regenerative medicine but also causes challenges for clinical applications. The ability for indefinite regeneration makes hPSCs similar to cancer cells. In addition, hPSCs have other cancer-like characteristics, like high telomerase activity and high expression of certain oncogenes. Transplanted hPSCs can in fact form teratomas or even malignant teratocarcinomas in animals. The tumorigenicity of hPSCs is the greatest obstacle to be overcome before applying transplantations in the clinics. So far many studies have already discovered hPSCs can harbour mutations and epigenetic changes that may change their growth, differentiation and tumorigenic capacity. The goal of our study was to determine whether common changes in epigenetic regulation associated with genomic instability of embryonic hPSCs could be identified. We studied genome-wide CpG methylation patterns in different hPSC lines with reduced representation bisulphite sequencing (RRBS). We used cells lines pre- and post-transformation to abnormal karyotype and hPSC lines that have never transformed. In addition we compared DNA methylation between hPSCs, embryonal carcinoma and T cell lymphoma cells. As a result we discovered thousands of differentially methylated regions between normal and abnormal hPSC lines. Some of these regions were differentially methylated between normal and abnormal karyotypes in all of the hPSC lines. Some of the changes had also affected transcription of the closest genes. The affected genes were linked to cell growth, proliferation, survival, differentiation, oncogenesis and maintenance of genomic integrity. One of the most interesting CpG site was hypermethylated in all of the abnormal and embryonal cancer cell lines compared to normal cells. This site localizes in a CpG island in the promoter of a gene functionally important for cellular defence against DNA damage. The hypermethylation of this site was associated with loss of active promoter mark H3K4me3 and silencing of transcription in abnormal cells. Our findings reveal that abnormal hPSCs do indeed harbour distinctive, cancer-like epigenetic patterns after the transformation process.

T-1372

DIFFERENTIAL ASSOCIATION OF CHROMATIN PROTEINS (D-CAP) IDENTIFIES BAF60A/SMARCD1 AS A REGULATOR OF EMBRYONIC STEM CELL DIFFERENTIATION

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Embryonic stem cells (ESCs) possess a distinct chromatin conformation maintained by specialized chromatin proteins. To identify chromatin regulators in ESCs, we developed a simple biochemical assay (D-CAP: Differential Chromatin Associated Proteins) using brief micrococcal nuclease digestion of chromatin, followed by LC-MS/MS. Using D-CAP, we identified several differentially chromatin-associated proteins between undifferentiated and differentiated ESCs, including the chromatin remodeling protein SMARCD1. SMARCD1-depletion in ESCs led to altered chromatin and enhanced endodermal differentiation. Gene expression and ChIP-Seq analyses suggested that SMARCD1 is both an activator and a repressor, is enriched at developmental regulators, and its chromatin-binding coincides with H3K27me3. SMARCD1-knockdown caused an extensive H3K27me3 redistribution and increased H3K4me3 levels around the TSS. One of the identified SMARCD1-targets in ESCs was Klf4. In SMARCD1-KD clones, Klf4, as well as H3K4me3 levels at the Klf4 locus, remained high, and H3K27me3 was abolished. These results propose a role for SMARCD1 in restricting pluripotency and activating lineage pathways by regulating H3K27 methylation.

GERMLINE CELLS

T-1374

CHARACTERIZATION OF MOLECULAR MECHANISMS DEFINING GERMLINE PROGENITOR CELL HETEROGENEITY AND FATE

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Maintenance of fertility in adult males is dependent on a pool of undifferentiated germline cells (spermatogonial stem/progenitor cells; SPCs) within the testis that self-renews and generates differentiating germ cells for production of spermatozoa. While all SPCs may possess stem cell potential, heterogeneity at the levels of cellular morphology and gene expression predicts differing propensities for self-renewal and differentiation. However, the molecular basis for these switches in SPC fate tendencies remains unclear. Here we define in detail the molecular signatures of self-renewing and differentiation-prone SPC populations and characterize key signalling pathways affecting adoption of these distinct cell states. Through use of a novel transgenic reporter line based on promoter elements of *Promyelocytic Leukemia Zinc Finger (Plzf)*, a gene highly expressed throughout the SPC pool, we could successfully mark the SPC population including long-term self-renewing cells. Importantly, by generating compound strains incorporating this and other distinct transgenic reporters demonstrating preferential activity in differentiation-prone SPC subsets we have isolated enriched fractions of self-renewing and differentiation committed SPCs. Subsequent analyses on sorted populations have allowed us to profile gene expression changes occurring upon transition between these distinct SPC states and suggested involvement of the mRNA translation machinery in the switch from self-renewing to differentiating fates. Strikingly, we found that differentiation-prone SPCs displayed elevated activation of the mTORC1 pathway, a key regulator of mRNA translation, when compared to SPCs with high self-renewal potential. Further, aberrant mTORC1 activation in vivo was found to promote adoption of a differentiation-prone SPC state, confirming a critical instructive role for this pathway in regulation of SPC fate. Continuing studies are aimed at elucidating key translational targets downstream the mTORC1 pathway in SPCs and their relevance to germline maintenance.

T-1375

EFFICIENT PRODUCTION OF TRANSGENIC MICE BY INTRA-CYTOPLASMIC INJECTION OF STREPTOLYSIN-O-TREATED SPERMATOZOA

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Many methods for efficient production of transgenic animals for biomedical research have been developed. Despite great improvements in transgenesis rates resulting from the use of intracytoplasmic sperm injection (ICSI), the ICSI-based spermmediated gene-transfer (iSMGT) technique is still not optimal in terms of sperm permeabilization efficiency and subsequent development. Here, we demonstrate that streptolysin-O (SLO) can efficiently permeabilize mouse spermatozoa, leading to improved developmental competence and high transgenesis rates in iSMGT embryos and pups. In particular, the most efficient production of iSMGT-transgenic embryos resulted from pretreatment with 5 U/mlSLOfor 30 min and co-incubation with 1.0 ng/ml of an EGFP expression vector. By incubating spermatozoa with Cy-3-labelled DNA, we found that fluorescence intensity was prominently detected in the head region of SLO-treated spermatozoa. In addition, blastocyst development rate and blastomere survival were greatly improved by iSMGT using SLO-treated spermatozoa (iSMGTSLO) as compared to freeze-thawed spermatozoa. Consistent with this, a high proportion of transgenic offspring was obtained by iSMGT-SLO after transfer into foster mothers, reaching 10.6% of the number of oocytes used (42.3% among pups). Together with successful germline transmission of transgenes in all founders analyzed, our data strongly suggest that SLO makes spermatozoa amenable to exogenous DNA uptake, and that the iSMGT-SLO technique is an efficient method for production of transgenic animals for biomedical research.

T-1376

EFFECTS OF CULTURE MEDIA ON SURVIVAL AND PROLIFERATION OF PORCINE GONOCYTES AND SPERMATOGONIAL STEM CELLS

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Gonocytes and spermatogonial stem cells (SSCs) are subpopulations of testicular stem cells in testis that have potency to differentiate into sperm. Identification and isolation of porcine gonocytes/SSCs is essential for studying their self-renewal and differentiation, mechanism of spermatogenesis and genetic modification. However, maintenance and proliferation of porcine testicular stem cells (pTSCs) in culture is limited by the lack of an appropriate in vitro culture system. Here, testes from juvenile (5 days old) and adult (11 months-4 years old) pigs were enzymatically dissociated using two digestion steps. Thereafter, porcine testicular cells were cultured in three different culture media, KSOM, MEM α and StemPro-34 up to 20 days. The highest proliferation of testicular cells was observed both in StemPro-34 (65-83%) and in MEM α medium (65-87%) for both age groups. The presence of pTSCs was proven by morphological observation and expression analysis of specific markers for undifferentiated germ cells (PGP 9.5; protein gene product 9.5) using RT-qPCR and immunostaining. Germ-like stem cells colonies were only observed in StemPro-34 medium. However, expression of the PGP 9.5 marker was significantly higher in MEM α medium compared to KSOM and StemPro-34 medium in both age groups. Supplementation of KSOM medium with 10% FCS, which led to a similar serum content as in MEM α, resulted also in similarly high expression of PGP 9.5. Thus, it can be concluded that higher expression of PGP 9.5 observed in MEM α was probably due to the higher serum content. Subsequently, the effects of hormones (FSH and testosterone) and growth factors (GDNF and IGF-1) in different concentrations were investigated in StemPro-34 as culture medium. However, none of the tested supplements had a significant effect on PGP 9.5 expression in culture. Although a long-term in vitro culture system in which pTSCs can be maintained and proliferated could not yet be established, the present study suggests that StemPro-34 medium is beneficial for formation of germ-like stem cell colonies and survival of porcine testicular cells. Further studies on the identification of factors that promote proliferation of porcine testicular stem cells are needed.

T-1377

NO DDX4-EXPRESSING FUNCTIONAL OOGONIAL STEM CELLS IN ADULT HUMAN OVARIES

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Whether or not oocyte regeneration occurs in adult life has been the subject of much debate. The generally accepted view that oocytes are not renewed in postnatal or adult mammalian ovaries has been recently challenged by reports of oogonial stem cell (OSC) isolation from adult mouse and human ovaries. In this study, we followed the reported protocol and isolated the putative OSCs by fluorescence-activated cell sorting (FACS) from adult human ovaries for further characterization. We dissociated ovarian cortical tissue biopsied from reproductive-age women to single cells and isolated a small population of cells by FACS using an antibody against DEAD box polypeptide 4 (DDX4), as previously reported. We did not, however, detect any DDX4 expression in the sorted "positive" cells by qPCR or by single cell mRNA sequencing, indicating that the reported DDX4 antibody based FACS method is not selecting DDX4 expressing cells. To investigate the stem cell properties of the isolated "positive" cells, we established in vitro cultures. Again, we found no DDX4 expression by qPCR or by immunocytochemistry, although both the cultured "positive" and "negative" cells appeared positive for DDX4 by FACS analysis. As a functional test, we injected the cultured cells into human ovarian cortical tissue samples and transplanted them into SCID mice. The injected cells survived in the grafts, but we could not observe any oocyte differentiation from the cells even after 4 weeks of transplantation. Based on our experimental evidence, we conclude that the human ovarian cells isolated by the DDX4 antibody based FACS method are neither specific DDX4-expressing cells nor are they functional germline stem cells that can regenerate oocytes. Therefore, more careful studies should be performed with stricter standards before characterizing these cells as OSCs.

TOTIPOTENT/EARLY EMBRYO CELLS

T-1378

SINGLE CELL TRANSCRIPTIONAL ANALYSIS: DELINEATING CELL LINEAGE AND PLURIPOTENCY DURING HUMAN BLASTOCYST FORMATION

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Characterizing embryonic development during the first and arguably the most critical week of human development is of great importance. During the first 7 days the human embryo divides to form the first three lineages: trophectoderm (TE), primitive endoderm (PE), and pluripotent epiblast cells (EPI). To date, limited information exists pertaining to the transcriptional landscape and what drives lineage segregation and pluripotency in humans. Using Smart-seq2 single-cell RNA-sequencing, we have now established a transcriptional road map consisting of ~1600 cells from 71 embryos, covering 8-cell stage to mature blastocyst at embryonic (E) day 7. Our data describe the temporal progression of lineage segregation where TE vs ICM segregation occurs in the transition from E4 to E5. GO-analysis identifies genes linked to cell-cell junctions and epithelial polarization within the TE progenitors and genes linked to stem cell maintenance, protein kinase cascade and WNT signaling in the ICM cells. Top genes include GATA2/3, PTGES, DAB2, TGFBR3, TEAD1 and NANOG, SOX2, IFITM, GDF3, BMP2 within the TE and ICM respectively. Concurrently at E5 we do detect a priming of ICM cells towards EPI and PE segregation, which progressively mature during E6 and E7. During this progression, GO-analysis points to genes linked to cell migration, epithelial morphogenesis and endoderm formation in emerging PE cells whereas the EPI cells express genes linked to stem cell maintenance and the Nodal, Notch and WNT signaling pathways. Top genes include: FN1, COL4A, GATA4/6, PDGFRA and SOX2, PRDM14, NANOG, FGF4 within the PE and EPI respectively. The functional importance of identified signaling pathways is currently being explored. The fundamental knowledge elucidated from this study is crucial in identifying mechanistic pathways underlying lineage segregation and the establishment of pluripotency, thus being of great importance for understanding human development and regenerative medicine.

T-1379

METABOLIC RESCUE THROUGH MTDNA GENOME CORRECTION IN PLURIPOTENT CELLS FROM PATIENTS WITH MITOCHONDRIAL DISEASE

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Mitochondria play a major role in energy production via oxidative phosphorylation (OXPHOS) that is dependent upon the expression of critical genes encoded by mitochondrial DNA (mtDNA). Germline mutations in mtDNA can cause fatal or severely debilitating disorders in children for which there is no cure. Clinical manifestations of mtDNA disease vary based on specific mutation and ratio of mutant to wild type (wt) mtDNA within each cell (heteroplasmy). To explore the feasibility of generating genetically corrected pluripotent stem cells (PSC) for future gene/cell therapies, we generated several induced pluripotent stem (iPS) cell lines from a MELAS patient caused by heteroplasmic 3243A>G mutation and from the Leigh syndrome patient caused by homoplasmic 8993T>G mutation. Due to spontaneous segregation of heteroplasmic mtDNA in proliferating fibroblasts, isogenic MELAS iPS cell lines were recovered containing exclusively wt mtDNA. These cells displayed normal metabolic function upon differentiation compared to MELAS iPS cells containing mutant mtDNA. As expected, all iPS cell lines from homoplasmic Leigh syndrome fibroblasts carried exclusively mutated mtDNA. We then simultaneously replaced mutant mtDNA and reprogrammed Leigh fibroblasts using somatic cell nuclear transfer (SCNT), and generated genetically rescued NT-ESCs. NT-ESCs displayed normal metabolic function compared to impaired oxygen consumption and ATP production in iPS cells. Moreover, NT-ESCs displayed normal expression both mtDNA and nuclear-encoded mitochondrial genes indicative of normal nuclear-to-mitochondrial interactions. We conclude that natural segregation of heteroplasmic mtDNA in individual iPS cells can be exploited for derivation of stem cells with exclusively wt mtDNA. Moreover, SCNT offers mitochondrial replacement while preserving the nuclear genetic contribution of patients with homoplasmic mtDNA disease.

T-1380

POTENT AND RAPID ANTIBACTERIAL ACTIVITY OF CONDITIONED MEDIUM FROM MOUSE EMBRYONIC FIBROBLAST FEEDER CELLS IN VITRO

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Mouse embryonic fibroblast (MEF) feeder layers prevent embryonic stem cell differentiation. Conditioned medium (CM) from MEF feeder layers contains proteins that participate in cell growth and differentiation, extracellular matrix formation and remodeling, in addition finding of many interesting proteins. The objective of this study was to test the hypothesis that MEF-CM possessed intrinsic antimicrobial properties. We studied for the first time the effect of MEF-CM on the bacterial growth of Gram-negative (*Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas aeruginosa* ATCC 9027), Gram-positive (*Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 122228) and clinical antibiotic-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria. The MEF-CM did not kill selected Gram-negative bacteria. Meanwhile, treated Gram-positive and MRSA bacterial lost their ability to divide long-term propagation in comparison to control medium. Moreover, MEF-CM exerted its antibacterial activity via a bactericidal mechanism, which the CM

killed *Staphylococcus aureus* ATCC 25923 through the disruption of their integrity membranes. Taken together, conditioned medium from mouse embryonic fibroblast feeder layers can be considerable potential for therapeutic application as novel molecules candidate for treating Gram-positive bacterial infections.

EMBRYONIC STEM CELL DIFFERENTIATION

T-1381

MODELING MOTOR NEURON DEGENERATION IN AMYOTROPHIC LATERAL SCLEROSIS USING EMBRYONIC STEM CELLS

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Pluripotent stem cell-derived neurons can be used to model diseases, elucidate mechanisms of neurodegeneration and perform drug screens in vitro. In Amyotrophic Lateral Sclerosis (ALS) a majority of motor neurons (MNs) in the spinal cord and brainstem, that innervate voluntary muscles, degenerates, while oculomotor neurons (OM MNs) in the midbrain are spared. The reasons for this differential vulnerability in disease are unknown. The goal of our studies was to derive MN populations with different vulnerabilities to degeneration in ALS from mouse embryonic stem cells (mESCs) and model their differential susceptibility to disease in vitro. Using extrinsic and intrinsic signals known to specify distinct MN populations along the anterior-posterior axis of the embryo, we could direct differentiation of our mESC cultures. Specifically, spinal MNs were patterned using retinoic acid (RA) and sonic hedgehog (Shh) and OM MNs using Shh and fibroblast growth factor 8. Forced expression of the intrinsic determinant Phox2A increased the yield of resistant OM MNs, while Olig2 further directed mESCs into spinal MNs. The identities of the MNs were confirmed by staining for subpopulation specific markers (a combination of Islet1 and Phox2A) and by electrophysiological recordings. Furthermore, we are developing in vitro ALS-like toxicity assays in which these MN populations should show differential vulnerability. We believe that these in vitro systems will aid in identifying future targets for the treatment of ALS, as they allow gain- and loss-of-function studies for candidate genes that could play a role in MN protection and differential vulnerability.

T-1382

MASS PRODUCTION OF HUMAN CARDIOMYOCYTES FROM ES AND IPS CELL AGGREGATES IN STIRRED BIOREACTOR VIA A ROBUST AND STEPWISE CHEMICALLY SIGNALING HIJACKED INTERLINE VARIABILITY

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Recent clinical studies using cardiomyocytes (CMs) from human pluripotent stem cells (hPSCs) offers a promising approach for treating patients with cardiovascular disease, which is the leading cause of death globally. Here we developed an integrated and simplified platform for large scale generation of highly homogenous hPSC-CM aggregates under stepwise chemically defined culture conditions which has resulted in high efficacy, reproducibility and universality. With this approach, approximately 100% of the cardiospheres showed spontaneous contractility, expressed lineage-specific markers and contained highly enriched (up to 90% cTNT+ and MHC+ cells) CMs that showed high functionality in vitro from multiple hPSC lines (5 hESC and 4 hiPSC) without cell sorting or selection. To the best of our knowledge, this is the first time that highly enriched CMs derived from hPSCs have been produced in a simplified batch and single unit operation using a scalable stirred suspension bioreactor. These hPSC-CMs may provide a potential cell source for cardiac regenerative therapy and research applications.

T-1383

IT'S ALL ELEMENTARY: COPPER TRANSPORT AND MITOCHONDRIAL ROS AS REGULATORS OF GERM LAYER SPECIFICATION

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Copper is an essential trace element, serving as a cofactor for a range of enzymes and proteins. The import of copper is facilitated by the high affinity solute carrier, Copper transporter 1 (Ctr1). Ctr1 is crucial to development as Ctr1-null (Ctr1-/-) embryos die in utero due to a gastrulation defect. This lack of mesoderm has been phenocopied in Ctr1-/- mouse embryonic stem (ES) cell in vitro differentiation. In contrast to mesoderm, we have recently found that Ctr1-/- ES can generate both endodermal and ectodermal lineages in vitro. While endoderm formation is unaffected by Ctr1 gene dosage, a lineage bias is observed during ectoderm formation. Ctr1-/- ES are capable of generating Nestin+ neural stem cells and NeuN+ neurons. Strikingly, Keratin 14+ surface ectoderm cell differentiation is greatly enhanced in the absence of Ctr1. This raised the question as to what copper- dependent processes are influencing Ctr1-/- ES differentiation? One candidate is the cuproenzyme cytochrome c oxidase, complex IV of the electron transport chain within mitochondria. We therefore assessed mitochondrial reactive oxygen species (ROS) generation, an indicator of mitochondria function and cellular stress. We used NpFR2, a novel fluorescent probe to assess mitochondrial ROS by flow cytometry. Mitochondrial ROS was reduced in undifferentiated Ctr1-/- ES was compared to wild type ES cells. After two days of mesodermal differentiation there was an even greater reduction of ROS generation in Ctr1-/- cells relative to Ctr1+/+ cells. No difference in ROS generation was observed in ectodermal conditions. The absence of Ctr1 reduces ROS production during mesoderm induction, implicating copper as a crucial component in gastrulation. Future directions aim to identify whether mitochondrial ROS, regulated by copper transporter levels, is responsible for the lineage bias observed during Ctr1-/- ectodermal differentiation.

T-1384

CEREBRAL ORGANOID CULTURE FROM EMBRYONIC STEM CELLS: A MODEL SYSTEM OF BRAIN DEVELOPMENT

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Brain size varies immensely among species, with that of humans being the largest and most complex relative to body mass. However, the mechanisms underlying this expansion remain unknown. Insights into this process have been gained through the identification of different neural progenitor subtypes that are preferentially expanded in the human brain. Characterization of these human specific features remains difficult due to the inaccessibility of human tissue. Recent studies have shown that human and mouse embryonic stem cells have the ability to form 3D structures called cerebral organoids that recapitulate many aspects of brain development. These features include the formation of distinct progenitor populations that promote cortical growth, and the generation of a range of cortical neuron subtypes with some signs of laminar organization. Our goal is to optimize organoid culture methods and use this system to elucidate the role of transcription factors hypothesized to function in regulating brain growth. We first compared two methods for human organoid production (Kadoshima et al. 2013; Lancaster and Knoblich 2014), and found the Kadoshima method more efficient, reproducible, and consistent in producing forebrain progenitors. We also established a modified protocol for mouse organoid production based on two methods (Eiraku et al. 2008; Nasu et al. 2012). Cerebral organoids were characterized at various time points and found to mimic developmental stages seen in human and mouse fetal tissue. These results establish cerebral organoids as an innovative tool for modeling normal and abnormal brain development in vitro.

T-1385

CENTROSOME FUNCTIONS IN DIFFERENTIATING CELLS: AN INSIGHT INTO BIOLOGY OF THE CENTROSOME USING HUMAN EMBRYONIC STEM CELLS

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Recent years have seen major progress towards the understanding of function of centrosome, an organelle acting as the major organizer of microtubule arrays. Furthermore, previously unexpected links between centrosome anomalies and a plethora of human diseases and developmental defects have been uncovered. This in turn raises the prospect of exploiting centrosome-related pathways for therapeutic applications. Moreover, recent evidence from animal models strongly suggests a context-dependent role for a centrosome in proliferation and differentiation. Hence, this raises an intriguing possibility of cell type-specific requirements for a correct number of centrosomes during proliferation and terminal differentiation. However, our current understanding of the role of centrosome in such processes is surprisingly sparse. Further, abnormalities in centrosome number and/or structure are often found in tumors, but direct causalities between numerical/structural centrosome abnormalities and cancerogenesis are yet to be established. It is clear that understanding of the different roles of centrosome in physiologically-relevant conditions is of general interest. However, the role of the centrosome in human cells has been so far studied using immortalized cancer cell lines, which often suffer from centrosomal and/or chromosomal alterations and deregulated cell cycle. Here we present our ongoing work on studying centrosome abnormalities and their functional consequences in hESCs. We use manipulation of gene expression of critical regulators of centrosome duplication cycle, namely Plk4 and STIL, to specifically alter the centrosome numbers. We are currently analyzing the impact of centrosome alterations induced in such manner on hESCs proliferation rate, survival, and ability to differentiate (EBs, neuronal precursors).

T-1386

IDENTIFYING CELL SURFACE MARKERS FOR THE ISOLATION OF PANCREATIC PROGENITOR CELLS

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Insulin administration is the current treatment for Type 1 Diabetes patients, but it is not a cure. Alternative therapies, which provide better glycemic control, and restore physiologically regulated insulin secretion, will help prevent long-term complications associated with the disease. Human Embryonic Stem Cells (hESCs) hold the potential to generate an unlimited supply of beta-cells that could be used for this very purpose. Several studies have demonstrated that it is possible to generate pancreatic progenitor cells (PPs) from hESCs, that give rise to functional beta-cells (and other mature pancreatic cells) when transplanted into mice. However, the percentage of PPs generated in vitro is cell-line dependent ranging from 6 to 80 percent. Increasing purity would be beneficial to generate a safer cell product for future clinical use. Our aim is to identify cell-surface markers that would allow us to purify the PPs. hESCs were differentiated using culture conditions which support development of either PPs or a polyhormonal population (PH) that does not generate functional beta-cells in vivo. The cells within the two populations, along with undifferentiated hESCs were used for the selective enrichment of N-glycoproteins and compared by mass spectrometry. We positively identified known markers of undifferentiated hESCs such as CD90, KDR and PTPRZ1, as well as novel markers of the PP and PH populations. Of particular interest, members of the FGF family of ligands and receptors, which are known to be important for pancreas development, were enriched in the PP population. Here we present validation of these markers by qPCR, flow cytometry, immunocytochemistry and immunoblotting. The identification of PP, PH and hESC-specific surface markers will allow us to prospectively isolate the PPs from contaminating cultures, either by positive or negative selection, and would provide a tremendous tool not only for cell therapy purposes, but also to understand beta-cell lineage commitment. Future work will focus on using these markers to enrich for PPs, and then determine their developmental potential through transplantation in immunocompromised mice. The enriched population may also be used to generate mature beta-like cells in vitro with greater efficiency.

T-1387

USING BONE MORPHOGENIC PROTEINS TO DIRECT SPINAL DORSAL INTERNEURON IDENTITY

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Distinct classes of neurons in the spinal cord are required to process sensory information from the periphery and execute motor functions. Both modalities must be restored to patients with injured spinal cords for them to effect a full recovery. However, although considerable progress has been made deriving spinal motor neurons in vitro, relatively little progress have been made directing stem cells towards spinal sensory neurons. Six classes of sensory interneurons (INs) arise in the dorsal spinal cord during development, in response to secreted signals from the roof plate (RP) at the dorsal midline. These signals include members of the Bone Morphogenetic Protein (BMP) family, which have been shown to direct neural progenitors towards dorsal fates. Surprisingly, the mode by which the BMPs specify interneuron identities remains unresolved. BMPs may direct cell fate in a concentration-dependent manner similar to Sonic hedgehog (Shh) the canonical morphogen that patterns the ventral spinal cord. However, unlike Shh, there are many BMPs present in the RP, including BMP4, BMP5, BMP6, BMP7 and Growth/Differentiation Factor (GDF) 7. Thus, an alternative hypothesis is that individual BMPs act in a signal-specific manner to direct distinct sensory IN fates in the dorsal spinal cord. We are distinguishing between these hypotheses by exposing mouse embryonic stem cells (mESCs) to different combinations and concentrations of the RP-resident BMPs. Our studies so far suggest that the different BMPs have distinct activities specifying dorsal IN identities. The ability to derive spinal sensory INs from mESCs will permit future studies examining how sensory function can be restored to paralyzed patients.

T-1388

RATIONAL DESIGN OF CELL FATE

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Direct reprogramming of somatic cell can serve as an important new source of human cells for modeling disease or for cellular-replacement therapies. Compared to iPSCs, direct reprogramming could obviate the need for the long reprogramming processes, bypass the maturation steps of embryogenesis and produce postnatal cell types. However, one major hurdle in this process is selecting the ideal reprogramming factors, which typically requires laborious testing for the optimal combination of transcription factors (TF) phase. We have previously designed a computational model named Core Expression Module Algorithm (CEMA), which identifies uniquely expressed genes in various cell types, by comparing sets of gene expression data from a host of cell types. This gene list was further narrowed to only include highly expressed TFs. For each cell type, 3 to 10 genes were found, representing a unique cell signature. We hypothesize that forced expression of these identified genes will derive lineage conversion to a specific desired cell type. Alternatively, CEMA defined TF sets could be used to program pluripotent stem cells (PSC) towards specific cell types. To test this we have engineered CEMA-identified sets for several cell types (keratinocytes, neural progenitor and endothelial cells) into an inducible lentiviral constructs and following infection of human fibroblasts or PSC, we induced expression by doxycycline (dox) admission. Our data suggests that expression of CEMA-selected TFs can induce morphology conversion and activate expression of endogenous markers. These changes were retained upon dox withdrawal, indicating that at least partial reprogramming can be achieved using ectopic expression of CEMA-selected genes. Similarly, hPSC engineered to express CEMA factors exhibit improved differentiation and maturation capacity. Further optimization of reprogramming/ programming conditions as well as a more comprehensive characterization of reprogrammed/ differentiated progeny is required. However, our results demonstrate a proof of concept for the feasibility of using CEMA (which will be an open database) for rational selection of factors for lineage conversion or direct differentiation, for the purposes of cell-based therapeutics, modeling human development, or modeling human diseases in a dish.

T-1389

L-PROLINE MEDIATED SIGNALLING ACTIVITY DURING THE DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS TO EARLY PRIMITIVE ECTODERM-LIKE CELLS

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Mouse embryonic stem cells (ESC) can be differentiated towards neurectoderm in the presence of exogenously added L-Proline. This amino acid acts as a novel growth factor stimulating differentiation to neural progenitors via embryologically relevant intermediate cell types. Initially, culturing ESCs with 400 µM L-Proline and 330 U/mL Leukaemia Inhibitory Factor (LIF) allows differentiation into a pluripotent population of early primitive ectoderm-like (EPL) cells. These cells are analogous to the in vivo primitive ectoderm in both gene expression and functional capacity. Further differentiation with L-Proline in the absence of LIF produces successive changes in gene expression consistent with the production of definitive ectoderm, then neurectoderm and, finally, mature neural cell types. We aim to understand the L-Proline-mediated signalling activity underpinning ESC differentiation, particularly from ESCs to EPL cells. qPCR confirmed that ESCs converted to EPL cells as measured by the increase in expression of *FGF5* to 7.3±2.9 fold (SEM; $P<0.05$) and the decrease in expression of *Nanog* to 0.6±0.1 fold (SEM; $P<0.05$) compared to an ES control. Western blot analysis shows that serum-starved ESC and EPL cells were responsive to L-Proline. E.g., in ESCs, phosphorylation of ERK1/2 increases 7-fold ($P<0.0001$), STAT3 2-fold ($P<0.05$) and S6 kinase 3-fold ($P<0.05$) compared to an ES control. However, in standard culture medium, the basal activity of these pathways did not differ between ESC and EPL cells. Instead, basal pathway activity changes upon further differentiation to multipotent cell types; STAT3 phosphorylation decreased to 0.10 ± 0.02 fold (SEM; $P<0.0001$) and S6 Kinase phosphorylation increased to 6.8 ± 2.3 fold (SEM; $P<0.05$). Various inhibitors revealed which pathways control specific aspects of cellular function, such as differentiation, proliferation, apoptosis and morphology changes. In

summary, L-Proline mediates the conversion of ESCs to EPL cells through activation of a number of signalling pathways. Blocking these pathways prevents this conversion. Studying these pathways will allow us to understand early embryogenesis and develop homogenous cell types for use in disease modelling.

T-1390

CELL POLARITY AND NEUROGENESIS IN EMBRYONIC STEM CELL-DERIVED NEURAL ROSETTES

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Embryonic stem cells (ESCs) undergoing neural differentiation form radial arrays of neural stem cells, termed neural rosettes. These structures manifest many of the properties associated with embryonic and adult neurogenesis, including cell polarization, interkinetic nuclear migration (INM), and a gradient of neuronal differentiation. We now identify novel rosette structural features that serve to localize key regulators of neurogenesis. Cells within neural rosettes have specialized basal as well as apical surfaces, based on localization of the extracellular matrix receptor β1 integrin. Apical processes of cells in mature rosettes terminate at the lumen, where adherens junctions are apparent. Primary cilia are randomly distributed in immature rosettes and tightly associated with the neural stem cell's apical domain as rosettes mature. Components of two signaling pathways known to regulate neurogenesis in vivo and in rosettes, Hedgehog and Notch, are apically localized, with the Hedgehog effector Smoothened (Smo) associated with primary cilia and the Notch pathway γ-secretase subunit Presenilin 2 associated with the adherens junction. Increased neuron production upon treatment with the Notch inhibitor DAPT suggests a major role for Notch signaling in maintaining the neural stem cell state, as previously described. A less robust outcome was observed with manipulation of Hedgehog levels, though consistent with a role in neural stem cell survival or proliferation. Inhibition of both pathways resulted in an additive effect. These data support a model whereby cells extending a process to the rosette lumen maintain neural stem cell identity whereas release from this association, either through asymmetric cell division or apical abscission, promotes neuronal differentiation.

T-1391

SIRT1 REGULATES THE DNA METHYLATION OF GERMLINE DEVELOPMENTAL GENES IN PLURIPOTENT STEM CELLS

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Proper DNA-methylation is required for normal development. Although embryonic stem-cells (ESCs) highly express all types of DNA methyltransferases (Dnmts), they exhibit the lowest levels of DNA-methylation. Here, we show that *Sirt1*, a NAD^+-dependent deacetylase prevents in murine ESCs the DNA-methylation selectively on imprinted and germline genes. Transcriptome and methylome analyses demonstrate that *Sirt1* null (*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 de novo Dnmts, and the treatment of 5-Azacytidine (5-Aza), a known Dnmts chemical inhibitor rescued abnormal DNA-methylation of affected Sirt1 target genes. In the embryoid body-based in vitro differentiation assay, *Sirt1* deficiency renders ESCs in an undifferentiated state, leading to delay in germline differentiation and defects of spermatogenesis, which were significantly rescued by reintroducing *Sirt1* cDNA. Thus, we suggest that *Sirt1* protects the DNA-methylation in germline genes in ESCs, thereby guaranteeing proper differentiation.

T-1392

EFFICIENT ISOLATION AND MAINTENANCE OF MESENCHYMAL PROGENITORS DERIVED FROM HUMAN EMBRYONIC STEM CELLS USING A POROUS MEMBRANE

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Pluripotent human embryonic stem cells (hESCs) acquire mesenchymal characteristics during the epithelial-mesenchymal transition (EMT) process. Here, we report a simple and an efficient isolation method for mesenchymal stem cells (MSCs) from hESCs undergoing EMT using a commercialized porous membrane transwell culture insert. Suspension culture of hESC colonies results in the formation of embryoid bodies (EBs), which adhered on the upper compartment of 8 μm porous membrane in the presence of EMG2-MV media. The population migrating through the permeable membrane to the lower compartment not only exhibited EMT markers but also expressed high levels of a panel of typical MSC surface antigen markers, and demonstrated multipotent differentiation capability. In addition, they have a prolonged proliferation capacity without characteristics and chromosomal changes. Furthermore, the isolated MSCs significantly enhanced cardiac functions in a rat model of myocardial infarction (MI) as measured by the left ventricle (LV) wall thickness (MI control, 32.9 ± 3.2% vs. hESCs-MSCs, 38.7 ± 2.4%), scar length (MI control, 46.1 ± 2.5% vs. hESCs-MSCs, 41.8 ± 1.3%), fibrosis area (MI control, 34.3 ± 1.6 % vs. hESCs-MSCs, 28.9 ± 3.5%) and capillaries density. Our findings demonstrate an ease with which hESCs-MSCs can be effectively isolated using the porous membrane, which overcomes the lack of availability of MSCs for therapeutic applications in various diseased animal models. Acknowledgments: This study was supported by grant (2011-0019487) from the Bio & Medical Technology Development Program of the National Research Foundation (NRF), grant (PJ00995602 and PJ00933303) from the Next-Generation BioGreen 21 Program of Rural Development Administration, and grant (PJ009103) from The Ministry for Food, Agriculture, Forestry and Fisheries, Cooperative Research Program for Agriculture Science & Technology Development, Rural Development Administration and, a grant of the Korea Healthcare technology R&D project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry for Health & Welfare (grant number : HI14C3365), all funded by the Korean government.

T-1393

SCALE UP OF DIFFERENTIATION AND CHALLENGE PROTOCOLS TO ENABLE FUNCTIONAL SCREENS IN EMBRYONIC STEM CELL DERIVED MOUSE MACROPHAGES

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Macrophages have key functions as regulators and effectors of innate immune responses and dysfunctional macrophages are associated with a wide range of severe diseases in humans. To facilitate the systematic experimental analysis of gene functions in macrophages we have adapted existing protocols to scale up the differentiation of the well characterised mouse embryonic stem (ES) cell line E14 (129P2/OlaHsd-derived) into CD14 and F4/80 positive macrophages in vitro. We present a screening platform in which 5 x 105 ES-cell derived macrophages (ESDMs) from up to eight genetically modified cell lines can be exposed to the same number of stimuli and cytokine and transcriptional responses measured in ~64 parallel assays in a single experiment. Using cytokine assays and RNA sequencing we observe robust responses of ESDMs to pathogens (Salmonella enterica serovar Typhimurium and influenza virus) and to pathogen derived agonists of different Toll-like receptors (e.g. LPS, CpG, poly (I:C)) and NOD1/2 (M-TriDAP), which show elements specific to the partially overlapping gene regulation networks activated by each stimulus. We anticipate that this screening platform will allow the efficient phenotyping of engineered loss-of-function and variant alleles in genetically modified ESDMs, which will be important for prioritising an increasing number of disease-associated genetic variants for in-depth analysis in mouse models.

T-1394

DIFFERENTIAL CILIOGENESIS ELICITS LINEAGE SPECIFIC RESPONSES TO DIFFERENTIATION CUES

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Human embryonic stem cells (hESCs) are capable of producing three germ layers. Many studies have been performed to reveal important signaling molecules and pathways driving hESCs to specific lineages. However, the molecular mechanism of lineage-specific interpretation of extracellular differentiation cues remains elusive. Here, we show that the transcription factor Nrf2, which normally coordinates cellular responses to endogenous and exogenous stresses, plays an important role in determining early lineage fates by directly controlling expression of key pluripotency factors such as OCT4 and NANOG. We showed that Nrf2 is inactivated in neuroectoderm (NE) cells, but not in mesendoderm (ME) cells and is regulated by autophagy in a lineage-specific manner. Increased autophagy activity during NE derivation allows de-repression from Nrf2, which is a prerequisite for NE determination. Further upstream, we identified the primary cilium as a key regulator of lineage-specific autophagy activity. Accordingly, ciliogenesis is specifically increased during NE differentiation, and primary cilia-mediated autophagy activation is necessary for NE fate determination by inactivating Nrf2 activity. Finally, we found that lineage-specific cell cycle patterns drive differential ciliation patterns during early hESC differentiation. Taken together, we identified the cilium-autophagy-Nrf2 axis as a novel regulatory mechanism for early lineage fate determination.

T-1395

CONSISTENT GENERATION OF EMBRYOID BODIES USING A NOVEL CELL CULTURE SURFACE

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Human pluripotent stem cells, including embryonic stem cells (ESC) and induced pluripotent stem cells, have great potential for modern medicine due to their ability to differentiate into cells of the three embryonic germ layers: ectoderm, mesoderm and endoderm. Under appropriate culture conditions, pluripotent stem cells aggregate spontaneously into three-dimensional, multicellular spheroids, known as embryoid bodies. This process of embryoid body (EB) formation represents a commonly used approach to initiate the differentiation of pluripotent stem cells in vitro. Here, we present Thermo Scientific Nunclon Sphera, a hydrophilic-polymer coated surface with a neutral charge, specifically developed to support the three dimensional culture of cells in suspension. We demonstrate 1) the surface's low binding properties, 2) the ability of this polymer-coated surface to form hESC-derived EBs of a consistent size using either serum-free- or xeno-free medium and 3) selective downstream analytical approaches.

T-1396

HEPARG ACELLULAR MATRIX FOR HEPATIC DIFFERENTIATION OF HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS

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Hepatocytes, the main cell type of the liver, are needed in the drug discovery for prediction of biotransformation pathways, possible drug-drug interactions, and hepatotoxicity of drug candidates. Pluripotent stem cells offer a valuable and limitless supply of hepatic cells with human origin for in vitro drug studies. In the majority of the current differentiation protocols stem cell fate is guided towards hepatic-like cells with stepwise growth factor treatment. However, it is known that not only soluble factors but also cell-cell interactions and cell-matrix interactions play an important role in the complex, multistage cell differentiation process. Especially, the optimal matrix for hepatic commitment is still poorly understood. The objective of our research was to use a human hepatic progenitor cell derived acellular matrix (ACM) as a culture platform for human

pluripotent stem cell differentiation towards hepatic cells. Human embryonic stem (hES) cells WA07 and H9-GFP and human induced pluripotent stem (hiPS) cells iPS(IMR90)-4 were first differentiated to definitive endoderm (DE) cells in standard Matrigel culture system using optimized growth factor cocktail. DE cells expressed CXCR-4 and HNF3B both on mRNA and protein level after six days of differentiation. Next, DE cells were seeded onto human liver progenitor HepaRG cell-derived ACM for further hepatic differentiation. On day 10-12, cells were positive for liver progenitor markers AFP, CK19 and for HNF4A which controls the initiation of expression of several key hepatic transcription factors. At the end of the differentiation cells showed hepatocyte-like morphology and expressed hepatic markers AAT and ALB in mRNA level but the expression level was remarkably lower compared to primary hepatocytes. HepaRG cell-derived ACM supports attachment and growth of DE cells, and also the hepatic differentiation of hES and iPS cells. However, differentiation needs to be further optimized to obtain mature hepatocytes which could be later used in drug studies.

T-1397

EFFICIENT PATTERNING OF HUMAN PLURIPOTENT STEM CELLS TOWARDS DISTINCT MESENDODERMAL SUBTYPES SIMPLY BY MODULATION OF THE BULK CELL DENSITY

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Human pluripotent stem cell (hPSC) differentiation can recapitulate early aspects of embryogenesis in vitro but the underlying processes are poorly understood and controlled. Here we introduce the bulk cell density (BCD; modulated by the medium volume) as a key regulatory factor directing early differentiation patterning of hPSCs. By modulating the BCD at fixed concentrations of the GSK3 inhibitor CHIR99021 (CHIR), hPSC were primed towards distinct primitive streak-like patterns. This effect was mediated by differential, BCD-dependent paracrine environments. For detailed systems analysis global gene expression profiling, lineage-specific flowcytometry as well as protein array- and mass spectrometry-based secretome assessment was performed. Our results show that 'high BCD / low CHIR' conditions favor priming towards definitive endoderm. Conversely, 'low BCD / high CHIR' results in late/posterior presomitic mesoderm priming along a primitive streak-like axis but entirely suppresses cardiac mesoderm formation. Strikingly, efficient cardiomyogenic differentiation is supported at both, 'low BCD / low CHIR' and 'high BCD / high CHIR' conditions and is characterized by a balanced intermediate gene expression pattern, essentially lacking exclusive expression of lineage regulators. Importantly, we show that the observed effect of the BCD is independent of the applied differentiation formats (including 2D and 3D static and dynamic conditions) and was applicable to all hPSC lines tested (ESC and iPSC). In addition, we identify specific paracrine signatures underlying the observed lineage specific priming, supporting the critical regulatory role of secreted factors in the process. Together, our results highlight the yet largely overlooked but highly decisive impact of the bulk cell density on hPSC differentiation. Monitoring and control of the BCD represents a novel, simple, but effective method to control hPSC priming, particular at early stages of lineage commitment. Our findings have strong practical implications for the definition and reproducibility of hPSC differentiation processes and their envisioned scale-up, which is prerequisite for the envisioned therapeutic and industrial application of hPSC derivatives.

T-1398

FUNCTIONAL INTEROGATION OF THE SENSORINEURAL CIRCUIT IN INNER EAR ORGANOIDS

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Sensory epithelia in the inner ear contain mechanosensitive hair cells that detect auditory and vestibular stimuli, which is then converted into electrical impulses and transmitted to the brain via sensory neurons. We recently established a 3D culture system capable of generating inner ear sensory epithelia from mouse pluripotent stem cells. Remarkably, the stem cell-derived tissue spontaneously developed into 1 mm-diameter epithelial cysts harboring vestibular-like hair cells that appeared to be innervated by sensory neurons—collectively known as *inner ear organoids.* Our approach uses small molecules and recombinant proteins to control BMP, TGFβ and FGF signaling in differentiating mouse embryonic stem cell aggregates. After a week of guided differentiation, aggregates are transferred to a defined medium to allow self-organization of inner ear organoids. Here we have characterized this culture system by investigating the functionality of the derived sensory neurons and hair cells. We used a reporter cell line that labels inner ear hair cells to aid electrophysiological recordings. On days 22-28, we dissected organoids and interrogated individual hair cells by patch-clamp recording. We stimulated hair cells that had prominent hair bundles using voltage steps and hair bundle deflections. Measurements from hair cells revealed large voltage-dependent potassium currents characteristic of immature vestibular hair cells. Furthermore, direct measurement of mechanotransduction currents suggested that hair cells acquired mechanosensitivity. Additionally, we found that the protein expression pattern observed in sensory neurons is indicative of pre- and post-synaptic development. Early in the culture, organoids produced Brn3a+ Islet1+ neuroblasts mimicking otic neurogenesis in vivo. Later, these neurons innervate hair cells with CtBP2+ PSD95+ synaptic ribbons. These results provide evidence that inner ear organoids faithfully produce properties of the sensorineural circuit formed between inner ear neurons and hair cells in vivo and likely reach a level of maturity similar to early postnatal mouse vestibular organs. Thus, our novel in vitro system should be suitable for investigation of mechanisms underlying normal and pathological development of the inner ear.

T-1399

EFFICIENT PRODUCTION AND FUNCTIONAL EVALUATION OF HEPATIC CLUSTERS DERIVED FROM HUMAN ES AND IPS CELLS USING SPHEROFILM

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Hepatocyte-like cells (HLCs) derived from human pluripotent stem cells have received extensive attention in the development of drug screening and toxicity testing. However, it has been reported that stem cell-derived HLCs showed hepatic functions that were too limited to be of use in drug screening and toxicity testing, possibly due to the lack of sufficient intercellular communication under conventional two-dimensional (2D) culture conditions. Therefore, a 3D differentiation system may overcome the in vitro limitation of 2D culture to produce stem cell-derived hepatocytes with mature metabolic functions. In this study, the feasibility of using a silicone-based spherofilm, specifically designed to produce spherical cell clusters, to generate uniformly sized 3D hepatic spheroids from hESCs was investigated. Hepatic spheroids generated on the spherofilm showed more homogenous size and shape than those generated in conventional low-attachment suspension culture dishes. Results of immunohistochemical analysis showed that expression of the mature hepatic marker albumin (ALB) increased over time during the hepatic maturation process. Furthermore, the 3D culture system mimicked the in vivo 3D microenvironment. Laminin, which is an important component of hepatic ECM, was expressed in hepatic spheroids. The results of immunohistochemical analysis indicated that the 3D culture environment is capable of generating an in vivo-like microenvironment. In addition, quantitative PCR analysis showed that the mature hepatic marker ALB and cytochrome P450 (CYP) enzymes CYP3A4 and CYP3A7 were expressed at higher levels in 3D culture than in 2D culture. This indicates that the 3D culture system is suitable for hepatic maturation and that our size-controlled 3D culture conditions might accelerate hepatic function. These results suggest that 3D hepatic spheroids significantly enhance metabolic maturation of hepatocytes derived from hESCs. This research was supported by the Bio&Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (No.2012M3A9B4028636 and 2012M3A9C7050139).
School of Life Science and Biotechnology for BK21 PLUS for Jong-Hoon Kim, Korea University.

T-1400

A LONG NON-CODING RNA REGULATES SOX2 TRANSCRIPTION IN AN ALLELE SPECIFIC MANNER

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The transcription factor Sox2 is a key player in regulating pluripotent and neural stem cell maintenance, and neural differentiation. These features make a proper and tightly regulated expression of Sox2 crucial for correct development. Since well-characterized distal regulatory elements do not fully corroborate the expression of Sox2 during neural development we searched for alternative regulatory mechanisms. Multiple presumably long non-coding RNAs are located in a 200 kb genomic region containing Sox2. We have investigated the role of some of these non-coding RNAs, with a special focus on the lncRNA Sox2 overlapping transcript (Sox2OT). We found that Sox2OT is predominantly transcribed in neural cells, transcription is induced upon neural differentiation of mouse ES cells, and that transcription levels of Sox2 and Sox2OT correlate in this in vitro model of neural differentiation. These results indicated that Sox2OT has a role in development and regulation of Sox2 transcription. To investigate this further we generated several knockin ES cell lines, in which Sox2OT is overexpressed. Analysis of these knockin lines revealed that transcriptional activity of Sox2OT regulates Sox2 in an allele specific way. Here we will present the results obtained, and discuss the possible mechanisms underlying the observed effects.

T-1401

UTILIZING IN VITRO-DERIVED CELL TYPES TO UNDERSTAND THE DIRECTIONALITY AND CELL TYPE SPECIFICITY OF REGULATORY POLYMORPHISMS IN SCHIZOPHRENIA

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Recent estimates by the World Health Organization reveal the global burden of psychiatric disorders exceeds that of cancer and cardiovascular disease combined. Despite vast unmet medical need, mechanisms underlying severe psychiatric disease like Schizophrenia remain unknown. As a result, the development of novel therapeutics has substantially lagged behind other disease areas in which innovative treatments are regularly introduced. Genome-wide analyses have linked variation in ~100 genomic regions to Schizophrenia. This provides for the first time an actionable list of candidate genes that potentially influence disease. In order to generate testable 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 alters the function of these genes in specific neural cell types. This information is critical for understanding disease mechanisms and for informed design of new drugs that aim to alleviate disease symptoms. Developing human stem cells as a model system provides an attractive approach to address these questions. Stem cells offer an experimentally tractable system that can be used to generate multiple neuronal cell types. We are generating numerous clinically relevant cells, including excitatory cortical neurons, inhibitory interneurons, dopaminergic neurons, oligodendrocytes, and microglia. By collecting cells at numerous timepoints we can observe changes in candidate gene expression over the course of development. We then systematically measure cell type-specific changes in gene expression to gauge the effects that disease-associated genetic variation might have on cellular function. Specifically, we need to determine whether genetic variation at each haplotype acts to increase or decrease the expression of candidate gene transcripts. In aggregate, this approach allows us to generate numerous disease-relevant human neuronal cell types and provides direct insight into the regulation of candidate gene expression in live neurons. This information will provide novel insight into the function of these genes in neurodevelopment, suggest how changes in gene expression might influence Schizophrenia, and ultimately reveal potential targets for downstream drug development.

T-1402

THE ROLE OF SOX2 REGULATORY REGIONS IN EMBRYONIC STEM CELL DIFFERENTIATION

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The *Sox2* transcription factor is required for embryonic stem cell (ESC) pluripotency and changes in the level of *Sox2* drive ESC down different developmental pathways suggesting that fine tuning *Sox2* transcription is critical for development. Our previous studies identified a distal enhancer, the *Sox2* control region (SCR) is required for *Sox2* transcription in ESC. Moreover, enhancer activity assays revealed a novel, more *Sox2* proximal enhancer, *Sox2* regulatory region 18 (SRR18) as well as known enhancers SRR1 and SRR2. Through CRISPR/Cas9-deletion analysis we established that the SCR is required for *Sox2* expression in ESC whereas SRR1/2/18 are dispensable. However, the role each of these regions has in ESC differentiation and lineage commitment has not been investigated. Using CRISPR/Cas9 genome editing we deleted each *Sox2* enhancer region: SRR1, SRR2, SRR18 and SCR. To investigate the effect of SCR position we deleted a 100 kb region, bringing the SCR downstream of *Sox2*. We monitored expression profiles for germ layer markers and pluripotency factors during ESC differentiation to embryoid bodies. Our results confirmed that SCR deletion dramatically reduces *Sox2* transcription and impairs neuroectoderm formation, whereas other enhancer deletions had little or no effect on *Sox2* expression and germ layer formation. Recent studies suggested that *Sox2* is down regulated by transcriptional repressive complexes binding to SRR2, however our results argue against this as SRR2 deletion did not affect *Sox2* down-regulation. Instead our results are consistent with *Sox2* down-regulation occurring through a loss of SCR activity. Repositioning the SCR did not greatly affect *Sox2* expression or ESC differentiation suggesting that SCR regulates Sox2 expression in a position-independent manner. Our results reveal the function of the SCR in regulating *Sox2* in both ESC and during early differentiation.

T-1403

SELF-ORGANIZATION OF POLARIZED CEREBELLAR TISSUE IN 3D CULTURE OF HUMAN PLURIPOTENT STEM CELLS

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During cerebellar development, the main portion of the cerebellar plate neuroepithelium gives birth to Purkinje cells and interneurons, whereas the rhombic lip, the germinal zone at its dorsal edge, generates granule cells (GCs) and deep cerebellar nuclei (DCN) neurons. However, it remains elusive how these components cooperate to form the intricate cerebellar structure. We previously reported that cerebellar neurons could be efficiently generated from mouse embryonic stem cells (mESCs) by recapitulating the self-inductive signaling microenvironment in 3D culture. mESCs formed isthmic organizer-like tissue in response to fibroblast growth factor 2 (FGF2) and insulin. Further inhibition of Shh signaling triggered the differentiation of the cerebellar plate neuroepithelium that expresses the Purkinje cell-progenitor marker Kirrel2 (also known as Neph3). On the other hand, the addition of bone morphogenetic protein (BMP) signals promoted differentiation into GCs and DCN neurons at the expense of Purkinje cells. Although we succeeded in induction of cerebellar neuronal components, 3D construction of cerebellar structures has not been so far recapitulated. Here, we found that a polarized cerebellar structure self-organizes in 3D human ESC culture. The self-organized neuroepithelium differentiates into electrophysiologically functional Purkinje cells. The addition of FGF19 promotes spontaneous generation of dorsoventrally polarized neural-tube-like structures at the level of the cerebellum. Furthermore, addition of SDF1 and FGF19 promotes the generation of a continuous cerebellar plate neuroepithelium with rhombic-lip-like structure at one end and a three-layer cytoarchitecture similar to the embryonic cerebellum. Thus, human-ESC-derived cerebellar progenitors exhibit substantial self-organizing potential for generating a polarized structure reminiscent of the early human cerebellum at the first trimester.

T-1404

MESP1 PROMOTES HUMAN CARDIOMYOCYTE DIFFERENTIATION BY SCALING CANONICAL WNT SIGNALING

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MESP1 is an important transcription factor that regulates heart development and is expressed transiently during early stage of cardiovascular differentiation in mouse and other model systems. To better understand its function during human cardiovascular differentiation, we generated a MESP1 reporter human embryonic stem cell line through transcription activator-like effector nuclease (TALEN) mediated homologous recombination. MESP1 reporter cells faithfully recapitulated the expression of endogenous MESP1 gene and high-throughput RNA sequencing revealed interesting change in global gene expression. Through growth factor and small molecule screening, we found that the activation of MESP1 relied on the strength of canonical Wnt signaling. Moreover, highly efficient cardiac microtissue differentiation can be achieved through optimizing MESP1-mTomato+ cell percentage at initiation stage of cardiovascular differentiation. Using an inducible overexpression system, we found that ectopic MESP1 expression downregulated β-catenin protein level. Through ChIP-seq, we identied several new down-stream target genes of MESP1 in human cardiovascular progenitor cells that contribute to the supression of canonical Wnt pathway. Finally, we showed that inducing MESP1 expression at appropriate time window could substitute canonical Wnt inhibition step and promote robust cardiomyocyte formation. Our work discovered new mechanisms of how MESP1 regulates cardiomyocyte differentiation in hESCs. Moreover, MESP1-mTomato reporter cells and inducible expression system can serve as valuable platforms to study the mechanism of cardiovascular cell fate decision in human.

T-1405

MIRNAS CONTROL THE DIFFERENTIATION OF ES CELLS INTO DOPAMINERGIC NEURONS

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Midbrain dopamine (mDA) neurons constitute a highly diverse neuronal population controlling important brain functions, such as motor action, cognition, motivation, and emotions. MDA neurons can

be broadly subdivided into two major anatomically and functionally groups, which form the substantia nigra (SN) and ventral tegmental area (VTA). While SN neurons selectively degenerate in Parkinson's disease, VTA neurons are implicated in other disorders including ADHD and autism. Efficient generation of midbrain dopamine neurons from embryonic stem cells offer important opportunities in cell replacement studies, drug discovery and disease modelling. Several protocols have been established for the derivation of dopamine neurons from embryonic stem (ES) cells. These protocols apply signalling molecules and transcription factors that are required for the generation of dopamine neurons in vitro. However, very little is known about the involvement of miRNAs in the directed differentiation of ES cells into the dopaminergic lineage. We have identified miRNAs that are selectively expressed in the dopaminergic lineage during ES cell differentiation. We will determine their role during the differentiation process using both mouse and human ES cells. Furthermore, we apply these miRNA to develop more efficient stem cell differentiation protocols and investigate whether these miRNA are also involved in the diversification of dopamine neurons into SN and VTA.

T-1406

OPTIMIZED ISOLATION OF HEPATOCYTES DERIVED FROM HUMAN ES CELLS USING EPCAM AND MODIFIED INDOCYANINE-GREEN

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Hepatocytes and hepatic progenitors derived from human ES cells may be a useful source for clinical application. Therefore, identification and purification of these cell types would be following important issues. There are very few candidate surface markers that can be used to identify and purify hepatic progenitor cells. In addition, indocyanine-green can be uptaken by mature hepatocytes, but cannot be applied for fluorescence activated cell sorting (FACS) due to its long emission wavelength. In the present study, we tested EpCAM as a potential marker for magnetic-activated cell sorting (MACS) of hepatic progenitors and also modified indocyanine-green into fluorescent indomonocarbocyanine for FACS-mediated sorting of mature hepatocytes after differentiation of human ES cells. Hepatic progenitor cells were sorted by MACS after incubation with anti-human EpCAM antibodies. After the final differentiation, the differentiated cells and mouse primary hepatocytes (control group) were incubated with indomonocarbocyanine and were sorted by FACS. MACS and immunocytochemistry data showed that approximately 45% of differentiated cells were EpCAM-positive cells. EpCAM-positive cells expressed α-fetoprotein, FOXa2, HnF4a, and CK18. Differentiation efficiency into albumin-positive cells was significantly higher in EpCAM-positive cells, compared to EpCAM-negative cells. Importantly, indomonocarbocyanine successfully stained cells that expressed ALB. Furthermore, FACS analysis data showed that the purity of hepatocytes that expressed albumin was significantly increased after purification of indomonocarbocyanine-positive cells. Our data demonstrated that human ES cell-derived hepatic progenitors can be efficiently isolated by MACS using EpCAM antibody. In addition, we also showed that indomonocarbocyanine can be successfully used to identify and purify mature hepatocytes using FACS. *This research was supported by the Bio&Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (No.2012M3A9B4028636 and 2012M3A9C7050139). School of Life Science and Biotechnology for BK21 PLUS for Jong-Hoon Kim, Korea University.*

T-1407

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

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Photoreceptor degeneration followed by degeneration of retinal pigment epitheliums (RPEs) is the major pathologic change in retinal degenerative diseases such as retinitis pigmentosa. The limited benefit of current treatments for retinal degenerative diseases has led to an increased interest in the use of cell-based transplantation therapy. We have differentiated RPE and photoreceptor precursors from human ESCs with the generation of cell masses of neural precursors, which we call 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. To investigate therapeutic potential of these differentiated RPE and photoreceptor precursors, we transplanted RPE and/or photoreceptor precursors into subretinal space of Royal College Surgeon rats. After 4 weeks of transplantation, histologic examination showed greater preservation of outer nuclear layer in the treated eyes. The transplanted eyes also showed less decrease in the amplitude of a-wave on electroretinography. It suggests that subretinal transplantation was well tolerated, without tumor formation or severe inflammation and the differentiated RPE and photoreceptor precursors from hESCs have potential to preserve visual function in the retinal degenerative rats.

T-1408

DISSECTING THE MOLECULAR MECHANISMS OF MESODERM INDUCTION IN HUMAN EMBRYONIC STEM CELLS

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In human embryonic stem cells (hESCs), OCT4, SOX2 and NANOG are at the core of an intrinsic gene-regulatory network controlling self-renewal and maintaining the undifferentiated stem cell state. However, these individual transcription factors could also actively participate in promoting transitions into specific cell fates. Acting upstream to these transcription factors, signaling pathways play a key role in directing the specification and differentiation of pluripotent embryonic stem cells into distinct lineages. This interplay of signaling pathways and the pluripotency gene-regulatory network is not well understood. We study the molecular mechanisms of mesoderm formation, focusing primarily on the role of BMP

and WNT signaling pathways in the process. An emphasis is put on identifying downstream targets of these cascades that could positively or negatively control the transition from the pluripotent state into the mesodermal lineage. We found that these pathways act in a highly synergistic manner to induce primtive streak genes while specifically repressing SOX2 in hESCs. Furthermore, controlled over-expression of SOX2 in hESCs specifically during primitive streak formation interfered with differentiation into the cardiac lineage, implying that SOX2 repression is a first key event in mesoderm induction. Furthermore, our mechanistic investigation suggests that while the two signaling cascades synergistically repress SOX2, they do so by employing distinct mechanisms. Our data thereby provides insights into key events underlying the transition form the undifferentiated pluripotent cell state into mesoderm, using hESCs as a model system.

T-1409

IMPACT OF IONIZING RADIATION ON THE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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Patients are increasingly subjected to diagnostic and/or therapeutic procedures based on ionizing radiation. However, this poses a threat to the early embryo in cases of inevitable or unintended exposure possibly leading to prenatal death, growth retardation, organ malformation, mental retardation or childhood cancer. A careful risk assessment is hampered by scarce epidemiological data and suboptimal model systems. However, human embryonic stem (hES) cells present a valuable tool to examine radiation effects on the early embryo and their underlying mechanisms. As these cells can differentiate into all cells of the body, we were interested in the ability of hES cells to form definitive endoderm (DE) after exposure to X-ray irradiation. Chromosomal aberrations were analyzed to determine the cytogenetic status of exposed and unexposed hES cells in addition to quantitative RT-PCR arrays evaluating pluripotency. DE formation was initiated by treatment with Activin A and Wnt3a under serum-free conditions leading to Foxa2^{high}/Sox17^{high}/Sdf1^{low} DE progenitors as assessed by quantitative RT-PCR and immunocytochemistry. Analyses of DE-specific microRNAs confirmed DE formation. X-ray irradiation of hES cells prior to differentiation initiation led to massive cells death. Our data suggest that surviving hES cells undergoing differentiation show an impaired DE formation judged by a decrease of Sox17 expression and an induction of Sdf1. This impairment could be connected to alterations in the TGF-beta and Wnt signaling pathway. Currently, the effect of carbon ions that are used in state of the art cancer therapies on the differentiation potential of hES cells is examined. We conclude, that hES cells surviving the radiation exposure maintain their differentiation capacity albeit with lower efficiency than unexposed cells possibly explaining the observed radiation-induced developmental retardations.

T-1410

CORTICAL INTERNEURON DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS (HPSC) IN 3D USING CUSTOMIZABLE HYDROGELS

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Efficient neuronal differentiation of human pluripotent stem cells into specific neuronal subtypes is an important step in human disease modeling of neurodegenerative diseases. Directed differentiation in 2D cultures towards cortical fate is challenging, as the cells lack 3D interaction and information. We have used a new 3D culture system to mimic in vivo differentiation, utilizing a fully synthetic, media dissolvable polypeptide that forms a hydrogel. Human PSC are induced to neuronal lineage by dual SMAD inhibition. After 10 days, obtained neuronal progenitors (NPCs) are mixed with the polypeptide, transferred into cell culture inserts and subsequently allowed to form a hydrogel. Hydrogels are kept in a CO2 incubator and cells are patterned to cortical fate using N2B27 medium supplemented with high concentration of sonic hedgehog for 9 days. Cells are viable in the hydrogels for longer time periods and form clusters, developing into expanding neurosphere-like structures. After 9 days in the hydrogel, cells express neuronal markers (i.e. Nestin and Tubulin III) and markers for cortical neurons (i.e. FoxG1). Cells have been further cultured in 3D hydrogels until day 65, when the protocol is terminated. Ongoing investigations at these late time points will clarify neuronal subtype identities as well as interconnectivity and plasticity of the sphere like structures.

T-1411

DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO GONADAL PRECURSOR CELLS

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Gonadal somatic cells (testicular Sertoli and Leyding cells, and ovarian granulosa and theca cells) develop from intermediate mesoderm-derived mesonephric coelomic epithelium, first as a bipotential *anlage* capable of developing into male or female direction. A number of transcription factors controlling gonadal development have been identified, but the regulatory networks involved in gonadal differentiation are not fully understood. The primary objective of this study was to establish an in vitro model for investigating human gonadal development using human embryonic stem cells (hESCs). H9 (XX) and FES29 (XY) hESC cells were first differentiated to a primitive streak-like stage with high doses of Activin A and CHIR-99021 (WNT agonist), whereafter the cells were shortly induced into intermediate mesoderm by WNT activation complemented with sequential activation and inhibition of BMP signalling by BMP7 and dorsomorphin, respectively. Then the cells were cultured in basal medium for an additional four days.

The sequential activation and inhibition of BMP activity induced several intermediate mesoderm markers such as *PAX2, LIM1* and *OSR1* at day 4 of differentiation as analyzed by quantitative real-time RT-PCR (qRT-PCR). At day 6-8 of differentiation the expression of bipotential gonadal genes *EMX2, LHX9, WT1, GATA4, DAX1* and *SIX1* was upregulated. Importantly, the expression of these markers decreased by continuous BMP activation, indicating the critical role of sequential BMP activity in bipotential gonadal cell induction. During the differentiation no ectodermal or endodermal differentiation was detected. In conclusion, we have established a protocol for differentiating hESCs into gonadal precursors and shown the importance of correct timing of BMP activity on differentiation. This protocol can be used as a platform for studying early development of human gonads.

T-1412

ROBUST REFERENCE GENES FOR NORMALIZATION OF GENE EXPRESSION DATA FROM DIFFERENTIATING HUMAN PLURIPOTENT STEM CELLS

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Normalization of gene expression studies requires stably expressed reference genes for proper calibration of samples and commonly, researchers use housekeeping genes (HKGs) for this purpose. However, several recent reports on studies on human pluripotent stem cells have shown that stem cells deviate substantially from somatic cells in their expression of HKGs, which may have important consequences for accurate normalization of stem cell experiments. We have investigated the expression of commonly used HKGs during differentiation of hPSCs using nine different global transcriptional datasets from public repositories and from in-house experiments in our lab. To cover a broad range of differentiation regimes, datasets from all three germ layers were selected for this study. Importantly, results show that common HKGs in somatic cells sometimes vary substantially during differentiation of hPSCs. This prompted us to search for alternative genes that show stable expression during hPSC differentiation, and can serve as suitable reference genes in studies of hPSCs. To remove background noise in the datasets, the 25th percentile of the genes with lowest expression values were filtered before the datasets were mined for stably expressed genes using coefficient of variation (CV) as stability measure. Genes with a low CV across the various differentiation procedures were considered as stably expressed, and therefore more suitable as reference genes in hPSC differentiation experiments. Results from this study reveal significant differences between somatic cells and stem cells that will have important implications on the selection of reliable reference genes for normalization of data from differentiating hPSCs. We identified sets of stably expressed genes that were stably expressed in each of the germ layers. Moreover, a small set of seven genes (EID2, TNFRSF13C, ZNF324B, CAPN10, RABEP2, LTB4R2, and CCDC108) was identified that show stable expression during all investigated differentiation regimes. We propose these genes as more reliable reference genes for normalization of differentiation studies on hPSCs.

T-1413

TRANSCRIPTION FACTOR DIRECTED DENDRITIC CELL DIFFERENTIATION FROM PLURIPOTENT STEM CELLS

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The application of dendritic cells (DCs) to prime responses to tumor antigens provides a promising approach to cancer immunotherapy. However only a limited number of DCs can be manufactured from adult precursors. In contrast, pluripotent embryonic stem (ES) cells would provide an unlimited source for DC production, however it remains a major challenge to steer directional differentiation because ES cell-derived DCs are typically immature with impaired immunogenicity. To characterize the ex vivo DC differentiation program, we have monitored the gene expression profile of 17 DC and macrophage specific transcription factors in mouse ES cell- and adult progenitor (bone marrow)-derived DCs. Our analysis revealed that three DC affiliated transcription factors (Spi-B, Runx3 and Irf4) were barely detected in ES cell-derived DCs. Interestingly gain of function analysis revealed that re-expression of these three factors exerted strong effects on the differentiation program. Unexpectedly, Irf4 negatively modulates, while Spi-B enhances the early myeloid blood cell development in ES cell-derived progenitors. Despite of the early stimulatory effects of the Spi-B, however the phenotype of the final DC products remained unaltered. Importantly sustained expression of Runx3 improves the ES cell-derived-DC maturation especially in the presence of LPS. These findings demonstrate that Runx3 directed cellular programming can enhance the cytokine driven DC development and support the notion that lineage determining transcription factors are important tools for the efficient production of antigen presenting cells from pluripotent stem cells.

T-1414

IDENTIFICATION AND ISOLATION OF HUMAN EMBRYONIC STEM CELL-DERIVED CARDIAC PROGENITORS

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Human embryonic stem cell (hESC) lines, derived from the inner cell mass of blastocyst-stage embryos can provide an unlimited source of differentiated cells. Among others hESCs have the capacity to differentiate into cardiac progenitors and later into cardiomyocytes. Here we introduce a method for identification and isolation of human embryonic stem cell-derived cardiomyocytes and cardiac progenitors and present an optimized culture condition for cardiac progenitors to enhance overall cardiomyocyte yield. The method utilizes the special characteristics of the CAG promoter, providing exceptionally high expression of the enhanced green fluorescent protein (EGFP) in hESC-derived cardiomyocytes, while its activity in other cell types emerging under in vitro conditions remains modest. This exceptionally high CAG-EGFP signal is also characteristic for cardiac progenitors, allowing the isolation of progenitor cells, which are still capable to divide and differentiate into atrial type cardiomyocytes and, to a lesser extent, into smooth muscle cells under 3D culture conditions. Enhanced survival of cardiac progenitors after isolation could be achieved by treatment with a

Rho-associated kinase inhibitor Thiazovivin, while in combination with, a β1- and β2-adrenoreceptor agonist Isoproterenol, resulted in enhanced cardiomyocyte yield. Our results suggest that purification of cardiac progenitors is a promising strategy to receive pure populations of cardiomyocytes even for large-scale production, supporting drug discovery and potential therapeutic applications.
This work was supported by the Hungarian Scientific Research Fund [NK83533]; Hungarian Brain Research Program [KTIA VKSZ_12, NAP-A-1.10] and by the National Research, Development and Innovation Office KTIA_AIK_12-1-2012-0025, KMR_12-1-2012-0112].

T-1415

TRACTION FORCES VIA INTEGRIN SIGNALING ARE NECESSARY FOR DEFINITIVE ENDODERM SPECIFICATION

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Morphogenic events during early development, including gastrulation, require force-mediated motility and coincide with initial fate specification of embryonic stem cells (ESCs), resulting in the formation of the definitive endoderm (DE) and mesoderm layers of the trilaminar embryo. Pluripotent embryonic stem cells exert low traction forces on their niche in vitro, suggesting a differentiation-mediated switch in contractility. However, the onset of contractility and extent to which force-mediated integrin signaling regulates fate choices is not understood. To address the requirement of tractions forces for differentiation, we examined mouse ESC specification towards DE on fibrillar fibronectin containing a deformation-sensitive FRET probe. DE induction was sufficient to cause an α5β1-integrin mediated decrease in the observed fibronectin FRET intensity ratio indicating the activation of traction forces. Inhibiting contractility with blebbistatin, an inhibitor of non-muscle myosin, resulted in an increase in the fibronectin FRET ratio but also decreased nuclear phospho-SMAD2 leading to reduced expression of the DE marker SOX17. By contrast ESCs maintained in pluripotency media did not exert significant tractions against the fibronectin matrix even in the absence of blebbistatin. When laminin-111 was added to fibrillar fibronectin matrices to improve DE induction efficiency, ESCs decreased their fibronectin traction forces in a laminin-dependent manner. Blocking laminin-binding α3-integrin restored fibronectin matrix deformation, reduced SOX17 expression, and SMAD2 phosphorylation, likely from compensation of inhibitory signaling from SMAD7 after 5 days in culture. These data imply that traction forces and integrin-signaling are important regulators of early fate decisions in ESCs.

T-1416

PREDOMINANT DIFFERENTIATION OF EGFP-TRANSGENIC MOUSE GS-2 ES-CELLS TO NEURAL CELL TYPES USING KSR SUPPLEMENT

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The cellular and molecular mechanisms of neural cell commitment and differentiation are not completely understood. Mouse embryonic stem cells (ES-cells) offer an excellent model system to study neural differentiation. We earlier derived EGFP-transgenic mouse GS-2 ES-cell line exhibiting all defining criteria of a typical ES-cell line. In this study, we developed a defined culture-system, using knockout serum replacement (KSR) supplement while forming GS-2 ES-cells-derived embryoid bodies (EBs). Post-attachment, EBs predominantly produced neural cell types, appearing from day 7 onwards. By day 10-11, 100% of EBs showed neural networks and their complexity increased during additional days of culture through day 18. Differentiated neural cell types were characterized by their morphology, morphometric analysis and molecular phenotyping using neurogenesis-associated markers such as NESTIN, PAX6, MAP2 AND TUBB3. By day 15, approx. 11% NESTIN$^+$ neural progenitors, 32% TUBB3$^+$ immature neurons, 21% MAP2$^+$ mature neurons, 51% GFAP$^+$ astrocytes and 4% MAG$^+$ oligodendrocytes were present in cultured EBs. Interestingly, removal of central EB mass from its peripheral outgrowth substantially increased NESTIN$^+$ cells (~35%) with no significant change in the percentage of TUBB3$^+$ cells. The enriched neural cell population was capable of forming neural network post-replating. Functional studies including patch-clamp analysis are under way. Molecular screening experiments using this in vitro neurogenesis model showed that the treatment of GS-2 ES-cells with FGF2 (10 ng/ml) on days 0-5, significantly increased the density of neural network with increased NESTIN$^+$ neural progenitor population. And, treatment of GS-2 ES-cells with NF-κB inhibitor *i.e.*, JSH-23 (10 μM) on days 0-5, completely abrogated neural differentiation. The spatio-temporal patterns of differentiation and its molecular mechanism remain to be examined. Taken together, we successfully established a defined and efficient ES-cell-derived in vitro neurogenesis model, providing opportunities in terms of enhancing in vitro neurogenesis using ES-cells and developing disease models, screening of molecules and in studying molecular regulation of neurogenesis.

T-1417

BIOACTIVE RECOMBINANT hWNT EXOSOMES IMPROVE HEMATOPOIETIC DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

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Wnts are a family of secreted glycoproteins that act as morphogens during development. They are composed of over 19 proteins that signal through multiple receptors (like Frizzled, Fzd). Over the past years our understanding of Wnt signalling has been substantially impeded due to the availability of stable active Wnt ligands. This is due to their complex post-translational modifications including lipidation. However, recent studies that have identified Wnts on secreted exosomes provide an opportunity to obtain stable bioactive Wnt ligands in-house. Here, we verified the activity of exosomes secreted from HEK293 cells, stably expressing human WNT11 or WNT3a. The hWNT11 and hWNT3a enriched exosomes were extracted using differential centrifugation protocol and active hWNTs were functionally assessed for: biochemical activity (CaMKII, beta-catenin and DVL2), TCF/Wnt reporter activity and hematopoietic differentiation from human pluripotent stem cell (hPSCs). The hWNT exosomes gave a bell-shaped dose response, where higher doses of hWNT11 and hWNT3a exosome extract inhibited CaMKII and beta-catenin activity in hPSCs respectively. Furthermore, hPSCs exposed to hWNT3a exosomes induced colony differentiation. Live cell confocal imaging on hiPSCs, stably expressing the TCF/Wnt reporter, showed increased GFP levels upon hWNT3a exosome treatment. Interestingly the hiPSC colony

displayed a variegated GFP expression pattern where high GFP responsive cells were asymmetrical positioned close to low GFP responsive cells. Human embryoid bodies (hEBs) exposed to hWNTs-exosomes also showed a dose dependent effect, with higher hematopoietic frequencies based on CD45+; CD45+ CD34+; CD31+ CD45- levels as previously described. We believe that hWNT on exosomes extract provide a formulation that is more physiological. Thus allowing a better understanding of the role of hWNT in hPSC self-renewal and differentiation.

T-1418

SKELETAL TERATOGENESIS THROUGH MODULATION OF WNT-SIGNALING VIA BETA-CATENIN/FOXO

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Tobacco use is a risk factor for osteoporosis, bone fracture and delayed bone healing, thus perpetuating poor bone health. Tobacco products not only contain numerous toxins and nicotine that can have adverse effects on bone, they also generate vast amounts of reactive oxygen species (ROS), which are capable of having detrimental effects on osteoblast function and generation. Because of this proposed mechanism of action, it is conceivable that tobacco products may harm developing fetuses and infants. The aim of this study was to understand the pathogenesis of tobacco-associated bone toxicity in developing cells as they differentiate into osteoblasts. Human embryonic stem cells (hESCs) were induced to differentiate into osteoblasts in the presence of tobacco products to examine osteotoxicity in vitro. Teratogenic doses of Snus tobacco extracts (STE) (0.1%) exhibited inhibition of osteoblast calcification in the absence of a cytotoxic effect. STE generated excessive ROS, both in the form of O_2^- and H_2O_2, during early stages of osteogenic differentiation. Though the high levels of ROS were coupled with an upregulation of stress-activated c-Jun N-terminal kinase (JNK), downregulation of nuclear activation of β-catenin and FoxO (transcriptional co-regulators of MnSOD) was found. Co-immunoprecipitation analysis further demonstrated that the interaction between β-catenin and FoxO was diminished. Our results demonstrate that STE damages specification of osteoblasts via beta-catenin/FoxO caused by accumulation of ROS. Therefore, the disruption of β-catenin/FoxO-mediated transcription should be preventable by antioxidants, suggesting a potential mechanism to ameliorate tobacco-associated birth defects.

T-1419

DERIVING SKELETAL MUSCLE PROGENITOR CELLS FROM HUMAN PLURIPOTENT STEM CELLS FOR TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy (DMD) is a fatal skeletal muscle disease affecting 1 out of 3500 new born males. DMD patients exhaust their endogenous muscle stem cells during the course of the disease, leading to progressive muscle degeneration. Thus, one promising strategy is to transplant skeletal muscle progenitor cells (SMPCs) into the patients to help replenish and maintain their musculature. In this regard, human pluripotent stem cells (hPSCs) are a superior source for obtaining an unlimited supply of SMPCs due to their self-renewal and differentiation capabilities. Only limited success has been reported on deriving SMPCs from hPSCs, and thus we aim to develop a defined and robust protocol to direct hPSCs towards SMPCs through multiple developmental stages by closely mimicking the environmental cues that are instrumental to skeletal myogenesis. We have found that transiently activating canonical WNT-β-catenin signaling drives hPSCs towards a presomitic mesoderm (PSM) fate. Subsequently, inhibiting BMP signaling promotes the PSM cells to become somitic cells, which are the common ancestors of most skeletal muscles. Moreover, manipulating BMP signaling in the somitic cells further differentiates them into distinct populations, which we hypothesize contain SMPCs with different migrating and repopulating capabilities in vivo. In order to isolate the SMPC populations, we are constructing CRISPR/Cas9 generated reporter hPSC lines containing SMPC markers. Once optimized, we will isolate and characterize the identity and functionality of the distinct SMPC populations both in vitro and in mouse models of DMD.

T-1420

EFFICIENT INDUCTION OF INNER EAR HAIR CELL-LIKE CELLS FROM MATH1-TRANSFECTED MOUSE EMBRYONIC STEM CELLS USING STROMAL CELL-CONDITIONED MEDIUM

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Hearing loss is mainly caused by loss of sensory hair cells (HCs) in the organ of Corti or cochlea. Although embryonic stem (ES) cells are a promising source for cell therapy, little is known about the efficient generation of HC-like cells from ES cells. Recently, we developed a simple and efficient technique, termed the HIST2 method, to obtain ES-derived HC-like cells with a relatively short period of cultivation, in which only conditioned medium from cultured ST2 stromal cells is used. Moreover, we demonstrated the differentiation of ES cells into inner ear hair cells using gene transfer of *Math1*, a key factor for induction of HCs during development of the organ of Corti or cochlea, and a Tet-on regulation system. ES cells carrying Tet-inducible *Math1* showed a high ability to differentiate into HC-like cells. In the present study, we examined more effective and efficient induction of HC-like cells from ES cells by combining *Math1* gene regulation by the Tet-On system with our HIST2 method. ES cells carrying Tet-inducible Math1, Math1-ES cells, were generated using a Tet-On gene expression system. Embryoid bodies (EBs) formed in the absence of doxycycline (Dox) for 4 days were allowed to grow for an additional 14 days in the dishes in the presence of Dox with HIST2 method. At the end of those 14-day cultures, approximately 30% of the cells in EB outgrowths expressed the HC-related markers myosin6, myosin7a, calretinin, Chrna9, and Brn3c (also known as Pou4f3), and showed formation of stereocilia-like structures, whereas few cells in EB outgrowths grown without Dox showed those markers. Combining *Math1* gene regulation with

HIST2 method achieved more effective and efficient induction of HC-like cells from ES cells.

T-1421 *see abstract in EMBRYONIC STEM CELLS AND CLINCIAL APPLICATION before T-1445*

EMBRYONIC STEM CELL PLURIPOTENCY

T-1422

IMPACT OF HYPOXIA AND OF METALLOPROTEINASE MMP1 ON LIF-DEPENDENT CELL PLASTICITY IN THE MES CELL MODEL

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Mouse ES cells are expanded and maintained pluripotent in vitro in the presence of LIF (Leukemia Inhibitory Factor), an IL6 cytokine family member which displays pleiotropic functions, depending on both cell maturity and cell type. LIF withdrawal leads to heterogeneous differentiation of mES cells and part of the differentiated cells also die by apoptosis. During the kinetics of LIF withdrawal, we have previously shown that cells enter a reversible (up to 36h) and irreversible phase of differentiation (from 48h). In this study we have set up an in vitro "plasticity test" in which we have investigated the effects of potential regulators and effectors of mES cell plasticity [eg: PI3K, Klf5, low oxygen (O2) concentration and MMP1]. We show that the PI3K signalling pathway, required for the maintenance of mES cell pluripotency, has no effect on mES cell plasticity. However, it displays a major role in differentiated cells where it stimulates the expression of the mesoderm marker (Bry) at the expense of endo (Sox17) and neuroectoderm (Nestin) markers. We show also that under low O2 concentration (3% O2), mES cells maintain plasticity and pluripotency potentials in vitro, despite lower levels of Pluri and Master gene expression in comparison to 20% O2. In addition, we show that the MMP1 metalloproteinase, which can replace LIF for maintenance of pluripotency, mimics LIF effects in the plasticity window, but less efficiently. (In collaboration with Nina Kirstein, Virginie Mournetas, Xavier Gauthereau, Anaïs Darracq, Sabine Broc, Dana Zeineddine and Mohamed Mortada).

T-1423

GLOBAL TRANSCRIPTIONAL AND EPIGENETIC PATTERNS IN ESCS AND EGCS ARE DRIVEN BY SEX RATHER THAN CELL TYPE

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Blastocyst-derived embryonic stem cells (ESCs) and genital ridge-derived embryonic germ cells (EGCs) represent two classic types of pluripotent stem cell lines. Despite remarkable similarities between ESCs and EGCs, the epigenetic equivalence of these cell types remains debated. For example, previous studies suggested that EGCs are globally hypomethylated relative to ESCs, mirroring epigenetic patterns in the germline. However, subsequent reports failed to identify differences between ESCs and EGCs. Here, we compared global DNA methylation and transcriptional signatures between isogenic ESC and EGC lines with the goal to define similarities and differences and to identify possible regulators underlying the purported DNA hypomethylation of EGCs. Surprisingly, we found that global DNA methylation patterns were indistinguishable between ESC and EGC lines of the same sex, with female cell lines showing global hypomethylation compared to male cell lines. Cell fusion experiments between EGCs or ESCs and somatic cells further showed that the ratio of X chromosomes to autosomes determines methylation levels in pluripotent cells. Mechanistically, we discovered a link between elevated expression of the X-linked gene, dual specificity phosphatase 9 (Dusp9), attenuated MAP kinase signaling and reduced protein levels of DNMT3A and DNMT3B in female pluripotent cell lines. Together, our data demonstrate that sex rather than cell type of origin of EGC and ESC lines dictates global epigenetic and transcriptional patterns in mouse pluripotent cell lines maintained in conventional culture conditions.

T-1424

THE TRANS-SPLICED LONG NON-CODING RNA, TSRMST, PROMOTE PLURIPOTENCY THROUGH WNT5A-MEDIATED INHIBITION OF IN VITRO DIFFERENTIATION

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We previously demonstrated that the lncRNA-tsRMST, forms repressive complex with NANOG and PRC2 complex to promote pluripotency maintenance through repressing lineage-specific transcription factors. By combining global gene expression profiling and API signaling pathway analysis of hESC with altered tsRMST expression, we show that tsRMST, can also affect extracellular signaling pathway, such as non-canonical WNT. This reveals that tsRMST is likely contributed to regulate pluripotency and early lineage differentiation of hESCs through additional mechanism aside from direct transcriptional regulation of the lineage-specific genes. Furthermore, we demonstrate that disruption of tsRMST expression in hESC lead to up-regulation of the components of non-canonical WNT and EMT pathways, which promote the in vitro differentiation of hESCs. We provide evidences showing that non-canonical WNT ligand WNT5A, activate non-canonical Wnt signaling through PKC phosphorylation and subsequently promotes EMT and induces mesendoderm differentiation in hESCs. Therefore, we have identified tsRMST as a key factor for regulating the interplay between intrinsic and extrinsic mechanisms required for the pluripotency maintenance and early lineage differentiation.

T-1425

A NOVEL AUTOREGULATORY LOOP BETWEEN THE GCN2-ATF4 PATHWAY AND L-PROLINE METABOLISM CONTROLS STEM CELL IDENTITY

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Increasing evidence indicates that metabolism is implicated in the control of stem cell identity. We have recently contributed to this emerging field showing that the nonessential amino acid (NEAA) L-Proline (L-Pro) acts as an epigenetic signal that promotes the conversion of mouse embryonic stem cells (ESCs) into mesenchymal-like, highly motile, invasive stem cells, which acquire metastatic potential in vivo. Despite its relevance, the molecular mechanisms underlying L-Pro -dependent control of ESC identity still remain largely unknown. Here, we provide mechanistic insights and demonstrate that ESC identity relies on a novel feedback loop that involves the L-Pro -dependent modulation of the Gcn2-Eif2α-Atf4 amino acid starvation response (AAR) pathway that in turn regulates L-Pro biosynthesis. This regulatory loop places the expression of L-Pro biosynthesis genes *(Aldh18a1 and Pycr1)* under the control of AAR/Atf4 pathway, which is in turn under the control of L-Pro availability. This L-Pro-AAR/Atf4-*Aldh18a1/Pycr1* autoregulatory loop thus maintains L-Pro as a growth limiting metabolites and generates a stress response in ESCs that restricts proliferation of tightly packed domed-like ESC colonies, and preserves ESC identity. Indeed, alleviation of this nutrient stress condition by exogenously provided L-Pro induces proliferation and modifies the ESC phenotypic and molecular identity towards that of mesenchymal-like, invasive pluripotent stem cells. Either pharmacological inhibition of the prolyl-tRNA synthetase or forced expression of Atf4 antagonises the effects of exogenous L-Pro. Our data provide unprecedented evidence that L-Pro metabolism and the nutrient stress response are functionally integrated to maintain mouse ESC identity.

T-1426

ADAPTATION TO SINGLE-CELL ENZYMATIC PASSAGING OF HUMAN PLURIPOTENT STEM CELLS IS BIMODAL AND THE INITIAL PHASE IS INDEPENDENT OF GENETIC ALTERATIONS

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Single-cell plating of human pluripotent stem cells (PSC) is often associated with cell death and low cloning efficiency, thus limiting their use for large-scale analyses or clinical applications. PSC plating efficiency can be strongly increased through a process commonly termed "adaptation". PSC adaptation has mechanistically been linked to karyotype alterations and, indeed, adapted PSC often display altered karyotypes. We recently described the temporal occurrence of genetic abnormalities in the human embryonic stem cell (hESC) lines HD291, HS306 and HD129 when cultured using single-cell enzymatic passaging. Here, we analyzed a wide range of data collected during the early phases of the adaptation of these three hESC lines. Specifically, we found that: (1) the central feature of hESC adaptation, i.e. increased single-cell plating efficiency, is a phenotype strongly induced in less than five passages (i.e., before or concomitantly with the appearance of chromosomal or sub-chromosomal abnormalities). (2) Although the three hESC lines accumulated different genetic abnormalities, they shared a common and marked "adaptation" transcriptomic profile. (3) While there was a clear "copy number" effect in adapted hESC, with copy number variations (CNV) modulating the transcription of genes located within these CNV (including those generated by karyotype abnormalities), the fold changes observed in the expression of many genes from the "adaptation" signature were much higher than those induced by the copy number effect. (4) In HD291 cells, a clone with a trisomy 12 overtook the culture between passage 15 and 30 and this event was associated with a striking cell population doubling increase, similar in magnitude to the increase observed during the initial adaptation process. These new data suggest that hESC adaption follow a bimodal path, with an initial phase occurring during the first five passages and independent of genetic abnormalities, followed by one or several genetic events that are selected by the culture conditions and further increase PSC adaptation.

T-1427

BROAD EXISTENCE OF PLURIPOTENCY FACTOR REGULATED TRANSCRIPT ISOFORMS WITH STAGE-SPECIFIC ALTERNATIVE FIRST EXONS IN MOUSE EMBRYONIC STEM CELLS

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Transcripts with stage-specific alternative first exon (SAFE) usage involve in the regulation of many biological processes, yet their presence and functions in embryonic stem cells (ESCs) are still largely unknown. In this work, we identify 137 mESC SAFE isoforms of 128 genes expressed in both ESCs and somatic cells. Functional analysis revealed that most genes participated in the regulation of stem cell regulated functions. The promoter regions of SAFE isoforms exhibit enriched H3K4me3 and Pol II binding as well as higher DNase I sensitivity in mESCs but not in somatic cells. We found an enrichment of Oct4, Sox2 or Nanog binding sites at the promoter regions of SAFE isoforms, and proved the transcription regulation of SAFE isoforms by these pluripotency factors experimentally. The expression of SAFE isoforms is activated during the reprogramming process of induced pluripotent stem (iPS) cells, and dynamically regulated in early stage embryos or during cell differentiation, indicating their functional importance in regulating pluripotency related cell features.

T-1428

A RAPID AND COST EFFICIENT METHOD FOR PASSAGING HES CELLS USING EDTA

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The maintance and differentiation of hES cells forms the basis for significant research in stem cell biology, as ES cells continue to provide a valuable benchmark for studies of pluripotency and the establishment of differentiation protocols. Due to their inherent propensity for differentiation, the maintance of undifferentiated cultures of hES cells demands more care and attention than does

the culture of most mammalian cells. Thus, the development of cost efficient protocols for the maintenance and passaging of hES cells is an important issue. We here describe a rapid, cost efficient and high yield method for passaging hES cell lines maintained on human foreskin fibroblasts (CRL2429), using EDTA instead of mechanical harvesting of colonies. The method requires less technical training and is more reproducible between different users. Even for a technician highly competent in passaging hES cells using conventional harvesting with a scalpel, our method competes on time, yield and quality. The method works well for transfer of colonies to feeder-free conditions (Matrigel and mTeSR-1) for utilization in downstream differentiation studies. Experiments were carried out on H9 and 429 hES cell lines. By systematically varying reagent concentrations and incubation times, we determined that the optimal conditions involved incubation with 0.1mM EDTA for 1 minute at 37°C. After removing the EDTA and adding new ES-medium a 5ml pipette is used to scrape off the fibroblast layer. The aggregate is triturated to release the hES cells into suspension which are then transferred to new dishes with fibroblast feeder layers. Cultures passaged by this method were compared to control cultures passaged by conventional scalpel harvesting. Assessment was made of colony morphology and pluripotency gene expression by TaqMan, flow cytometry and immunohistomchemistry using laser confocal scanning microscopy. Results demonstrated that the EDTA method is reliable for at least 20 passages at a relatively high passage number (p63). The clones showed stable expression of pluripotency markers, both at mRNA and protein level.

T-1429

ACTL6A PROTECTS EMBRYONIC STEM CELLS FROM DIFFERENTIATING INTO PRIMITIVE ENDODERM

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Actl6a (actin-like protein 6A, also known as Baf53a or Arp4) is a subunit shared by multiple complexes including esBAF, INO80, and Tip60-p400, whose main components (Brg1, Ino80, and p400 respectively) are crucial for the maintenance of embryonic stem cells (ESCs). However, whether and how Actl6a functions in ESCs has not been investigated. ESCs originate from the epiblast (EPI) that is derived from the inner cell mass (ICM) in blastocysts, which also give rise to primitive endoderm (PrE). The molecular mechanisms for EPI/PrE specification remain unclear. In this report, we provide the first evidence that Actl6a can protect mouse ESCs (mESCs) from differentiating into PrE. While RNAi knockdown of Actl6a, which appeared highly expressed in mESCs and downregulated during differentiation, induced mESCs to differentiate towards the PrE lineage, ectopic expression of Actl6a was able to repress PrE differentiation. Our work also revealed that Actl6a could interact with Nanog and Sox2, and promote Nanog binding to pluripotency genes such as Oct4 and Sox2. Interestingly, cells depleted of p400, but not of Brg1 or Ino80, displayed similar PrE differentiation patterns. And mutant Actl6a with impaired ability to bind Tip60 and p400 failed to block PrE differentiation induced by Actl6a dysfunction. Finally, we showed that Actl6a could target to the promoters of key PrE regulators (e.g., Sall4 and Fgf4), repressing their expression and inhibiting PrE differentiation. Our findings uncover a novel function of Actl6a in mESCs, where it acts as a gatekeeper to prevent mESCs from entering into the PrE lineage through a Yin/Yang regulating pattern.

T-1430

THE EFFECT OF JQ1/BRD4 INHIBITION IN THE SELF RENEWAL OF ESCS

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Pluripotent stem cell (PSC)-associated master transcription factors and chromatin remodeling complexes are critical determinants of cellular pluripotency. Recently, it has been described that large numbers of PSC-specific transcription factors are driven by the unusual large enhancers, so called super enhancers where Brd4, a member of the bromodomain and extraterminal domain (BET) family, is highly enriched. Previously it was also reported that the inhibition of Brd4 could lead the differentiation of PSCs via suppression of brd4 dependent transcriptional elongation or unknown mechanism. To define underlying mechanism of Brd4 inhibition in PSCs, we used a small molecule, JQ1. In contrast to previous studies, we show that both mouse and human PSCs lose their self-renewal capacity and eventually die via apoptosis upon JQ1 treatment but JQ1 treatment could not induce differentiation of PSCs into any specific lineages. Furthermore, the somatic fibroblasts and adult stem cells (neural stem cells) are resistant to JQ1 treatment and thus, maintain their cellular identities. Taken together, our data indicate that JQ1 could selectively eliminate PSCs by disrupting self-renewal circuitry of PSCs without inducing differentiation, suggesting the potential application of JQ1 in the clinical translation of PSCs.

T-1431

A GENOME- WIDE RNAI SCREEN OF TRANSCRIPTION FACTORS TO IDENTIFY CRITICAL REGULATORS OF HUMAN EMBRYONIC STEM CELL IDENTITY

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Human embryonic stem cells (hESCs) derived from human blastocysts are capable of unlimited self- renewal and differentiation into any cell types in the body. Transcription factors are key cellular components that control cell identity maintenance and developmental processes. Here we present the result of RNAi screening for critical regulators in hESCs. The combination of high- throughout screening using an OCT-GFP reporter and naked eye observation of cell morphology uncovers a list of factors indispensible for the maintenance of hESC identity. Among identified factors, PHB, a highly conserved protein, is highly expressed in undifferentiated hESCs, and its expression decreases during differentiation. Loss of PHB causes hESC massive differentiation, and severely disrupts histone (H) modifications. Using PHB specific immunoprecipitation- mass spectrometry approaches, we find that PHB can interact with HIRA, which is involved in H3.3 deposition and epigenetic landscape establishment in the pluripotent state. Furthermore, in contrast to mouse ESCs, HIRA RNAi induces conspicuous differentiation and aberrant histone modifications in hESCs. It implies that differential regulation mechanisms might exist in the establishment of bivalent modifications between the naive and primed pluripotency. Interestingly, hESC differentiation induced by RNAi of either PHB or HIRA is accompanied by the impairment of the Krebs cycle. Addition of a Kreb cycle produced

metabolite reverts both differentiation and altered histone modification phenotypes induced by PHB or HIRA deficiency in hESCs. Mechanistically, we show that PHB plays an essential role in the maintaining of an appropriate epigenetic state at the promoter of the enzyme responsible for the production of the Kreb cycle metabolite. Therefore, this study uncovers an unexpected role for PHB and HIRA in the metabolism and epigenetic regulation in hESCs and emphasizes the critical role of the metabolism in the maintenance of hESC properties. In addition, we are able to show that PHB sufficiently promotes reprogramming efficiency combined with Yamanaka factors. Together,our study links the epigenetic and metabolism regulation to the cell fate determination in hESCs.

T-1432

ESTABLISHMENT AND DIFFERENTIATION OF PLURIPOTENT STEM CELLS FROM CLONED RABBIT BLASTOCYSTS

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Our previous studies have successfully established putative rabbit embryonic stem (ES) cell lines with dome-shaped colonial morphology by inhibitor treatment during culture. The present study was aimed to establish somatic cell nuclear transferred (SCNT) embryo-derived ES cells, i.e., ntES cells from cloned rabbit embryos at the blastocyst stage. Firstly, we investigated the development of cloned rabbit embryos reconstructed with or without enhanced green fluorescence protein (eGFP) transgenic fibroblasts. The blastocyst rates in $eGFP^-$ and $eGFP^+$ groups were 27.4% and 23.9%, respectively, compared to that from parthenogenetic embryos (43.1%). One ntES cell line was established from $eGFP^+$ embryos (1/17, 5.9%) and three ntES cell lines were derived from $eGFP^-$ embryos (3/17, 17.6%). All of the ntES cell lines retained alkaline phosphatase activity and expressed ES cell specific markers Oct-4, Nanog, SSEA-4, TRA-1-60 and TRA-1-81. Moreover, the expression of *Oct-4, Nanog,* and *Sox-2* in ntES cell lines was confirmed by RT-PCR. The differentiation capacity of ntES cells *in vitro* and in vivo were examined by the capacity of forming embryoid bodies (EBs) and teratomas, and these ntES cells could differentiate into all three germ layers both in vitro and in vivo. In conclusion, the pluripotent ntES cell lines could be derived from cloned rabbit embryos and these cells express pluripotent stem cell markers and retain their capability to differentiate into various cell lineages of all three germ layers. Further confirmation on their competency of directed differentiation to desired tissue cell lineages is undergoing.

T-1433

CHARACTERIZATION NR5A2 IN EMBRYONIC STEM CELLS

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Although Oct-4 is one of the most intensively studied factors in mammalian development, no cellular genes capable of replacing Oct-4 function in embryonic stem (ES) cells have been found. Recent data show that the nuclear receptor Nr5a2 is able to replace Oct-4 function in the reprogramming process; however, it is unclear whether Nr5a2 can replace Oct-4 function in ES cells. In this study, the ability of Nr5a2 to maintain self-renewal and pluripotency in ES cells was investigated. Nr5a2 localized to the nucleus in ES cells, similar to Oct-4. However, expression of Nr5a2 failed to rescue the stem cell phenotype or to maintain the self-renewal ability of the ES cells. Furthermore, compared to Oct-4-expressing ES cells, the Nr5a2-expressing ES cells showed a reduced number of cells in S-phase, did not expand normally, and did not remain in an undifferentiated state. Ectopic expression of Nr5a2 in ES cells was not able to activate transcription of ES cell-specific genes and gene expression profiling demonstrated differences between ES cells expressing Nr5a2 and Oct-4. In addition, ES cells harboring Nr5a2 were not able to form teratomas in nude mice. Taken together, these results strongly suggest that the gene regulation properties of Nr5a2 and Oct-4 and their abilities to confer self-renewal and pluripotency of ES cells differ. The present study provides strong evidence that Nr5a2 cannot replace Oct-4 function in ES cells.

T-1434

GLUTAMINE HAS AN IMPORTANT ROLE IN MAINTENANCE OF SELF-RENEWAL IN MOUSE EMBRYONIC STEM CELL THROUGH MTOR-DEPENDENT DOWNREGULATION OF HDAC1 AND DNMT1/3A-INDUCED OCT4 EXPRESSION

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Although glutamine (Gln) is not strictly an essential amino acid, it is considered a critical substrate in many key metabolic processes, which are able to control many physiological functions and are involved in regulating early embryonic development. Thus, we investigated the effect of Gln on regulation of mouse (m)ESC self-renewal and related signaling pathways. Gln deprivation decreased Oct4 expression as well as cell cycle regulatory proteins expression. Furthermore, Gln deprivation decreased c-Myc mRNA expression, but significantly increased c-Jun and c-Fos. However, Gln addition retained the cell cycle regulatory proteins and Oct4 expression level of mESCs, which were blocked by compound 968 (glutaminase inhibitor). Gln stimulated Akt phosphorylation which subsequently elicited PKCε translocation from cytosol to membrane without intracellular $Ca2+$ influx, which was inhibited by compound 968. Inhibition of Akt and PKC blocked Gln-induced Oct4 expression and proliferation. Gln also stimulated mTOR phosphorylation in a time dependent manner, which abolished by PKC inhibition. In addition, rapamycin (mTOR inhibitor) blocked Gln-induced Oct4 and c-Myc expression. Furthermore, Gln increased cellular population of both Oct4 and BrdU positive cell, suggesting that Gln mediates both proliferation and maintenance of self-renewal in mESCs. Gln induced decrease in HDAC1 but not HDAC2, which were inhibited by compound 968, PKC, and mTOR inhibitors. Consistently, Gln

addition stimulates nuclear export of HDAC1, which increased global histone acetylation and methylation. In experiment to investigate the role of Gln in hypomethylation of Oct4 promoter, Gln decreased DNMT1 and DNMT3a expression, which were blocked by PKC and mTOR inhibitors. Gln addition significantly reduced methylation of Oct4 promoter region, which were blocked by rapamycin, but synergistically increased by trichostatin A pretreatment. In conclusion, Gln stimulated mESCs proliferation and maintenance of undifferentiation status through epigenetical transcription regulation via Akt, PKCε, mTOR, signaling pathway.

T-1435

SPECIFIC CELL CYCLE PATHWAYS RESTRICT THE DISSOLUTION OF PLURIPOTENCY STATE IN HUMAN EMBRYONIC STEM CELLS

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During differentiation, human embryonic stem cells (hESCs) have to shut down the regulatory network conferring pluripotency in a process we designate as pluripotent state dissolution. Strickingly, from a high-throughput RNAi screen to look for factors that restrict the dissolution of pluripotency in hESCs, we detected a strong enrichment of specific cell cycle genes involved in S phase DNA replication and G2 phase progression. Further genetic and chemical validations showed that the S and G2 phases play a deterministic role in pluripotency state dissolution; in contrast G1 phase did not seem to affect pluripotency dissolution although it was previously linked to lineage specification. Moreover, the deterministic effect of the S and G2 phases on dissolution of pluripotency is due to their intrinsic propensity towards pluripotency maintenance, associated with active pathways in these two phases. Interestingly, we find that S phase associated pathway activates the well-known cell fate regulator, p53, to sustain TGFβ pathway activation and NANOG expression in the absence of self-renewal signal. Our study thus functionally demonstrates that the pluripotency network is hardwired to the cell cycle machinery. It also suggests the importance of balanced cell cycle phases and pathways in regulation of embryonic stem cell fate, in contrast to a G1 centric view on ESC fate determination prevailing in the literature.

T-1436

EMERGENCE OF DISTINCT SPATIAL EXPRESSION PATTERNS IN HUMAN PLURIPOTENT STEM CELL COLONIES OF CONFINED SIZES

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During early development, stem cells organize into strictly defined spatial configurations through coordinated proliferation and differentiation. Recapitulating developmental organization events in vitro would allow us to study spatial patterning in the human system and provide new insights into the earliest stages of intrinsically controlled organogenesis. These insights may also explain challenges in human pluripotent stem cell (hPSC) differentiation wherein factors such as endogenous signals and mechanical forces in the surroundings of differentiating cells in vitro can influence their cell fate trajectory. To control microenvironmental variation, we use micro-contact printing of extracellular matrix proteins to restrict hPSCs attachment to predefined spots and assess Oct4 and Sox2 expression in pluripotency maintaining and differentiation inducing conditions. By automated computational analysis of replicate spots, we quantified distinct spatial expression patterns emergent in different media conditions and variable sized colonies. These patterns were divided into three categories - gradually decreasing expression towards the edge, a ring of high expression around the center and stable expression throughout the colony. These differences were quantified by averaging the expression of cells in ten ring-shaped bins within each colony. Bins were compared to the central bin and showed differences in expression ranging from 40 % to 120 % relative the center. To explore if this spatial heterogeneity extends to other systems, we used our analysis pipeline to probe blood induction from hPSC-derived hemogenic endothelium. These cells display spatial organization of VECAD and CD34 expression where accumulation of these markers are observed in colony centers independent of colony size. In summary, colonies of hPSCs display spatial heterogeneity, which may play a role in hPSC differentiation. Thus, an increased understanding for heterogeneity would not only contribute to our understanding of spatial morphogenesis, but may aid in the development of efficient differentiation protocols. Our experimental platform and analysis framework will facilitate future investigations of the molecular underpinnings by which these patterns arise and how they can be manipulated to alter stem cell fate.

T-1437

THE BASIS AND CONSEQUENCES OF RECURRENT EPIGENETIC ABERRATIONS IN HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) often acquire molecular abnormalities in culture that might restrict their biomedical potential, but may also provide insight into hPSC biology. While several genetic defects have been shown to provide hPSCs with selective advantage, little is known of similar cases involving epigenetic aberrations. To explore this issue we focused on genomic imprinting, whereby parent-of-origin epigenetic modifications differentiate the maternal and paternal alleles and regulate the allele-specific expression of imprinted genes. First, we compared hPSCs from distinct origins, namely embryonic stem cells (ESCs), nuclear-transfer ESCs (NT-ESCs) and induced pluripotent stem cells (iPSCs), and showed that although normal imprinting patterns are generally preserved, both NT-ESCs and iPSCs display comparable frequencies of gene- and clone-specific imprinting aberrations. Some aberrations occur more commonly than in ESCs, suggesting that they may be associated with reprogramming. As a second approach, we performed a global imprinting analysis in 28 hPSC lines, including 15 parthenogenetic ESC (pESC) lines, derived from unfertilized human oocytes, as well as 13 normal hPSC lines as controls. We reasoned that advantageous aberrations would be more readily discovered in parthenogenetic cells, if involving expression of a paternally-expressed gene (PEG)

despite the absence of a paternally-inherited allele. As expected, RNA-Seq analysis revealed that most PEGs were not expressed in pESCs. Nonetheless, a striking proportion (~33%) of genetically-independent pESC lines exhibited upregulation of two co-regulated PEGs residing at the same imprinted locus. In these cell lines, the expression levels of both genes strongly correlated with DNA methylation levels, resembling those in bi-parental cells. As this particular locus is exceptionally stable in normal hPSCs, the prevalence of this aberration suggests that parthenogenetic cells may benefit from reactivation of the silent paternally-inherited allele. We propose that the epigenetic status of this imprinted locus may direct hPSC self-renewal, illustrating that recurrent epigenetic aberrations in hPSCs may bear important functional outcomes.

T-1438

DERIVATION AND CHARACTERIZATIONS FOR TEN XENO FREE SNUHES CELL LINES

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Human embryonic stem cells (hESCs) represent important cell resources and hold tremendous promise for cell-based therapy as well as basic scientific research. One of the crucial requirements to enable to clinical use of these cells is to eliminate the risk of xeno-transmitted infections and immunoreactions caused by animal origin products in conventional cell culture system. We have established ten xeno-free SNUhES cell lines from 136 frozen embryos by using a mechanical dissection method in gradual culture conditions. Derived hESC lines were characterized by DNA fingerprinting, HLA & ABO typing, karyotype, AP staining, immunocytochemistry analysis for pluripotent cell surface markers (SSEA-3, SSEA-4, Tra-1-60, Tra-1-81, Oct-4), RT-PCR for undifferentiated gene expression (SOX2, Nanog, hTERT, Rex-1), mycoplasma detection test, in vitro differentiation capacity using embryoid body (EB) formation and Neu5Gc detection by FACs. Two hESC lines (SNUhES32 & SNUhES33) were established from phase I xeno-free culture condition (human foreskin fibroblasts (HFFs) cultured by IMDM basal medium containing FBS + xeno-free medium + humanized cell substrate) and eight cell lines (SNUhES34, 35, 36, 37, 38, 39, 40 & 41) were established from phase II (HFFs cultured by IMDM medium containing human serum (HS) + xeno-free medium + humanized cell substrate). Particularly, SNUhES34 and SNUhES35 are sibling. All cell lines were identified by DNA fingerprinting, HLA & ABOtyping and karyotype and were successful for cryopreservation & thawing. Currently, these cell lines were registered with the National Stem Cell Bank in Korea. We have derived ten xeno-free SNUhESC lines under phase I and phase II xeno-free culture conditions. These hESC lines can be used for research but also clinical application in cell therapy. This research was supported by the Bio & Medical Technology Dvelopment Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (2012M3A9C6049722)

T-1439

FEEDER LAYER CONTRACTILITY REGULATES ESC FATE VIA ALTERATIONS IN COMPOSITION AND ORGANIZATION OF THE SECRETED MATRIX

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Embryonic stem cells (ESCs) decision to remain pluripotent in vitro is directed by the interplay between extrinsic microenvironment and the cytokines added to the culture media. Mouse embryonic fibroblasts (MEFs) are known to maintain ESCs in their self-renewal state via secretion of multiple factors. However, the importance of physical crosstalk between MEFs and ESCs remains incompletely understood. Contractility being one important regulator of self-renewal in ESCs, we address the importance of MEF-ESCs interaction by perturbing the contractility of MEFs by culturing them on gelatin-coated dishes at varying densities. We show that increasing the gelatin-coating density alters the contractility and cortical elasticity of MEFs leading to differences in composition of ECM secreted (MEF-derived matrix (MDM)), with greater collagen I secretion at higher gelatin-coating. When plated on MEFs cultured on gelatin substrates at varying density, mouse ESCs tend to form smaller colonies with high Oct4 levels on MEFs with higher contractility, with Oct4 levels closely related to the colony size. Interestingly, similar results are observed when ESCs are cultured directly on the MEF-derived matrices. Treatment of MEFs with contractility altering drugs like blebbistatin, ML-7 and nocadazole lead to alterations in MDM composition and organization, which in turn, influenced ESC colony size and Oct4 expression. Taken together, these data demonstrate the role of MEF contractility in regulating ESC fate and the potential of MDM and drug altered MDM in directing lineage specific differentiation in the absence of exogenous morphogens.

T-1440

REGULATION OF PLURIPOTENCY BY NAT1 IN MOUSE EMBRYONIC STEM CELLS

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Nat1 is essential for 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, 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 and in cell culture with serum + LIF were undifferentiated and had a rounded, dome-like morphology that is similar to the ground state of 2i-treated WT ES cells. In the present work, to study the relationship between Nat1 function and these phenotypes, we examined how Nat1 regulates pluripotency in mES cells. We revealed that Nat1-/- mES cells upregulated several pluripotency-associated genes compared with WT mES cells. After reintroduction of Nat1, rescued mES cells exhibited gene expressions

and morphology similar to WT mES cells. We found that Nat1 was localized in the cytoplasm but not in the nucleus of mES cells. Lastly, we identified Nat1-binding proteins that regulate translation. These results indicate that NAT1 influences pluripotency through cooperation with NAT1-binding proteins in the cytoplasm and indirect transcriptional regulation of pluripotency-associated genes.

T-1441

THE EXPRESSION AND ROLE OF ARYL HYDROCARBON RECEPTOR IN EMBRYONIC STEM CELLS AND EMBRYOID BODIES

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Aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor which belongs to the bHLH/PAS family. Upon activation by its ligand, AHR translocates to the nucleus and controls the expression of a diverse set of genes. Initially, AHR was known as a major mediator of toxicity of various environmental contaminants. In the following studies, however, the important role of AHR was established in cancer as well as in normophysiology, including regulation of cell cycle, importance in reproductive system etc. There are also data indicating the importance of AHR in the regulation of development and functioning of cells with high developmental potential, for example liver, neural, hematopoietic and breast cancer stem cells/progenitors. However data concerning the expression and role of AHR in human embryonic stem (hES) cells and their differentiating counterparts - human emryoid bodies (hEB) - are limited. First, we determined that AHR is expressed and activated in pluripotent hES cells, as seen by western blotting and immunofluorescence analysis, respectively. Moreover, the persistent environmental contaminant and most potent ligand of AHR - TCDD - did not have any effect on the pluripotency of hES cells. A previous study on murine embryonic stem (mES) cells showed that the expression of AHR is absent in their pluripotent state and increases as differentiation in embryoid bodies proceeds. Our experiments, however, indicate that AHR is indeed expressed in pluripotent hES cells but its expression of both mRNA and protein is downregulated in differentiating embryoid bodies. In our ongoing experiments we aim to elucidate the role of AHR in the pluripotency of hES cells as well as describe the mechanisms by which it is modulated during non-directed differentiation.

T-1442

RNAI SCREEN REVEALS A ROLE OF THE SMALL SUBUNIT PROCESSOME AS A REGULATOR OF PLURIPOTENCY IN EMBRYONIC STEM CELLS

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Embryonic stem cells (ESCs) provide the tractable model system for the cell fate commitment in early development, and have a great potential for medical application. Substantial advancement has been made in molecular regulatory networks including transcription factors, epigenetic regulators, and chromatin remodeling complex. However, less attention has been paid to the role of post-transcriptional and translational regulation in ESCs. To gain a comprehensive insight about the role of RNA binding proteins (RBPs), which are important players responsible for all the processes related with RNA transcripts, in pluripotency network, we applied RNAi screen for the RBPs expressed in mouse ESCs. Novelty and the specific advances: We find 16 RBPs required for ESC maintenance, which include splicing regulators, noncoding RNA interacting proteins, and ribosome biogenesis factors. Unexpectedly, we discover components of Small Subunit Processome (SSUP), which is required for 18S rRNA processing, are upregulated in mESCs and contribute critically to ESC self-renewal through translational enhancement. Furthermore, SSUP components are required to keep the open structure of nucleolar chromatin, which is important for maintaining ESC identity. Our study uncovers novel RBP regulators in ESCs, and reveals the multifaceted role of SSUP in supporting ESCs.

T-1443

MSX2 MEDIATES ENTRY OF HUMAN PLURIPOTENT STEM CELLS INTO MESENDODERM BY SIMULTANEOUSLY SUPPRES-SING SOX2 AND ACTIVATING NODAL SIGNALING

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How BMP signaling integrates into and destabilizes the pluripotency circuitry of human pluripotent stem cells (hPSCs) to initiate differentiation into individual germ layers is a long-standing puzzle. Here we report muscle segment homeobox 2 (MSX2), a homeobox transcription factor of msh family, as a direct target gene of BMP signaling and a master mediator of hPSC entry from pluripotency to mesendoderm. Enforced expression of MSX2 suffices to abolish pluripotency and induce directed mesendoderm differentiation of hPSCs, while MSX2 depletion impairs mesendoderm induction. MSX2 acts as a direct target gene of the BMP pathway in hPSCs that can be synergistically activated by Wnt signals during mesendoderm induction. Furthermore, MSX2 destabilizes the pluripotency circuitry through direct binding to the SOX2 promoter and repression of SOX2 transcription, while MSX2 induction of mesendoderm lineage commitment requires simultaneous suppression of SOX2 and activation of Nodal signaling. Interestingly, SOX2 does not merely lie downstream of MSX2 but instead can promote the degradation of MSX2 protein, suggesting mutual antagonism between the two lineage-specifying factors in the control of stem-cell fate. Together, our findings reveal critical new mechanisms of destabilizing pluripotency and directing lineage commitment in hPSCs.

T-1444

HUMAN STEM CELLS WITH STABLE AND AUTHENTIC MOU-SE ESC-LIKE NAÏVE GROUND STATES OF PLURIPOTENCY

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Human pluripotent stem cells (hPSC) share developmental, biochemical, and epigenetic commonalities with "primed" rodent post-implantation epiblast stem cells (EpiSC). Unlike mouse embryonic stem cells (mESC), primed EpiSC possess a less primitive and more restricted pluripotency with variable directed differentiation potencies. Stable reversion to an authentic mESC-like naïve ground state may improve the utility of primed hPSC. Although mESC stably revert to a ground state with only GSK3β and ERK inhibition (2i), thus far only meta-stable naïve hPSC requiring complex anti-apoptotic cocktails, ectopic transgene expressions, or primed epiblast growth factors have been described. Herein, we evaluated the impact of human induced pluripotent stem cell (hiPSC) derivation method on amenability to naïve reversion. We demonstrate that optimization of classical WNT and ERK modulation was both sufficient and necessary for stably converting a repertoire of hESC and non-integrated hiPSC lines to naïve pluripotency. Supplementation of LIF/2i with a small molecule WNT pathway axin stabilizer (LIF/3i) synergized with GSK3β inhibition to enhance activated β-catenin to levels that permitted stable reversion of primed hPSC lines to clonogenic mESC-like dome-shaped colonies. These naïve-reverted hPSC maintained stable growth kinetics, normal karyotypes, and SSEA4/TRA-1 antigen expression for >30 passages. Moreover, naïve-reverted hPSC adopted authentic mESC signaling pathways (e.g., LIF/JAK/STAT3; BMP4 proliferative responsiveness), acquired classic epigenetic configurations and gene expressions distinctive of mESC-like naïve pluripotency (e.g., increased NANOG, KLF2, STELLA, NR5A2, DNMT3L, decreased XIST), clustered closely with LIF-2i-reverted mESC, and supported robust tri-lineage teratoma formation and directed differentiation. Interestingly, although large cohorts of hPSC reverted to naïve phenotypes, long-term stability was attained only for hiPSC lines with evidence of more effective reprogramming and reduced lineage priming. Thus, high-fidelity reprogramming may be a prerequisite for stably maintaining the human naive ground states under classical conditions. The derivation of authentic naive hPSC greatly impacts human developmental biology and regenerative medicine.

EMBRYONIC STEM CELL CLINICAL APPLICATION

T-1421 *This poster board is located in a different topic area in the poster hall*

IMMUNOSUPPRESSIVE EFFECTS OF HUMAN PLURIPOTENT STEM CELL-DERIVED VENTRICULAR CARDIOMYOCYTES TOWARD HUMAN DENDRITIC CELLS

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Recent advances in directed cardiac differentiation of human pluripotent stem cells (hPSC) provide great promises in cell-based regenerative therapies for incurable cardiovascular diseases. However, immune rejection remains a major obstacle to consider. Dendritic cells (DCs) play a central role in the development of both alloimmunity and transplantation tolerance. In the present study, human DCs from peripheral blood cocultured with human embryonic stem cell (hESC)-derived ventricular cardiomyocytes (VCMs) during LPS activation (hESC-CM/LPSDCs) displayed a distinct "semi-mature" phenotype with a lower expression of the DC maturation marker CD83. The ability to secrete IL-12 was also reduced in hESC-CM/LPSDCs. Interestingly, hESC-CM/LPSDCs exhibited an abolished ability to stimulate allogeneic T cell proliferation and the activated T cells were hyporesponsive to further stimulation. Although there was no increase in the secretion of indoleamine 2,3-dioxygenase (IDO) and TGF-β, an increased IL-10 production was detected in VCM/LPSDCs with an augmented ability to promote CD4+CD25+Foxp3+ regulatory T cells (Treg) differentiation. Similar immunomodulatory properties were seen with hPSC-VCMs differentiated from several different lines, including HES2, H7 and an in-house hiPSC-PB2, suggesting that our observations were not line-specific. Taken collectively, we conclude that hPSC-VCMs suppress DC ability to trigger effective T cell response, and hence tolerance may be achieved with minimal immune suppressive regimen after transplantation.

T-1445

IMPROVING THE RESTORATION OF VISUAL FUNCTION IN RCS RATS BY TRANSPLANTING A MONOLAYER OF RPE CELLS DERIVED FROM HUMAN EMBRYONIC STEM CELLS

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Replacement of defective retinal pigment epithelium (RPE) by RPE cells derived from human pluripotent stem cells provide a novel approach to a rational treatment of forms of blindness that affect

the RPE. Transplantation of a polarized RPE monolayer as a sheet seems to be more promising than injection of RPE cell suspension. We recently developed, under clinically compatible conditions, a tissue-engineered product (TEP) consisting of RPE cells derived from human Embryonic Stem (hES) cells disposed on a biocompatible substrate (denuded human amniotic membrane). Here, we have compared the visual recovery of RCS rats after transplantation of RPE cells in suspension vs. TEP. RCS rats (N=47) were grafted at day 28 postnatal (P28) with either a cell suspension of RPE cells, or the TEP. Photoreceptor functionality and survival were evaluated at different times (from P60 to P118) after transplantation through electroretinography (ERG) and optokinetic tests. We successfully developed a surgical procedure to graft the TEP in the subretinal space of P28 RCS rats by wrapping the TEP in a thin gelatin layer. We demonstrated by measuring the b wave amplitude after 72 days (P90 rats) that TEP engraftment provide better ERG responses (198.9 ± 29.4 µV) compared to the cell suspension injection (95.8 ± 67.7 µV; p=0.0001) and dystrophic control (30.1 ± 25.0 µV; p=0.0001). Visual acuity evaluated through optokinetic responses in P118 RCS rats indicate that, compared to the SHAM (gelatin alone, 0.268 ± 0.104 c/d), injection of RPE cell suspension (0.444 ± 0.093 c/d; p=0.001) or TEP (0.496 ± 0.085 c/d; p<0.0001) improved performance of dystrophic eyes. Our optokinetic test results indicate that injection of hES-derived RPE cells as a cell suspension or as a monolayer (TEP) ameliorate the functionality and/or the survival of photoreceptors in transplanted RCS rats. ERG recording clearly demonstrated that TEP engraftment leads to a better visual improvement in RCS rats, and that this recovery was maintained for a longer period of time. Further morphologic and histologic studies are ongoing to assess the effect of transplanted cells on host photoreceptor degeneration.

T-1446

VERSATILE PLURIPOTENT STEM CELL CULTURE PLATFORMS FOR REGENERATIVE MEDICINE AND DRUG DISCOVERY

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Efficient generation of differentiated mature cells from human pluripotent stem cells (hPSCs) is a vital issue for stem-cell-based regenerative medicine and pharmaceutical applications. Conventionally employed colony-type culture methods often result in low cell yields and heterogeneous cell populations. To optimize current culture methods, we analyzed core signaling pathways that underlie epithelial-to-mesenchymal transitions (EMTs), cellular heterogeneity, and various hPSC growth patterns. We further developed new cell growth platforms based on non-colony type monolayer (NCM) on Matrigel, which were mediated by various Rho-associated kinase (ROCK) inhibitors. These ROCK inhibitors include Y-27632, thiazovivin, Y-39983 (ROCK I inhibitor), and phenylbenzodioxane (ROCK II inhibitor). Human pluripotent stem cells cultured under NCM conditions retained the pluripotent state and the capacity to differentiate toward the three germ layers and their derivatives. However, microarray analysis of mRNA gene expression indicated that a subset of genes that are related to mesoendodermal differentiation have been altered, suggesting that these altered gene expression might influence the efficiency of directed differentiation. We further modified our basic NCM protocols without the use of ROCK inhibitors. One of such NCM methods is to cultivate hPSCs on defined extracellular proteins such as the laminin isoform 521 (LN-521) in the presence or absence of E-cadherin. Modified NCM methods provided versatile platforms for genetic analysis of hPSCs and their differentiated cells. We found that NCM-based cell cultures are efficient for transfection of small oligonucleotide-based microRNAs and short-hairpin RNAs, DNA plasmids, and lentiviral particles. Moreover, we have used genetically modified cells for high throughput (HTP) assays in a 384-well format for drug discovery and directed differentiation. Thus, NCM-based methods overcome the major shortcomings of colony-type culture, particularly suitable for producing large amounts of homogeneous hPSCs for future clinical applications, drug discovery, and disease modeling.

T-1447

GENOMIC INSTABILITY OF HUMAN PLURIPOTENT STEM CELLS: DEVELOPMENT OF A HIGH-SENSITIVITY SCREEN UTILISING DROPLET DIGITAL PCR AND FISH-BASED CHROMOSOME POSITIONING

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For human pluripotent stem cells (hPSCs) to be approved for therapeutic use, both sensitive and meaningful methods must be developed to assess product safety. An area of concern is genomic instability and the effect it may have on cellular phenotype, differentiation potential and malignant transformation. Whilst methods such as molecular karyotyping and array comparative genomic hybridization do exist for the analysis of genomic copy number variation, they are likely unsuitable for fine-scale, high-throughput studies. Droplet Digital PCR (ddPCR) enables highly sensitive absolute quantification of nucleic acids without need for standard curves and can be used for copy number variation and rare event detection, reportedly as low as one mutant copy in one million wild-type targets. Here ddPCR will be utilised to determine the origin and selection of aneuploidy hPSCs in culture. Additionally, differences in the genomic stability of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) will be characterised. Of particular focus will be chromosome 20, specifically BCL2L1 with its anti-apoptotic/pro-cell survival roles. Optimisation of ddPCR using AMELX and AMELY probe-based assays with titration of female DNA into male DNA has shown the ability to detect at least a 6.25% deviation from the initial 50:50 AMELX:AMELY ratio, with the assay serving as a model for the detection of low level aneuploidy sub-clones in hPSC cultures. Assays for housekeeping genes including RPP30 and ALB are being optimised as copy number variation reference standards. Further assay optimisation included the use of restriction enzyme digestion to improve data quality by increased droplet segregation in the 2-D plots. Chromosome and gene positioning in interphase nuclei has been demonstrated to influence the regulation of gene expression. Fluorescence in situ hybridization (FISH)-based analysis will determine the impact of aneuploidy on chromosome territory positioning in hPSC nuclei, modelled initially in cell lines containing supernumerary

X chromosomes. Combined with molecular analysis by ddPCR, this should translate into a rapid, high-sensitivity screen for the assessment of hPSCs for regenerative medicine, including those now being accepted by the UK Stem Cell Bank at NIBSC.

T-1448

ADAPTATION OF CLINICAL GRADE FEEDER-DEPENDENT HUMAN EMBRYONIC STEM CELL LINES TO FEEDER-FREE GROWTH CONDITIONS TOWARDS CELL THERAPY FOR ALS

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Human embryonic stem cells (hESCs) hold a great promise in clinical cell therapy applications. Their unique characteristics of unlimited expansion and ability to differentiate to all cell types, makes them perfect candidates as a source for variety of cell therapies. In order to pave their road into the clinic, we have found that a platform for large scale clinical expansion of hESCs in cGMP feeder-free growth conditions is obligatory in terms of reproducibility, robustness and cost-effectiveness. For this, we have developed a process of adaptation of cell grown with feeders to feeder-free growth conditions. We have thawed frozen sub-colonies (from manual cut) directly onto wells coated with vitronectin, human serum (HS) or vitronectin plus HS, all with the same media for feeder-free growth conditions. Cultures were inspected daily. After 7 days on HS, several hESC colonies with typical morphology were observed. On vitronectin, the cells were flatter and more elongated presenting morphologies of differentiated cells. The cells were passaged using single cell dissociation into new wells with the same coating and grew for further 5 days. The cells that came from the HS-coated wells gave again the typical hESC morphology and allowed further expansion to yield hundreds of frozen ampules as a master cell bank. We have characterized these latter cells by testing their gene expression, by FACS and immunofluorescence, as well as for karyotypic stability. More than 95% of the cells express pluripotency genes including SSEA4, TRA-1-60 and OCT4. The cells were also shown to keep their differentiation potential as tested by their ability to develop into astrocytes. This study shows that clinical grade cell lines that were established few years ago, prior to the "feeder-free" era, can be adapted to current standards and be used for industrial applications. The importance of this development is the fact that it significantly shortens the time and costs to get into the clinic with hESCs. Eliminating the need to create and characterize feeder cell banks and using feeder-free growth conditions that have high expansion folds compared to feeder dependent cultures allowed us to accelerate our program using hESC-derived astrocytes for ALS cell therapy.

T-1449

COMPREHENSIVE WHOLE GENOME SEQUENCE ANALYSIS OF HUMAN EMBRYONIC STEM CELLS REVEALS INNATE DISEASE SUSCEPTIBILITY

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Human embryonic stem cells (hESCs) have the potential to give rise to any cell type in the body and are increasingly used to model human diseases. It is widely accepted that genotypic variability is a major driver of phenotypic variability, including the manifestation of disease phenotypes observed in cellular models of human disease. However, the genetic makeup of hESCs have not been extensively studied. This fact limits the ability to rationally choose cell lines to use for disease modeling and therapeutic transplantation, and likely contributes to conflicting reports from laboratories studying different cell lines. To address this critical gap in our knowledge, we collected nearly 100 hESC lines from collaborators across the globe that were reported to be karyotypically normal and are listed on the National Institutes of Health Human Embryonic Stem Cell Registry. We cultured these cells under similar conditions and sequenced their genomic DNA to >30x coverage using Illumina next generation sequencing. We observed that hESCs are extremely rich in genetic variation at the level of copy number variants (CNVs) and simple nucleotide variants (SNVs). These variants sometimes disrupted gene function, including developmentally important and disease-linked genes that might compromise the ability of certain cell lines to be differentiated into cell types of interest or to be used for therapeutic transplantation. In addition to rare loss-of-function variants, we characterized the common genetic variants carried by these lines. Based on the known associations of some of these variants to disease, we predicted the inherent susceptibility of different hESC lines to obesity, Chron's disease, schizophrenia, and rheumatoid arthritis. Overall, the comprehensive analysis of whole genome sequencing data should aid in the selection of cell lines for chemical screening and therapeutic transplantation as well as in the interpretation of phenotypic data obtained from hESC-derived cell types.

TISSUE ENGINEERING

T-1450

INCREASE OF RECEPTORS ASSOCIATED WITH FUNCTIONALITY IN MELANOCYTES SUBJECT TO CONDITIONS IN VITRO

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Vitiligo impacts on a large scale the quality of life of the sufferer. To optimize the methods of expansion of melanocyte that ensure their functionality and lead to improved efficacy of cell therapy is a challenge for the scientific community. Skin biopsies from areas not exposed to sunlight were obtained from ten healthy individuals after they had signed informed consent forms. After enzymatic and mechanical digestion, the melanocytes, which were grown in selective media, were isolated. The first sample was obtained when the melanocytes achieved an 80% confluence in T25 boxes. The trypsinizated cells were plated for later expansion and for a second measurement at the moment of 80% confluence. The samples obtained in the two periods of cultivation were characterized by immunohistochemistry for the melanocyte receptors HMB45, Melan A and C -Kit. The positive characterization of the receptors in the cultures of melanocyte were: HMB45; 9% first pass, 61.8% second

pass, C -kit; 9% first pass, 36.6% second pass, Melan A; 30% first pass, 80.8% second pass. The average increase between the two passes was: HMB45 53%, C -kit 28 %, Melan A 51%. The receptors associated with functionality increased their expression in the second pass; the culture of melanocytes is constituted as an alternative for treating extensive air.

T-1451

INVESTIGATION OF ENDOTHELIAL PROGENITOR CELLS CO-CULTURED WITH MESENCYMAL STEM CELLS IN 3-D BONE SCAFFOLDS: AN ATTEMPT TO MANUFACTURE A VASCULARIZED BONE CONSTRUCT

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Besides a biocompatible scaffold and an osteogenic cell population, engineered-bone construct requires appropriate blood vessel to overcome nutritional problems and oxygen transport in the 3-D construction. We hypothesized that adding endothelial progenitor cells (EPCs) into the bone construct developed from mesenchymal stem cell (MSCs)-seeded scaffold may enhance bone formation and contribute in angiogenesis within the construct. For this purpose, MSCs and EPCs were isolated from canine bone marrow, culture-expanded and characterized by flowcytometry using the known surface markers. Then, about 5×10^5 cells from triple passaged EPCs, MSCs and 1:1 ratio of MSCs/EPCs (co-culture) were seeded on 5×5×5mm porous cube of b-TCP-PLGA scaffolds (developed in our lab) in an osteogenic medium. Three weeks after culture initiation, vascularization and bone formation were examined with qPCR and immunohistochemistry using an assortment of appropriate markers. The expression levels of bone-specific genes including Collagen type I, osteopontin, osteocalcin and runx2 observed to be significantly higher in the MSC/EPC co-culture compared to that of pure MSC and EPC culture ($p<0.05$). On the other hand, the expression levels of the endothelial markers including, KDR, VEGFR2 and vWF tended to be statistically higher in the EPC culture and MSC/EPC co-culture than MSC alone culture ($p<0.05$). In this regard there was no difference between EPC culture and MSC/EPC co-culture. Immunohistochemical analysis confirmed the data obtained by qPCR. Furthermore our preliminary data on the implantation of MSC/EPC co-cultured constructs in animal model of bone defect was promising. Taken together, it seems that the presence of EPC in bone scaffold containing MSC enhances MSC bone differentiation. Co-culture of MSC with EPC in 3-D scaffold would be an appropriate system for creating a vascularized bone construct for applications in bone regeneration.

T-1452

OPTOGENETIC CONTROL OF BIOARTIFICIAL CARDIAC TISSUE FROM INDUCED PLURIPOTENT STEM CELLS

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Electrical stimulation is a widely used approach in cardiac tissue engineering for the enhancement of tissue maturation. However, tissue damage due to faradaic reactions might occur during electrical stimulation. We aimed to overcome these limitations by constructing a light-sensitive bioartificial cardiac tissue (BCT) generated from murine induced pluripotent stem cell (miPSC)-derived cardiomyocytes expressing channelrhodopsin, a light-activated cation channel. Cardiomyocytes were generated from a transgenic miPSC line expressing channelrhodopsin under control of the chicken β-actin promoter. BCTs were prepared by mixing cardiomyocytes and mitotically inactivated mouse embryonic fibroblasts. Light-induced contraction forces were measured in a custom made bioreactor system. Light stimulation of BCTs was performed through a royal blue (470 nm) high power LED. Stimulation triggers were generated by the bioreactor amplifier with software-controlled stimulation duration and frequency. Threshold light intensity required to obtain stable 1:1 pacing was determined. The effect of long-term (14 days) light stimulation of BCT was tested. Light-inducible BCTs showed spontaneous beating activity, indicating that expression of channelrhodopsin did not result in a leaky membrane current. The magnitude of light-induced contractions was found to depend on two factors: light intensity and stimulus duration. Beating frequency analysis showed that the frequency could be controlled with 1:1 capture up to 300 bpm. After long-term light stimulation for 14 days, immunostaining showed improved cellular alignment and sarcomeric organization in BCTs compared to unstimulated controls. Accordingly, light stimulation resulted in increased contraction forces (0.792 ± 0.048 mN vs. 0.473 ± 0.026 mN in unstimulated controls) similar to conventional electrical stimulation (0.676 ± 0.044 mN). No side effects were observed after long-term light stimulation and microarray expression analysis revealed differentially regulated genes for both stimulation methods. We have shown light stimulation can be used as an alternative approach to electrical stimulation of cardiac constructs and the underlying mechanisms of improvement of cardiomyocyte maturation and tissue function are currently further investigated.

T-1453

MSC-SOURCED EXOSOMES AS THERAPEUTIC AGENTS FOR WOUND HEALING AND SKIN REGENERATION: FROM SCALED PRODUCTION TO FUNCTIONAL REGENERATIVE OUTCOMES IN VITRO AND IN VIVO

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Mesenchymal stem cells (MSCs) are characterized by their self-renewing capacity and their ability to differentiate into chondrocytes, adipocytes, and osteocytes. This makes them attractive starting materials for tissue engineering and regenerative medicine applications. While the recognized regenerative properties of stem cells show promise in bioengineering dermal constructs for repairs, there is limited evidence that direct injection of stem cells for skin healing has beneficial effects. It has been suggested that much of the observed benefit of these stem cell injections arises from stem cell-secreted factors carried in discreet microvesicles called

exosomes. These small vesicles contain bioactive components related to wound healing and present a potential new allogeneic therapy for dermal repair and regeneration. Here, we present our findings on the physical and functional characteristics of MSC exosomes relevant to wound healing and tissue regeneration. Data is presented on the miRNA profile of these exosomes, their ability to mediate cell migration and incorporate into the recipient cell membrane, and their ability to down-regulate STAT3 phosphorylation. Scalable production of exosomes was accomplished using a hollow-fiber bioreactor, whereby the total bioreactor yield was approximately 10-fold more than T225 flask controls based on exosome number and at a 10-fold higher concentration as well. We evaluate the application of exosome preparations sourced from MSC and other stem and progenitor cell populations in rodent models of wound healing and skin repair; preliminary, proof-of-concept data will be presented. Having successfully developed scalable exosome production, isolation procedures, and in vitro and in vivo assays to functionally characterize these particles which are secreted by the cultured MSCs and other cell populations, our goal is to leverage the regenerative and healing properties of adult stem cells by developing exosomes as a non-surgical and non-cellular therapeutic or cosmeceutical agent for skin repair.

T-1454

PREPARATION AND CHARACTERIZATION OF CARDIAC STEM CELLS BASED CELL SHEET FOR CARDIAC REGENERATION

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For the last decade, cell therapy has emerged as one of the most promising therapies for patients suffering from post-MI heart failure. In contrast to direct cell injection novel cell transplantation methodology based on cell sheets transplantation has been proposed to recover damaged heart function. We here investigated the integration and survival of scaffold-free cardiac stem cells-based sheets and their potential for myocardial regeneration. After coronary artery ligation in rats syngeneic c-kit+Lin-cardiac stem cells (CSC) marked with vital fluorescent dye (Cell Tracker CM-DIL) were grafted by epicardial placement of CSC sheets generated by using temperature-responsive dishes. Cell sheets neovascularization and integration to underlying myocardium, transplanted CSC proliferation and differentiation were assessed by immunofluorescence analysis of myocardial frozen sections obtained 14 days after transplantation. Immunofluorescence analysis of CSC sheets before transplantation has shown that CSC in sheets interact with each other via connexin 43, produce extracellular matrix proteins, proliferate and express of the cardiac transcription factor Gata 4. Histological analyses of heart frozen sections revealed that on day 14 the CSC sheet grafts had produced thick tissues with a high-cell density, and promoted vascularization. Proliferation and migration to underlying myocardium of marked CSCs from sheets were observed. Part of transplanted CSC showed signs of differentiation to cardiomyocytes and endothelial cells. Transplantation of CSCs in cell sheets resulted in cells survival, proliferation, migration and differentiation, and was associated with neovascularization and structural integration of cell sheets in myocardium. These data strongly support the potential of CSC sheet transplantation for the treatment of damaged heart.

T-1455

SAFETY AND EFFECTS OF HYDROXYAPATITE NANOPARTICLES IN MESENCHYMAL STEM CELLS

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Natural bone encompasses nanosize blade-like crystals of hydroxyapatite developed in adjacent connection with collagen (Col) fibers. The cells included the Nanoparticle spheres of hydroxyapatite (N-HA) in the cytoplasma through phagocytosis. There is no literature report of cytotoxicity and genotoxicity in bone marrow mesenchymal stem cells (MSCs) whose nuclear membrane status is cultured in a N-HA medium. N-HA, dispersed in reconstituted fibrous Col, were prepared in three weight ratios of 75:25, 65:35 and 50:50 (N-HA:Col). MSCs from rabbits were seeded and cultured on the N-HA/Col and HA/Col microbeads and characterized. The cultured MSCs on the N-HA were prepared for the cytoplasm and nuclear membrane examination by Transmission Electron Microscope (TEM). N-HA were distributed evenly throughout the Col matrix and aggregated to microbeads as determined by scanning electron microscopy. Electron and confocal microscopy showed that the MSCs spread and attached to microbeads via focal adhesions, while staining for F-actin and DNA revealed the presence of stress fibers. The phenotype of the MSCs in the flow cytometry was remained as CD11a-, CD44+, and CD90.1+. The optimal weight ratio was 65:35 of N-HA for the normalized alkaline phosphatase activities. The cytoplasmic and nuclear membranes of MSCs were intact while the blade-like N-HA was phagocytosed in endosomes of the cytoplasma. MSCs are capable of proliferating and differentiating in appropriate combinations of N-HA/Col. The osteogenic ability of MSCs was enhanced by N-HA. There are no cytotoxity or genotoxicity found. Thus it is a promising composite for future clinical applications.

T-1456

HDAC INHIBITION AND ELECTRICAL CONDITIONING ACT ON COMMON PATHWAYS THAT DRIVE MATURATION OF HESC-DERIVED VENTRICULAR CARDIOMYOCYTES AND CARDIAC MICROTISSUES

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Ventricular cardiomyocyte derived from human pluripotent/embryonic stem cells (hESC-VCMs) are considered as an unlimited source for cell-based heart therapies. However, hESC-VCMs are physically smaller and can only generate forces that are substantially weaker than adult-VCMs. Furthermore, action potential, electrical propagation and Ca2+-transients are most consistent with immature CMs. Epigenetic regulations are implicated in embryonic development and indeed, the epigenetic state of hESCs is dynamic over the course of human cardiac differentiation and can be modulated by the introduction of transient HDAC inhibition, which promotes H3K4-trimethylation and amplifies the expression levels of specific genes important for CM functions. Separately, by providing proper physical and environmental cues, including electrical

stimulation and 3D-tissue engineering, can also facilitate maturation of hESC-VCMs. Here, we conjectured that synergistic effects exist by combining HDAC inhibition with electrical and physical conditioning. hESC-VCMs cultured with electrical stimulation increased gene expression of RYR2, TRDN, SCN5A, KCNA4, KCNH2, MYH6 and MYH7, and treatment with an HDAC inhibitor - valproic acid (VPA) showed increased expression of genes encoding for Ca2+-handling proteins, ion channels and contractile proteins. However, combined treatment did not further amplify the expression level of these genes, suggesting that HDAC inhibition and electrical stimulation may act on common pathways. Interestingly, electrical stimulated hESC-VCMs had lower spontaneously firing frequency and increased membrane capacitance, indicating hypertrophic growth and physical maturation. 3D-cardiac microtissues (CMTs) were generated from hESC-VCMs. While no significant difference in force was detected between control and VPA-treated CMTs, there was a higher percentage of CMTs that can be electrically stimulated at a high frequency with VPA treatment. Furthermore, the force-frequency relationships of each individual CMT under VPA-treatment was found to be less negative when compared to the controls, signifying maturation. Based on these results, we conclude that synergisms exist among pro-maturation stimuli which act on common as well as distinct pathways to intricately regulate maturation.

T-1457

HUMAN MESOANGIOBLASTS AND DECELLULARIZED MATRIX TO DEVELOP OF ARTIFICIAL OESOPHAGUS FOR CONGENITAL DEFECTS

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Tissue engineering has been proposed as a therapeutic alternative to oesophageal substitution for congenital defects. This interdisciplinary field comprises the use of (i) biomaterials (scaffolds) and (ii) stem cells in order to create a construct able to restore normal function of the tissue of interest. Decellularised matrices are ideal scaffolds for tissue engineering because they maintain extracellular matrix (ECM) information. However, successful engineering of functional smooth muscle of the gut with decellularized matrices and subsequent re-cellularization have not been established yet. Human mesoangioblasts (MABs) were used to repopulate the muscular layer as they are able to differentiate into smooth muscle. This tissue engineering approach has the purpose of building a differentiated oesophageal muscularis externa combining cells with acellular ECM in a dynamic 3D tissue culture. A tailored bioreactor provided optimal nutrient exchange, oxygenation and pulsatile stimulation. Primary MABs were injected into the muscular layer of acellular rat oesophagi and cultured in dynamic vs static conditions. Injection of MABs displayed successful and consistent cell engraftment and migration from the injection sites after 9 days of culture. Seeded matrices cultured in dynamic condition showed improvement in cell survival, migration and homogeneous distribution within the scaffold. An optimized combination of proliferating and differentiating medium allowed cell expansion and subsequent differentiation towards smooth muscle, with 30% of cells expressing Ki67 and 45% positive for smooth muscle marker SM22 after a minimum of 9 days of dynamic culture. Pulsatile culture seemed to improve cell migratory properties with evident amelioration of cell orientation with pre-existing ECM structures. In conclusion, we identified MABs injection into the muscle layer followed by 3D dynamic culture as a successful combination for effective re-colonization of decellularized oesophageal matrix. The bioreactor seemed to be a key factor in supporting and improving cell engraftment, proliferation, migration, homogeneous distribution and differentiation. Future work will include maximization of cell differentiation to obtain a functional fully engrafted scaffold suitable for in vivo transplantation.

T-1458

HEPATIC DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN A PERFUSED 3D POROUS POLYMER SCAFFOLD FOR LIVER TISSUE MODELLING

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Due to limitations of primary hepatocytes regarding availability and maintenance of functionality, stem cells are an attractive cell source for liver tissue engineering. Our approach for engineering liver tissue is to culture and differentiate hiPS cells in a 3D porous polymer scaffold built-in a perfusable bioreactor. hiPS-derived definitive endoderm cells were seeded into the scaffold and differentiated according to a Cellartis® protocol developed by Takara Bio Europe AB. Compared to conventional batch cultures, a similar gene expression was observed for albumin, α-fetoprotein, hepatocyte nuclear transcription factor 4α, the transcription factor CAR, the transporter protein permeability glycoprotein ABCB1 and the CYP enzymes CYP3A4, CYP3A5 and CYP3A7. The impact of flow rate was investigated and indicated that the expression of CYP genes were increased by higher flow rates, while Alfa-fetoprotein and KRT7 were down regulated by higher flow rates. This indicates that frozen DE cells could directly be turned in situ into cells with hepatocyte like expression profile under correct flow condition.

T-1459

ENGINEERING EXTRACELLULAR MATRIX FOR LIVER TRANSPLANTATION

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Severe hepatic failure is the result of long-term liver injury. Liver transplantation is the only efficient treatment, but is currently limited by organ shortage. The demand for new livers continues overcoming the availability. In this context, the creation of a bio-artificial liver might solve this clinical problem. This work aims to produce a 3D liver recellularized scaffold with intact components

of extracellular matrix (ECM) and vascular system. Wistar rats were used to liver excision surgery. Twenty minutes before this procedure, heparine was administrated. Livers were perfused through portal vein using an infusion pump at 4 ml/min with water for 1 hour followed by Triton X-100 for 30 min and SDS 1% for 12h. After total decellularization, livers were washed with distilled H_2O for 30 min to remove residual SDS and then were preserved at 4 °C for 7 days. To analyze the ECM integrity post decellularization protocol, DAPI, H&E, sirius red, DNA quantification, electronic scanning microscopy and immunohistochemistry assays against collagen type I, III, IV, laminin and fibronectin, were performed. Toluidine blue was used to examine the vasculature. For recellularization, approximately 10^9 HEPG2 (Human hepatocyte carcinoma) and 10^9 endothelial cells were injected through portal vein and allowed to attach for 2 hours at 37°C. Cells were continuous perfused with medium and FBS 10% using an infusion pump at 4ml/min for 3 and 7 days. DAPI, H&E, sirius red, DNA quantification, electronic scanning microscopy, ELISA and immunohistochemistry assays against albumin and CK-18 were performed. Toluidine blue showed that the vascular system was totally preserved. Macroscopy, microscopy and histological staining showed that the decellularization process preserves the structure and components of the ECM. After 7 days, cells were detected in the decellularized tissue. Our decellularization method was efficient removing resident cells and preserving the liver's ECM and vascular system. After 7 days HEPG2 cells were able to adhere on liver 3D scaffold generating new perspectives on building bioartificial liver.

T-1460

NEOVASCULARIZATION IN 3D KIDNEY TISSUE USING ADIPOSE STEM CELLS

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The adipose-derived stem cells (ASCs) are the most common adult stem cells populations utilized in tissue engineering and regenerative medicine. The ASCs have ectodermal, endodermal and mesodermal potentials and their capacity for reprogramming into stimulated pluripotent cells have been proven in vitro and in vivo studies. We used CD29+/CD34+/Sca-1+ cell populations in 3d kidney tissue cultures to stimulate vascularization. The stem cells were obtained from liposuctioned adipose tissue, after were isolated, purified and characterized (CD29+/CD34+/Sca-1+/ CD146+), and they were applied into twenty-five kidney tissue cultures. We observed neovascularization in a period of 4 days in all cultures using ASCs and HGF and VEGF ($p < 0.001$) compared with the neovascularization observed in cultures with ASCs, which was detected after 8 days. Furthermore, we found a rehabilitation of renal parenchyma, implying their potential use in animal models. The ASCs have many applications, but the principal purpose must demonstrate the functionality and benefit in the patients.

T-1461

PERIPHERAL BLOOD-DERIVED MESENCHYMAL STEM CELLS: CANDIDATE CELLS RESPONSIBLE FOR HEALING CRITICAL-SIZED CALVARIAL BONE DEFECTS

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Postnatal tissue-specific stem/progenitor cells hold great promise to enhance repair of damaged tissues. Many of these cells are retrieved from bone marrow or adipose tissue via invasive procedures. Peripheral blood is an ideal alternative source for the stem/progenitor cells thanks to its ease of retrieval. We present a co-culture system that routinely produces a group of cells from adult peripheral blood. Treatment with these cells enhanced healing of critical-sized bone defects in the mouse calvarium, a proof of principle that peripheral blood-derived cells can be used to heal bone defects. From these cells, we isolated a subset of CD45- cells that have a fibroblastic morphology. The CD45- cells were responsible for most of the differentiation-induced calcification activity and were most likely responsible for the enhanced healing process. These CD45- fibroblastic cells are plastic-adherent and exhibit a surface marker profile negative for CD34, CD19, CD11b, Lineage, c-kit while positive for Sca-1, CD73, CD44, CD90.1, CD29, CD105, CD106, and CD140α. Furthermore, these cells exhibited osteogenesis, chondrogenesis, and adipogenesis capabilities. The CD45- fibroblastic cells are the first peripheral blood-derived cells that fulfill the criteria of mesenchymal stem cells as defined by the International Society for Cellular Therapy. We name these cells, "blood-derived mesenchymal stem cells" (BD-MSCs).

T-1462

GENERATION OF CNS MYELIN IN VITRO USING THE 3D CO-CULTURE MICROFLUIDIC PLATFORM

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The brain is the most complex organ due to its intricate functions. Its complexity, once brain damaged, it is hard to recover. Now many research groups are studying to figure out the how brain function works. Especially the myelin sheath is an important structure for rapid conduction of electrical signals in the CNS. When the damaged myelin sheath occurs that multiple sclerosis and other dymyelinating disorders. The Neural stem cell (NSCs) is another key for remyelination in the brain. NSCs is able to self-renewal and differentiate into neuron, astrocyte and oligodendrocyte. Using the multipoitency of NSCs, we were induced axonal myelination by controlling its surrounding condition. Here we developed the myelin sheath in three-dimensional (3D) hydrogel incorporating microfluidics platform. NSCs and neurons were co-cultured inside the platform for three weeks to study axonal myelination. The microfluidic platform fabricated the conventional MEMS soft-lithography method. This microfluidic platform has benefited for its micro-size scale, it has a high media to cell ratio and the chemical gradient firmly sustain to culture time. The designed microfluidic platform has one hydrogel area and four media channel around gel area. And we use two cells for myelin sheath generation. The NSCs and neuron isolated from mouse embryo. For the 3D culture conditions, NSCs mixed with Extracellular matrix (ECM) hydrogel and insert to the platform and attaching the neuron around the hydrogel. Only the axon growth into the hydrogel and interaction with embedded NSCs. During the culture time, we adjusting some surround conditions of NSCs. We mixed with two types of ECM changing the cellular composition and also changing the media condition surrounding NSCs for mimic the brain environment. Based on controlled the micro-environment, we successfully generation myelin. From the result, we optimized the ECM hydrogel and cell component, we successfully NSC differentiation to oligodendrocyte

linage and axon myelination. We expect that these results can use the base tool for neuroscience that brain disease models and drug research.

T-1463

IN VIVO IMPLACATION OF TISSUE ENGINEERED VASCULAR GRAFT USING NEW BORN PIGLET PERIPHERAL BLOOD DERIVED STEM CELLS: A PROOF OF CONCEPT STUDY

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Lack of growing and remodelling potential of the available grafts is the bottleneck of current congenital heart defect (CHD) treatment in paediatric surgery. Tissue engineering is a very promising approach to solve this problem. We used newborn piglet peripheral blood derived mesenchymal stem cells (pbMSCs) to tissue engineer vascular grafts and test these grafts into a large animal model. Peripheral blood was collected from new-born piglets less than 12 hours following birth. The pbMSCs were isolated, expanded and characterised in vitro. Cells were then seeded onto the decellularised porcine small intestinal sub-mucosa at density of 1×10^6 cells/cm^2. After incubation in a bioreactor for 10days, the cell-seeded scaffold was shaped into a conduit and implanted into the left pulmonary artery of 12-15 kg piglets. At 6 months after surgery, echocardiography (ECG) was carried-out and grafts were harvested and analysed by histology, scanning electron microscopy (SEM), and immunohistochemistry. The pbMSCs displayed fibroblast-like morphology and were capable of differentiating into adipo-, osteo-, chondro- and especially smooth muscle cell-phenotypes. One porcine cell-seeded and one acellular grafts were implanted into the left pulmonary artery of two piglets. Both piglets recovered well and grew at normal rate. The ECG showed that the blood flow velocity was normal in recellularised graft, but was higher in the acellular graft suggesting a normal patency in the recellularised graft and a narrowing in the acellular graft. SEM images of the luminal side of the recellularized graft demonstrated a confluent cell layer similar to the left pulmonary artery luminal cell layer. The acellular graft exhibited a patchy luminal cell layer. Immunocytochemistry staining of recellularized graft sections revealed endothelial-like cells in the lumen and a multi-layer of smooth muscle-like cells within the vessel wall. The acellular graft showed little endothelial-like and smooth muscle-like stainings. This in vivo study provides a proof of concept that perinatal blood provide a very promising stem cell source to use in graft tissue engineering for potential paediatric cardiac surgery. These stem cells are capable to produce a live graft with remodelling potential when seeded onto a decellularised scaffold.

T-1464

HUMAN VENTRICULAR CARDIAC TISSUE STRIPS ENGINEERED FROM PLURIPOTENT STEM CELLS AS A SUPERIOR PREDICTIVE MODEL OVER CELLS OR CLUSTERS FOR PHARMACOLOGICAL SCREENING OF CONTRACTILE EFFECTS

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Traditional discovery and development of novel drugs and therapeutics for heart diseases continue to be an inefficient and expensive process. Due to the lack of appropriate human models, cardiotoxicity has indeed been a common leading cause for withdrawal, even for non-cardiovascular (e.g. cancer drugs). Although such traditional animal models as rodents, dogs and pigs are accessible, major species differences in both the anatomy and function exist. Human pluripotent stem cells (hPSC) have been proposed to fill this gap, but conventional 2D cultures and experiments with single cells or disorganized clusters inadequately recapitulate the human cardiac phenotype. To address these for facilitating drug discovery/screening, we propose to employ our engineered human ventricular cardiac tissue strips (hvCTS, ~1-cm), made out of ~10^6 hPSC-derived ventricular cardiomyocytes (VCMs) whose single-cell properties such as electrophysiology (action potential, Ca^{2+} handling), transcriptome, proteome etc have been extensively characterized. Under baseline conditions, hvCTS, with a morphological appearance resembling that of native human trabecular muscle, developed dynamic tension at the mN range. VCMs within the tissue showed aligned myofibrils and registered sarcomeres. As a validation step, we subjected hvCTS to drugs with known cardiovascular effects, followed by direct measurements of any changes in contractile forces as gauged by optically tracked movements of the posts between where the tissue was suspended. When treated with the Ca^{2+}channel blockers, the developed forces of hCTS dose-dependently decreased. The logEC_{50} values were -7.233±0.39, -5.896±0.44, -5.61±0.25, -5.248±0.40 for verapamil, nifedipine, mibefradil and bepridil respectively, resembling those previously reported in human trabecular muscle[1]. The anthracycline doxorubicin also elicited a dose-dependent decrease in developed tension after 96 hrs of administration. We conclude that hvCTS can recapitulate known effects of cardiovascular drugs with accurate prediction of drug potency and EC_{50}. Combining with patient/disease-specific induced pluripotent stem cell, hvCTS offers a flexible 3D in vitro platform for facilitating pre-clinical drug testing and bench-to-bedside translation of novel cardiac therapies.

T-1465

CARTILAGE REPAIR USING SCAFFOLD-FREE TISSUE ENGINEERED CONSTRUCT DERIVED FROM SYNOVIAL MESENCHYMAL STEM CELLS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Most of patients with rheumatoid arthritis (RA) involve cartilage damage due to severe synovitis. Recently the progress in medication for RA allows the patients into remission before their massive joint destruction. Even under the controlled condition through medication, there is progression in chondral damage with time. Therefore, it is important to protect cartilage along with systemic medication. As a treatment option of cell-based therapy in cartilage repair, we have developed a tissue engineered construct (TEC) composed of synovial mesenchymal stem cells (SMSC) and extracellular matrix

synthesized by them and its safety and efficacy in cartilage repair is clinically being tested in Osaka University Hospital. The purpose of this study was to evaluate the characteristics of SMSC and the TEC derived from RA patients with and without biologics, in comparison with those derived from trauma patients. 19 patients who had knee surgery were divided into 3 groups (6 post-trauma (PT), 7 RA with no biologics (nonBio-RA), 6 RA with biologics (Bio-RA)). We analyzed the following items. 1: Proliferation of SMSC. 2: Weight and volume of TEC, 3: Cytokine expression by the TEC, 4: Chondrogenesis of TEC. In every item tested, there was no significant difference detected data suggesting the inferiority of SMSC or TEC from RA groups than from PT regardless of medication (1: $p>0.05$ in all combination between the groups, 2: Weight / volume; $p= 0.68$ / 0.57, 3: Gene expression of IL-1β / IL-6 / IL-10; $p=0.82$ / 0.89 / 0.21, 4: GAG component; $p=0.34$, Gene expression of COL2 / SOX9 / ACAN; $p=0.85$ / 0.65 / 0.89). TEC derived from RA patients have similar characteristics in proliferation, cytokine expression and chondrogenic differentiation capacity as compared with that from trauma patients and thus could be feasible to cartilage repair.

T-1466

LIVER TISSUE ENGINEERING IN SWINE LYMPH NODES BY CELL TRANSPLANTATION

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Cell transplantation has been a promising alternative to organ transplant. Usage of diseased organ as a niche for donor cells maintenance is occasionally challenging. We have focused on lymph nodes for ectopic cell transplantation. We have previously demonstrated that primary hepatocytes injected directly into a single lymph node (LN) can generate an ectopic liver and rescue a tyrosinemic mouse model from lethal liver failure. To validate the clinical relevancy and feasibility of generating an ectopic liver in patients with liver disease, we generated a swine model of liver disease. After total portacaval shunts and hepatectomy, an autologous population of hepatocytes isolated from the removed lobe was transplanted back into the LNs of the animal. 1-2 months later, autopsies were performed. Almost all of the injected LNs demonstrated hepatocytes engraftment with vascularization. Here, we provide the first report describing the use of a LN as a site for liver cells transplant and demonstrating quarter-coin sized engraftments (over 4 mm thickness) in a large animal model. Histologically the ectopic livers show the similarity to the healthy liver. To determine biliary function in the ectopic liver, we measured the level of genes expression related to synthesis of bile acids from cholesterol and exocrine of bile acid. The mRNA levels of CYP7A1, ABCB1 and ABCC2 in ectopic liver were same level as those in normal liver. These genetic results were consistent with the bile acids level in the ectopic liver which was equivalent to that in the healthy liver. These findings suggested that hepatocytes in ectopic liver could metabolize cholesterol to bile acids and secret them to corridors connected through CK19+ bile ducts. Bile efflux from the ectopic liver might be absorbed actively or passively into portal flow by the connective tissue. Otherwise we speculate that CK19+ cells might connect functionally lymphatic system (CK19+ bile ducts in the ectopic livers were adjacent to LYVE1+ lymphatic vessels) so that bile could go to systemic venous circulation through the thoracic duct. This approach is providing a new concept to use the LN as an in vivo bioreactor in which to regenerate functional organs.

T-1467

INTRAVENOUS DENTAL PULP STEM CELL THERAPY IN A MOUSE MODEL OF ISCHEMIC STROKE

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Human stem cells harvested from the dental pulp of adult human teeth (DPSC) appear to have an intrinsic ability to form neurons and interact with the nervous system. Stroke, an acute interruption of blood flow to the brain, results in neuronal death in the area directly affected by the loss of blood supply. We have recently published a study showing DPSC, delivered at 24 hours post-stroke directly into the brain of affected animals, enhanced neuro-behavioural outcomes in a rodent model of stroke, and showed transplanted DPSC migrated to the site of the infarct, and had differentiated into neurons and astrocytes. The aim of this study is to investigate a less invasive route of delivery for the DPSC -intravenous delivery- the effect on neuro-behavioural outcomes, and investigation into the processes by which DPSC exert their beneficial effects on the stroke brain. Mice underwent occlusion of the middle cerebral artery (MCAo) to replicate the most common clinical ischemic stroke. 24 hours post MCAo, mice received an injection of 1x106 human DPSC or control media via the dorsal tail vein. Mice were run on a battery of neuro-behavioural tests to assess motor function, balance, ataxia, skilled limb movement and sensory deficits weekly for 4 weeks. Injected DPSC were retrovirally transduced to express GFP and Luciferase. Mice were imaged for bioluminescence twice during the duration of the experiment to non-invasively "track" where the transplanted DPSC were residing within the whole animal. After 4 weeks, immunohistochemical analysis of transplanted DPSC was carried out, with co-localisation of GFP (DPSC) and neuronal/glial markers, as well as investigation into ultra-structural interactions between transplanted DPSC and the stroke affected region of the brain. The hypothesis of this study is that intravenous delivery of DPSC at 24 hours post-stroke will improve neuro-behavioural outcome in a murine MCAo stroke model. The mechanism by which DPSC help improve neuro-behaviour is suggested to include several mechanisms of action acting through paracrine secretion of factors from DPSC rather than direct cell replacement.

T-1468

AUTOLOGOUS EXPANDED CULTURED CHONDROCYTES FROM THE ILIAC CREST APOPHYSIS IN THE TREATMENT OF PHYSEAL BARS IN CHILDREN

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This Institutional review board approved pilot study was carried out to treat the children with large physeal bars, also known as growth plate arrest, following infection or trauma in early infancy

or childhood. The physeal bar was resected and the resultant defect in the growth plate was replaced with autologous cultured chondrocytes from the Iliac crest apophysis. Recruitment in the study included 5 children with 8 physeal bars. GMP protocol was standardized as a first step of this study and CTRI registration was carried out prior to the study. Five children with a mean age of 3 years (range 2-10 years) underwent a mean of 266.2 million (range 30 to 460 million cells) autologous cultured expanded chondrocytes transplant in the eight physeal bar (5 distal femurs and 3 proximal tibias) of a size greater than 30% of the physis. The bars were excised as per the established surgical techniques. Preoperative investigations included CT scans to assess the bar size. Children were followed up with length measurements, scanograms, radiographs and MRI scans. Minimum duration of follow up following the index surgery is 2 years and maximum 32 months. Growth was restored in six out of the eight physis which received the transplant. Till date none of the children have faced any complication related to either cartilage harvest or the index surgery. One child who underwent excision and replacement of the medial half of damaged upper tibial growth plate developed the arrest of adjacent area of central growth plate after one year. This required treatment with excision of the arrested area and methyl methacrylate replacement and growth resumed after the intervention. This is the first study to report the short term results of autologous chondrocyte transplantation in physeal arrests in children. This study followed a successful autologous chondrocyte transplant study in a large animal model study. The study paves the way for a new treatment for growth plate arrests in children.

T-1469

SCAFFOLDS BASED BONE TISSUE ENGINEERING: USE OF CHITOSAN HYDROXYAPATITE AND POLYCAPROLACTONE

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Tissue engineering (TE) is an emerging multidisciplinary field involving biology, medicine, and engineering. In TE, biomaterials replicate the biologic and mechanical function of the native Extra Cellular Matrix (ECM) found in tissues by serving as an artificial ECM and provide a 3D space for cells to adhere, proliferate and differentiate to form new tissues with appropriate structure and function. In our present study, we used various biopolymers to prepare scaffolds that help in osteogenesis, to help in treating bone defects. Chitosan, hydroxyapatite and polycaprolactone were taken to prepare scaffolds in various formulations which are FDA approved. These 3D scaffold constructs were prepared by freeze drying method. These scaffolds were characterized in vitro for their physical and biological properties, like porosity, water retention capacity, mechanical strength, degradation time, cell proliferation and differentiation capacity, etc. In vivo studies were performed to study the efficiency of these scaffolds under natural conditions (calcification and bone formation). In vitro characterization revealed scaffold with composition as 50% CHT/ 40% HAP/ 10% PCL fared better than other compositions in terms of porosity, water retention capacity, mechanical strength, degradation time, cell proliferation and differentiation capacity of scaffold. In vivo studies were performed on Wistar rats by transplanting scaffolds at ectopic sites for 12 weeks. Analysis was performed at various time points of transplantation of scaffolds. Results of X-ray photography and H & E Staining of extracted scaffolds showed no inflammatory bodies and bone spicules formation. To conclude, the scaffold implant system used in present study due to its better requisite properties could be a candidate for bone tissue regeneration. This will help in replacing the need for autologous bone grafts used today and substantiate its use for future clinical application.

T-1470

SERUM FREE AND FEEDER CELLS FREE ORAL MUCOSA EPITHELIAL CELL SHEET ENGINEERING

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Limbal Stem Cell Deficiency has been successfully treated with grafting of oral mucosa epithelial cell sheet, expanded ex vivo in presence of feeder cells and bovine serum. The ex vivo cell culture conditions is a critical step in a safe and successful tissue engineering for future human autologous transplantation. Currently, animal serum and xeno-feeder cells are used to stimulate the growth of cells, with the disadvantage of possible serious side effects for the patients. In the present study, we engineered oral mucosa epithelial OMECS with four different serums free culture media, in absence of feeder cells: SFM1, SFM2, SFM3 and SFM4. These engineered cell sheets were compared to cell sheets cultured in presence of serum and feeder cells. Using SFM1, 2 and 3, OMECS grew slower than OMEC cultured with SFM4 or with Serum and feeder cells. OMECS, cultured with SFM1, 2 and 3 never reached confluence and did not differentiate to engineer a multilayer cell sheet. Using SFM 4 culture media and serum/feeder cells conditions of culture, OMECS reached confluence and differentiate in a multilayer cell sheet. The phenotype of engineered cell sheets with SFM 4 was compared to the cell sheets engineered with serum/feeder cells, using immunocytochemistry. H&E staining showed that with SFM4 OMECS grew to form a stratified multilayer cell sheets similar to OMECS grown in conditions with serum and feeder cells. In addition the expression of stem cell marker p40, ABCG2, p75, adhesion molecule marker E-Cadherin, and intermediate filaments specific of OMECS CK4, CK13 markers were similar in cell sheet engineered with SFM4 compared to cell sheet engineered with serum/feeder. In conclusion, we demonstrated that it is possible to engineer multilayer oral mucosa epithelial cell sheet in absence of serum and feeder cells, which is a necessity for future safe and successful oral mucosa epithelial cell sheet treatment of the ocular surface diseases.

T-1471

DEVELOPMENT OF A NANOFIBROUS MINERALIZED ELECTROSPUN SCAFFOLD FOR MUSCULOSKELETAL TISSUE ENGINEETING

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Fibrous scaffolds that recapitulate the extracellular matrix (ECM) have been utilized for tissue regeneration demonstrated potential for guiding stem cell differentiation. Nanofibrous scaffolds fabricated

by electrospinning allow the creation of tailored, functional scaffolds on-demand. However, traditional electrospun nanofibers have some inherent drawbacks due to the nature of the electrospinning process. Conventional eletrospun nanofibers are comprised of dense, interconnected networks of fibers that affect the final performance of the biomaterials and are often limited in terms of their ability to provide a biomimetic environment. To generate a highly porous electrospun nanofibrous PCL scaffold (HP-ePCL), we have devised a simple, modified electrospinning technique. This study is aimed to evaluate the electrospun nanofibers created by the proposed electrospinning technique. A variety of electrospun poly(ε-caprolactone) (PCL) nanofibrous scaffolds can be created by both traditional, and modified electrospinning processes, and then further modified by the addition of simulated body fluid (SBF). The unique deposition of mineral onto the HP-ePCL was confirmed by scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDS) analysis. Human adipose-derived stem cells (hADSC) were cultured on the different SBF-treated elctrospun fibrous scaffolds and differentiated into osteoblasts. Image analysis and alamar blue assay indicated a significant increase of hADSC adhesion and proliferation on the highly porous electrospun PCL scaffolds. Subsequent analysis of osteogenic potential by via gene expression analysis and alkaline phosphatase (ALP) activity also demonstrated that the HP-ePCL made by the modified electrospinning process is more favorable for the osteogenic differentiation hADSCs. Additionally, results of alizarin red S staining at day 14 showed improved deposition of mineralized matrix on the HP-ePCL scaffold compared to the traditional PCL electrospun scaffold. Therefore, this study indicates that the facile scaffold fabrication method described in this study is a promising approach to prepare a conducive scaffold for musculoskeletal tissue engineering.

T-1472

ADIPOSE DERIVED STEM CELL-BASED TISSUE ENGINEERED SMALL DIAMETER BLOOD VESSEL

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Cardiovascular disease (CVD) is the most common cause of death in worldwide and there is clear need for improved and alternative treatments. Tissue engineering and regenerative medicine showed high promises for diseases that involve damaged tissues or organs. Among the diversity of CVD, arteriosclerosis causes a high demand of replacement vessels. Unfortunately, the seemingly relatively simple structure of a medium-sized artery proved difficult to recreate in the lab. We set out to combine the highly biocompatible and creepless poly-1, 3-trimethylene carbonate (PTMC) with smooth muscle cells (SMC) derived from adipose tissue-derived stromal cells (ADSC) to engineer contractile bioartificial arteries in vitro. M&M: Human ADSC were cultured on TCPS (Tissue Culture-treated Polystyrene), glass and glass coated with PTMC for 2D experiments. Differentiation to SMC was induced by adding TGF-β1 to the culture medium for one week and were assessed by analyses of gene and protein expression of SMC markers, while function was investigated through a collagen contraction assay. Tubular interconnected porous PTMC scaffolds were loaded with non-differentiated ADSC and ADSC-derived SMC and cultured for 14 days. Cultured tubes were analyzed similarly as for the 2D experiments. After one week stimulation with TGF-β1, ADSC expressed SMC markers αSMA, SM22α, calponin, SM-MHC II as mRNA and protein. In terms of expression levels, the differentiation of ADSC to SMC was as efficient on PTMC as compared to the controls (TCPS and glass). ADSC-derived SMC showed a higher contraction than without differentiation, albeit not significantly different. Furthermore, TCPS-cultured ADSC had a higher initial contraction compared to glass or PTMC-cultured ADSC or ADSC-derived SMC. However, after 48h cells cultured on all surfaced and independent of TGF-β1 stimulation had reached the same, maximal, contraction. To induce circumferential alignment and further maturation of ADSC-derived SMC the seeded scaffolds were cultured in a Bose pulsatile flow reactor for 14 days. Our findings show that tubular porous PTMC scaffolds can be used as a scaffold to engineer functional bioartificial artery substitutes, since PTMC support ADSCs adhesion, proliferate and differentiate to SMCs.

T-1473

CULTURE OF HUMAN STEM CELLS ON THERMORESPONSIVE NANOBRUSH SURFACES

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Thermoresponsive surface prepared using thermoresponsive polymers with low critical solution temperatures (LCSTs) is attractive candidates for cell culturing because cells can be detached from the surface without applying an enzymatic digestion method and, instead, by decreasing the temperature, e.g., to 4 °C, which enables cell aggregates or cell sheets to be obtained. In this study, we designed the thermoresponsive nano-brush surfaces for human stem cell culture (human adipose-derived stem cells [hADSCs] and human pluripotent stem cells [hPSCs]). Using RAFT polymerization, we prepared the coating copolymers having polystyrene and (a) thermoresponsive poly(N-isopropyl acrylamide), PNIPAAm, (b) biocompatible and hydrophilic polyethylene glycol methacrylate (PEGMA), and (c) polyacrylic acid (PAA) where bioactive oligopeptide (oligo-vitronectin) can be conjugated via carboxylic acid of PAA. The coating copolymers prepared had narrow molecular weight distribution (PDI is less than 1.8) and were characterized by NMR and FTIR. The coating surface density was analyzed by XPS and SPR measurements. hADSCs were cultured on the surface coated with copolymers containing PNIPAAm, PEGMA and PAA conjugated with oligo-vitronectin. The optimal surface composition where hADSCs can attach and detach by decreasing temperature was investigated. Furthermore, human embryonic stem cells (WA09) and human induced pluripotent stem cells were cultured on the surface coated with these copolymers. We investigated whether hPSCs could maintain their pluripotency on the surface coated with these copolymers for long period (>passage ten) and whether hPSCs could be easily detach from the surface by decreasing the temperature.

T-1474

DEVELOPMENT OF A NEW BIOMATERIAL ASSOCIATED WITH HUMAN MESENCHYMAL STEM CELLS AND KERATINOCYTES FOR USE AS A SKIN SUBSTITUTE

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Tissue engineering (TE) is an important tool for skin regeneration. Currently available treatments are insufficient to prevent scar formation and promote healing of the patient. Therefore, the current study has aimed to produce a cutaneous substitute with a PDLLA polymer as a biomaterial. For this proposal, scaffolds were constructed by the electrospinning technique and divided into 3 groups: 1) PDLLA, 2) PDLLA/NaOH (PDLLA scaffolds hydrolyzed with a solution of NaOH 0.75M) and 3) PDLLA/Lam (also hydrolyzed with NaOH and in which the protein laminin was linked by covalent binding). They were constructed with 2 different fiber diameters, with the smallest at the top of the scaffold. Mesenchymal stem cells were then seeded onto the bottom of the scaffold and, after 24 hours, skin keratinocytes were seeded on the other side. The groups were evaluated for cell adhesion on the day of the seeding and on days 7, 14 and 21 for cell viability analysis. From day 7, the scaffolds were submitted to an air/liquid system culture. As a result, cell adhesion was greater in the PDLLA/Lam scaffolds with absorbance of 2.268± 0.494, in comparison with 1.264±0.473 for the control (PDLLA scaffold) and 1.159±0.120 for the PDLLA/NaOH scaffold. On day 7 of the viability analysis, the absorbance for the PDLLA scaffold was 1.148±0.411, the PDLLA/NaOH group was 1.380±0.501 and the PDLLA/Lam was 1.990±0.255. On day 14, absorbance for groups 1, 2 and 3 were 1.032±0.169, 0.755±0.016 and 1.636±0.313, respectively. On day 21, the results were 2.204±0.317, 1.437±0.024 and 2.811±0.477, respectively for groups 1, 2 and 3. In general, in terms of the biological analysis, the PDLLA/Lam group showed the best results for cell adhesion and viability tests. The presence of both types of cells was observed through histological analysis in the evaluation of all the groups up to 21 days of cultivation. Moreover, it was clearly observed that the cells occupied all the structure of the scaffolds in all groups. In conclusion, the PDLLA scaffolds, mainly the PDLLA/Lam groups, showed good results for the co-cultivation of the cells, with good cell adhesion and the presence of viable cells. These biomaterials were capable of providing support for the growth of the cells, indicating that they can be suitable biomaterials for use in TE.

T-1475

SCREENING SYSTEM ESTABLISHMENT FOR POTENTIAL ANTI-AGING AGENT USING UVA-INDUCED AGING OF HUMAN DERMAL STEM/PROGENITOR CELLS

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Tissue specific stem cells were identified in adult stromal tissues. Ultraviolet (UV) radiation induces photo-aging on human skin. Because of UVA irradiation, it is thought that aging phenomenon such as skin elasticity reduction, wrinkle formation, and retardation of wound healing were occurred. In the present study we found that reduction of human dermal stem/progenitor cells (hDSPC) functionality by UVA irrdation. We enriched hDSPCs from normal human dermal fibroblasts. To develop the screening system for potential anti-aging agent using hDSPCs, we treated UVA in the hDSPCs. To evaluate whether the stemness of hDSPCs is down-regulated in UVA treated hDSPCs, we measured the expression levels of SOX2, NANOG, and S100B, which are well-known representative dermal stem/progenitor cell markers. We observed that UVA-irradiated hDSPCs had lower expression levels of those markers compared with non treated hDSPCs. Furthermore UVA irradiation reduces the multipotency of hDSPCs to differentiate into adipocytes, chondrocytes, and osteoblasts. In this study, we suggest that chronic aging model by UVA treated hDSPCs can be used for new screening system for anti-aging agents.

T-1476

MURINE ES/IPS CELL-DERIVED IN VITRO LIVER MODEL ON A MICRO-FLUIDIC DEVICE

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Liver is a vital and multiple functional organ involved in metabolism, detoxification and protein synthesis. However, it is impossible to maintain multiple hepatic functions and also to keep structural hepatic polarity in a long culture of primary hepatocytes. Polygonal and multipolar hepatocytes in liver are surrounded by sinusoids, bile canaliculus, and adjacent hepatocytes. It is only in the context of hepatic tissue architecture that hepatocytes can express their specific and multiple functions. Hepatocyte polarity exerts a major influence on the physiology of the cell. We established a unique system of in vitro liver model derived from murine ES/iPS cells, *i.e.,* $IVL^{mES/iPS}$. The $IVL^{mES/iPS}$, consisting of not only hepatocytes, but also endothelial networks, together with cardiac mesoderm differentiation, was induced after the embryoid body formation. To confirm cellular polarities of the $IVL^{mES/iPS}$, dichlorofluorescein diacetate (CDFDA) was added into the $IVL^{mES/iPS}$. In liver, CDFDA is incorporated into hepatocytes via OATP2 which expresses at apical side, and afterward CDFDA was hydrolyzed by cytoplasmic esterase to green fluorescent CDF, and which is excreted to bile canaliculus via MRP2. CDF was observed to be accumulated at the boundary of the cells in the $IVL^{mES/iPS}$, indicating that bile canaliculus-like space was formed in the $IVL^{mES/iPS}$, but not in primary hepatocyte culture. Second, we tried to activate urea cycle by addition of L-ornithine in the $IVL^{mES/iPS}$ or liver perfusion system. Urea production increased and ammonia decreased in a dose-dependent manner with respect to the amount of L-ornithine both in the $IVL^{mES/iPS}$ and the liver perfusion system, but not in primary hepatocyte culture. Architectural and functional properties in the $IVL^{mES/iPS}$ were quite similar to those in the liver perfusion system, but different from those in the culture of primary hepatocytes. Third, we made an in vitro flow system to culture the IVL^{mES} on a micro-fluidic device (IVL^{mES} chip). Hepatic activities were much higher in the culture of primary hepatocytes with flow than that without flow, furthermore the activity of the IVL^{mES} chip with flow was the highest in others. The IVL^{mES} chip has great promise to be useful for drug metabolism and pharmacokinetics in liver as an alternative to animal experiments.

T-1477

MODELLING HEART FAILURE IN HUMAN ENGINEERED HEART MUSCLE

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Tissue engineering of heart muscle from human pluripotent stem cells holds great potential for in vitro drug screening and disease modeling. Here we sought to optimize the engineered heart muscle (EHM) technology for applications in simulations of a heart failure phenotype by (1) optimizing the non-myocyte component in EHM and (2) establishing conditions of neuro-humoral overstimulation under chemically defined culture conditions. We generated EHM from cardiomyocytes (hES2) with undefined non-myocytes (hES2-derived) and defined non-myocytes (primary fibroblasts). Total cell number and cardiomyocyte content was not different between EHM assembled with undefined and defined non-myocyte populations (2.5x105 vs 2.3x105 total cells, 1.4x105 vs 1.1x105 cardiomyocytes, n=10). Defined EHM developed higher forces with lower variability between experimental series (defined: 9.8±2.8 nN/cardiomyocyte, undefined: 4.7±4.1 nN/cardiomyocyte; mean±SD). We observed an enhanced response to isoprenaline (47±4 % vs 21±4 % force increase in defined vs. undefined, n=10, $p<0.05$) with increased inotropy and lusitropy. Next, we tested if defining cell composition and non-myocytes will also reduce variability of EHM from other stem cell lines compared to hES2 and observed a similarly reduced variability of mean force developed by EHM constructed from cardiomyocytes derived from H7-ESC and hiPSC-G1 (generated in the lab from gingiva fibroblasts). We then treated defined EHM with increasing concentrations of norepinephrine (NE, 0.001-1 µM) for 7 days under serum-free conditions to model pathological catecholamine levels in heart failure. Pathological NE (>0.01 µM) concentrations led to decreased force of contraction, cardiomyocyte death, and cardiomyocyte hypertrophy. BNP release was increased and the inotropic response to isoprenaline blunted; both are classical clinical hallmarks of heart failure. Defining the non-myocyte component allowed for construction of EHM with highly comparable function from various pluripotent stem cell lines. Together with the demonstrated susceptibility of EHM to catecholamine-toxicity this opens the door for modelling heart failure in personalized, i.e., iPSC-based EHM.

T-1478

EXAMINATION OF KERATIN BIOMATERIALS FOR CELL ADHESION AND ADIPOSE-DERIVED STEM CELLS DIFFERENTIATION

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Keratin biomaterials derived from human hair have raised researcher's interests due to the intrinsic ability to interact with different types of cells and the potential to serve as a controllable extracellular matrix protein which can be used in studies of cell mechanism and cell-matrix interaction. However,the interaction between keratin and stem cells has not been well reported. In the present study, we investigate the effect of keratin biomaterials to the growth of porcine adipose stem cells (pASCs) as well as a series of selective cell lines. pASCs on keratin coating demonstrated enhanced adhesion, proliferation and differentiation. Evaluation of genetic markers showed that adipogenic and osteogenic differentiation of pASCs can be successfully induced. In vitro studies of coatings and scaffolds also support keratin's potential for cell adhesion including 3T3 fibroblasts, MDCK (Madin-Darby canine kidney cells), and MG63 osteoblasts. Therefore, the combination of keratin biomaterials with stem cells may provide additional benefits in various bioengineering approaches.

T-1479

MAKING ENGINEERED CEREBELLUM FOR BRAIN REGENERATIVE MEDICINE

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The cerebellum is involved in the coordination of voluntary motor movement, balance and equilibrium. Thus, elucidating the function and development of the cerebellum not only improves our understanding of the mechanisms of higher brain function, but may also suggest strategies for developing new therapies for cerebellum-related disorders. The cerebellum develops over a long period of time, extending from the early embryonic period until the first postnatal years. This protracted development makes the cerebellum vulnerable to a broad spectrum of developmental disorders, ranging from the Dandy-Walker and related mal-formations to medulloblastoma, a neoplasia of granule precursor cells. Cerebellum is likely to become a major platform to investigate how making engineered whole cerebellum for brain regenerative medicine. A new approach to treatment involves the use of three-dimensional biological scaffolds made of allogeneic extracellular matrix. The studies described here illustrate the feasibility of engineering whole cerebellum in vitro when acellular matrices are combined with efficient recellularization strategies. These scaffolds can act as an inductive template for functional tissue and organ reconstruction after recellularisation with mouse fetal cerebellar progenitor cells. These cerebellar progenitor cell were deliver to a biologic scaffolds composed of mouse postnatal extracellular matrix (ECM) in vitro to construct remodeling of cerebellar tissues. We performed patch-clamp recordings to evaluate the electrophysiological phenotype of the cerebellar granule neurons after 50 days. Whole-cell patch-clamp recordings confirmed that the granule neuronal cells exhibited properties of functional mature neurons. About 65% (n=21 out of 32cells recorded) of the induced granule neurons fired mature action potentials in response to depolarizing current injection, and expressed the voltage-gated inward Na+ and outward K+ currents. The cerebellum scaffolds also showed the ability to differentiate progenitor cells into Purkinje's cells and astrocytes. This preliminary work demonstrates the biocompatibility of cerebellum scaffolds and supports the potential for engineered whole cerebellum for brain regenerative medicine

T-1480

BACTERIAL CELLULOSE BEHAVIOR AS EXTRACELLULAR MATRIX (ECM) WITH HUMAN EXFOLIATED DECIDUOUS TEETH (SHED) STEM CELLS

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Bacterial cellulose (BC) has become established as a remarkably versatile biomaterial and can be used in a wide variety of scientific applications, especially for medical devices in orthopedics and dentistry. The Nanoskin membrane (bacterial cellulose from Gluconacetobacter xylinus) mimics the role of Collagen in creating an extra cellular matrix at the wound site, which facilitates cell migration and activates the intracellular signaling pathways that regulate the various steps of wound re-epithelization and granulation. In this work, the Nanoskin (bacterial cellulose membrane) were modified by adding hyaluronic acid in the bacterial culture medium and with SHED stem cells with possible application for several Medicine Area. Viability and Microscopy study with human dental pulp stem cell using modified and unmodified bacterial cellulose scaffolds for regenerative medicine were analyzed in this work. MTT viability assays show higher cell adhesion in unmodified bacterial cellulose and modified bacterial cellulose/ hyaluronic acid scaffolds over time with differences due to fiber agglomeration in bacterial cellulose/gelatin. Confocal Microscopy Images show that the cells were adhered and well distributed on the fibers of both tested membranes.

REGENERATION MECHANISMS

T-1481

CXCL7 SECRETED BY NK CELL LEADS TO INCREASED MSC RECRUITMENT

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For injury repair, specific progenitor cells are recruited and have to move coordinately, in a highly regulated manner. We have previously described that NK cells lead to increased Mesenchymal Stem/Stromal Cells (MSC) recruitment. NK cells are lymphocytes associated with the immune response against certain viruses and tumors and also with immunoregulatory functions, particularly through secretion of soluble factors and interaction with other cells. NK cells are one of the first immune cell populations to arrive at an injury site, are involved in uterine tissue remodelling in pregnancy and may contribute to wound healing. Here, we describe the mechanism behind NK cell-mediated MSC recruitment. Assays were performed with bone marrow human MSC and NK cells freshly isolated from buffy coats. It was found that chemokines are essential for this MSC recruitment. Experiments performed with supernatants from NK cell - MSC co-cultures showed that the soluble mediators produced by the cells are sufficient for an increase on MSC recruitment. Antibody arrays and ELISA assays confirmed that NK cells secrete CCL5 (RANTES), previously described to have a role in macrophage-mediated MSC recruitment, and revealed for the first time that human NK cells secrete CXCL7 (NAP-2), but not CXCL12 (SDF-1), more commonly associated with MSC chemotaxis. Boyden chamber assays showed that CXCL7 induces MSC migration in a dose-dependent manner. Furthermore, MSC express CXCR2, a chemokine receptor that recognizes CXCL7. Thus, NK cells secrete different chemokines that can stimulate MSC recruitment, including the chemokine CXCL7 that can be recognized by CXCR2, leading to chemotaxis of MSC. This capacity of NK cells to recruit MSC points towards a new role for this cell population in regulating tissue repair/regeneration. *We would like to thank FEDER -Programa Operacional Factores de Competitividade - COMPETE and FCT - Fundação para a Ciência e a Tecnologia (project EXPL/BIM-MED/0022/2013).*

T-1482

THE REGENERATIVE POTENTIAL OF THE AMNIOTIC FLUID STEM CELL SECRETOME

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Several works suggest that stem cells might exert therapeutic beneficial effects due to their paracrine potential, rather than by trans-differentiation when transplanted in vivo. Growing interest has been driven to the characterisation of the stem cells secretome and of the stem cell-derived microvesicles (MVs), including exosomes, as mediators of such modulatory effect. Human amniotic fluid stem cells (hAFS) have been recently described as immature mesenchymal progenitors with a distinct proteomic profile and significant paracrine potential. Therefore, we aim at characterising the hAFS secretome and their MVs (hAFS-MVs) to reveal candidate molecules for future drug therapy. hAFS were isolated for the expression of c-kit from left over samples for prenatal diagnosis and cultured in normoxia (20% O2) versus hypoxia (1% O2) for 24h in serum-free medium to improve the secretion of MVs, including exosomes. MVs were obtained by ultracentrifugation of the hAFS-conditioned medium (hAFS-CM) and were characterised by transmission electron microscope, western blot and flow cytometry. The small and microRNA (miRNA) content within the hAFS-MVs was evaluated by Agilent technology and miRNA PCR array analysis. Rat H9c2 cells were purchased from ATCC and used to evaluate in vitro the effect and uptake of hAFS-MVs. hAFS cells showed to secrete vesicles ranging in size from of 100 to 1000 nm. MVs were detected in control conditions while hypoxic preconditioning led to enrichment for specific exosomal markers like CD81. Similarly, the analysis of small RNAs within the hAFS-MVs isolated under hypoxic conditions showed an up-regulation of specific miRNAs involved in regenerative and repair processes. Uptake analysis using H9c2 cells suggested the role of hAFS-derived MVs as biologically mediators of paracrine effects. This is the first study showing that hAFS cells can actively secrete MVs in their conditioned medium with hypoxic

preconditioning being a promising strategy to improve the efficiency of their isolation within the hAFS-CM. Although preliminary, these encouraging findings suggest a novel translational approach based on exploiting the paracrine potential of the hAFS secretome for future therapy.

T-1483

AN IN VIVO SELECTION STRATEGY FOR THE IDENTIFICATION OF NOVEL CYTOKINES INDUCING CARDIAC RETENTION OF MURINE MESENCHYMAL STROMAL CELLS AFTER MYOCARDIAL INFARCTION

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Numerous studies have shown that transplantation of mesenchymal stromal cells (MSCs) in the infarcted heart improves cardiac function through paracrine mechanisms. However, the injected cells are gradually lost at the site of injury, thus liming the window of therapeutic efficacy. To identify novel factors able to improve engraftment and long-term survival of MSCs, we developed an innovative Functional Selection (FunSel) procedure aimed at the in vivo identification of effective molecules. A collection of 80 murine cytokines cloned into AAV2 vectors was used for the batched transduction of MSCs ex vivo, followed by intracardiac administration of the cells in non-ischemic or ischemic conditions. Three weeks after injection, when most cells were normally lost, viral genomes were recovered from the few persisting cells and their relative abundance was analyzed by next generation sequencing. We expected that protective factors mediating cell retention would be enriched over the others. Interestingly, among the in vivo selected cytokines, we found genes already associated with MSC homing and survival, such as Ccl2 and Ccl7. Unexpectedly, however, the most effective factor in mediating cardiac MSC retention was Cardiotrophin1 (Ctf1), a pro-hypertrophic cytokine never associated with this property before. We found that the effect of Ctf1 was the consequence of the induction of MSC survival through STAT3 activation and, most notably, the promotion of MSC adhesive properties by the dynamic activation of Focal Adhesion Kinase (FAK). In vivo, AAV2-Ctf1-transduced MSCs preserved cardiac function and reduced infarct size after myocardial infarction, as assessed by echocardiography, histology and molecular analysis. These effects were strictly correlated with the persistence, for at least two months, of the Ctf1-expressing cells in the healing hearts. These results support the feasibility our the in vivo FunSel approach and identify Ctf1 as a powerful cytokine supporting cell survival and adhesion in the ischemic myocardium and providing benefit after cardiac damage.

T-1484

RECONSTITUTION OF HEMATOPOIESIS FROM A SMALL NUMBER OF REPOPULATING CELLS SURVIVING IN IRRADIATED MICE

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Murine hematopoietic tissue consists of ~300 million of cells and daily generates ~250 million of blood cells. All these cells are derived from a small pool of immature cells that are hierarchically organized into stem cells, multipotent progenitors and developmentally restricted progenitors. Irradiation with a dose of 6 Gy interrupts production of blood cells and reduces pools of hematopoietic stem and progenitors cells (HSPCs) to ~0.1% of their normal size. This small number of HSPCs reconstitutes damaged hematopoiesis under control mechanisms exerted by the tissue stroma (microenvironment). Mice irradiated with 6 Gy and control mice were examined for blood cell count, bone marrow cellularity and myeloid/lymphoid ratio, immature cells negative for lineage markers (Lin- cells) expressing c-Kit receptor (c-Kit+ cells), and positive/negative for Sca-1, CD150, CD48 and CD71 antigens. The examination period was from 8 -180 days after irradiation. When production of blood cells resumed about day 10 after irradiation, a small number of immature Lin-c-Kit+ cells generated a large number of blood cell precursors. The representation of immature cells with immunophenotypes characteristic for various types of HSPCs in normal bone marrow was markedly changed. Lin-Sca 1+c Kit+ (LSK) CD150+/-CD48+ cells, representing progenitors in normal bone marrow, greatly dominated over stem cells and multipotent progenitors. The cells expressed a low level of c Kit receptors and, in contrast to normal LSK cells, uniformly expressed transferrin receptor (CD71). Total number of hematopoietic cells and production rate of blood cells recovered about day 20 after irradiation. In parallel, a dramatic change in the immunophenotype of immature cells occurred. Immature cells never fully recovered to normal and remained significantly inferior in a transplantation based assay when compared to corresponding cells from normal bone marrow. We conclude that specific "regeneration" progenitors are induced in a heavily damaged bone marrow. The progenitors have a significant self-renewing capacity to expand their numbers despite a vast pressure for their differentiation into vital blood cells. The cells may be also fundamental for later reconstitution of pools of slowly proliferating progenitors and stem cells.

T-1485

THE SECRETOME OF HUMAN DENTAL PULP STEM CELLS STIMULATES NEURITE OUTGROWTH AND NEURONAL DIFFERENTIATION OF SH-SY5Y NEUROBLASTOMA CELLS

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Following injury to the central nervous system, endogenous repair of the damaged tissue is limited. Neural stem cells would be the ideal candidate for cell-based therapies in neurodegenerative diseases. However, due to isolation difficulties, additional stem cell sources with a neurogenic differentiation potential or with the ability to stimulate endogenous neurogenesis are required. Human dental pulp stem cells (hDPSC) are an attractive alternative stem cell source for neural stem cells due to their neuroectodermal origin and isolation simplicity in addition to their neurogenic differentiation potential and ability to secrete neurotrophic factors. This study focused on the paracrine effect of hDPSCs on neurite outgrowth and ultrastructural adaptations of SH-SY5Y neuroblastoma cells as a means of investigating neuronal regeneration. To induce neuronal

differentiation, cells were exposed to retinoic acid for 5 days, followed by a 7 day incubation in the presence of the conditioned medium of hDPSCs or 50 nM BDNF to stimulate neuronal maturation. Analysis of neurite outgrowth after ß-III tubulin staining demonstrated a significant increase in the fraction of neurite bearing cells and average neurite length compared to SH-SY5Y cells that were not exposed to conditioned medium or BDNF. Furthermore, the ultrastructural appearance of SH-SY5Y cells exposed to conditioned medium or BDNF is characterized by the presence of multiple electron dense vesicles associated with the cytoskeleton along the course of the neurites and increased vesicular release in the extracellular space. Although these result do not prove functional maturation of the differentiated SH-SY5Y cells, they demonstrate the applicability of this cell line to easily study paracrine effects in vitro. Subsequent studies should focus on the neuroprotective effects of the hDPSC-secretome in in vitro models for neuronal damage such as glutamate excitotoxicity and oxygen-glucose deprivation.

T-1486

EXOSOMES DERIVED FROM WHARTON'S JELLY MESENCHYMAL STEM CELLS ENHANCES ANGIOGENESIS AND MIGRATION OF ENDOTHELIAL CELLS

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Exosomes are a group of small vesicles released from cells via exocytosis pathway. These small extracellular vesicles can function as carriers of proteins and genetic materials. Therefore, exosomes have been recognized as a potent mediator involved in many biological functions. Mesenchymal stem cells (MSCs) present as a promising tool for a variety of degenerative diseases. Most of these pathologies are related to insufficient blood flow supply. MSCs can renovate the damaged tissues by the paracrine effect. The effect of MSC-derived exosomes on angiogenesis remains to be explored. In this study, exosomes from Wharton's Jelly MSCs (WJ-MSCs) were isolated and characterized. WJ-MSCs derived exosomes exhibited a dominate angiogenic potential by facilitating the migration of endothelial cells and stabilizing the tube-like structures of human umbilical vein endothelial cells (HUVEC) in vitro, and the precise molecular mechanisms that trigger angiogenesis by WJ-MSCs derived exosomes will be further elucidated. Through this study, we may provide a new insight into angiogenic therapies with stem cell-derived exosomes.

T-1487

SIRT2 CONTROLS SKELETAL MUSCLE REGENERATION BY REGULATING AMPK PATHWAY

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Aging is the phenomenon that a number of functions are broken down as time goes on. One of the classic method can prevent aging is a calorie restriction (CR). CR is known extends life span by inducing anti-aging effect. Sirtuins are reported that it is increased the expression when calorie is restricted and known well as a longevity gene. Among the seven members of the sirtuin, SIRT1 has been the most extensively studied. A lot of studies have already been revealed about roles of AMPK-SIRT1-PGC-1α axis in skeletal muscle. However, the roles of SIRT2 in skeletal muscle have received surprisingly little attention although it is also worth consideration as a possible regulatory gene. Thus, we investigated the role of SIRT2 whether it is related with regulation AMPK-PGC-1α axis in skeletal muscle regeneration. SIRT2 wild type (WT) and knock-out (KO) mice were used. To induce injury, notexin injection was performed in the both of gastrocnemius muscle and sacrificed the muscle sample at 3, 14, 28 days after notexin injection. On histological findings, the average size of centronucleated myofibers is much smaller in KO group than in WT group in 14d samples. The expression of AMPK signaling related genes, including PGC-1α and cyclin D1 were reduced in KO group than in WT group. And then, to validate muscle regeneration, the expression level of myogenic regulatory factors (MRFs) were analyzed. All MRFs were decreased the expression in KO group in 3d samples. We demonstrated that inhibition of SIRT2 is negative effect on recovery of skeletal muscle by repressing gene expressions of AMK pathway, cell cycle and MRFs. In conclusion, we suggest that SIRT2 is possible regulatory gene in skeletal muscle regeneration.

T-1488

SYNAPTIC INTEGRATION OF STEM CELL-DERIVED MOTOR NEURONS TRANSPLANTED TO THE MOUSE HYPOGLOSSAL NUCLEUS

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Injuries and diseases in the central nervous system (CNS) often lead to permanent loss of function due to limited regenerative capacity. Therefore, means to replace lost neurons are required to efficiently restore function. One approach is to transplant cells with potential to generate new neurons. However, inefficient integration of such grafted cells with host tissue constitutes an important obstacle before clinical applications can be fully considered. This project focuses on transplantation and subsequent integration of motor neurons (MNs), a cell type commonly affected in CNS injuries and neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS). MNs expressing eGFP under the Hb9-promotor were generated by directed differentiation of mouse embryonic stem cells. Cells were transplanted to the hypoglossal nucleus of young adult mice by stereotaxic technique. Graft survival and axonal projections were assessed at 1, 2 and 4 weeks post transplantation. Synaptic inputs onto soma and proximal dendrites of resident and grafted cells were characterized and quantified using immunohistochemical markers for cholinergic (VAChT), glutamatergic (vGLUT2), GABAergic/glycinergic (VIAAT) and serotonergic presynaptic terminals. On resident MNs, GABA/glycinergic terminals were the most abundant type, followed by glutamatergic and then cholinergic terminals. Serotonergic fibers were observed throughout the hypoglossal nucleus, but did not appear to connect to MNs. In order to assess the level of synaptic integration, we are in the process of comparing the composition of excitatory and inhibitory synaptic inputs onto grafted cells to that of resident MNs. Formation of glutamatergic synaptic terminals on grafted MNs was detected as early as 1 week post transplantation. Moreover, different methods to boost synaptic integration (e.g. preconditioning lesion), survival and axonal outgrowth (e.g. neurotrophic small molecules) of the graft are

being evaluated. In summary, we used a mouse model for studying engraftment and synaptic integration of transplanted stem cell-derived MNs. By employing different methods for stimulating synapse formation on grafted cells, we hope to improve functional integration and overall outcome for transplanted neurons.

T-1489

INJURY-INDUCED CORTICAL NEUROGENESIS ENHANCED BY COMBINATION OF CELL DEATH BLOCKADE AND BDNF TREATMENTS IN THE ADULT SUBCALLOSAL ZONE

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Neurogenesis occurs spontaneously in some brain regions, including the subventricular zone (SVZ) of the lateral ventricle and subgranular zone (SGZ) of the dentate gyrus in adult brain regions. We recently identified that subcallosal zone (SCZ) also contains neural stem cells (NSCs) and they spontaneously produce neuroblasts although these neuroblasts all eliminated by programmed cell death. However, following brain injury the number of SCZ-derived neuroblasts survived, and migrated toward the injured brain region. In this study we further characterized the long-term fate of migrated neuroblasts in the injured cortex region. Within 1 month, most newly produced cells in the injury penumbra differentiated into oligodendrocytes and astrocytes, but neuroblasts were all degenerated by apoptosis. Overexpression of anti-apoptotic Bcl-xL in the SCZ NSC resulted in a progressive increase in the number of neuroblasts, but neuronal maturation was limited and they became highly atrophied. Infusion of neurotropic factor, brain derived neurotropic factor (BDNF) into the injured cortex further promoted maturation of adult-borne neurons with sign of circuit integration. These results indicate that combination of anti-apoptotic therapy and infusion of appropriate tropic factors may be an efficient means to promote brain repair using endogenous neural stem cell. *This research was supported by the Brain Research Program through the National Research Foundation (NRF) funded by the Korean Ministry of Science, ICT & Future Planning (NRF-2012M3A9C6049933, NRF-2011-0019212, and NRF-2013R1A1A3011896).*

T-1490

HUMAN NEURAL STEM CELL TRANSPLANTATION AMELIORATE ALZHEIMER-LIKE PATHOLOGY IN A MOUSE MODEL VIA MULTIPLE MECHANISMS

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Alzheimer's disease (AD) is a dire neurodegenerative disorder that commonly occurs in elderly adults. AD manifests with extracellular deposition of amyloid-β (Aβ), intraneuronal accumulation of hyperphosphorylated tau, and inflammation in the brain. Neural stem cells (NSCs) generated from developing and adult mammalian brains are defined by their ability to self-renew, give rise to glial and neuronal cell throughout the neuraxis, and reconstruct developing or damaged CNS. When transplanted into a diseased brain, human NSCs have advanced clinical approaches providing replacement and protection of neural cells against degenerated or injured stimuli after preferential extensive migration to and engraftment within lesions. To investigate the therapeutic effects in AD of human NSCs isolated from an aborted human fetal telencephalon at 13 weeks of gestation, these cells were intracerebroventricularly transplanted into the NSE promoter-controlled APPsw expressing transgenic mice as an AD mouse model. Most grafted cells, migrated and engrafted around the injection site at 7 weeks post-grafting, remained immature, and others differentiated into neural cells. Furthermore, human NSC transplantation elevated the spatial memory showing that tau phosphorylation and amyloid-β (Aβ) 42 were significantly reduced, and microgliosis and astrogliosis were markedly attenuated in the transgenic mice. In detail, human NSC transplantation induced activation of increased neurotrophins-mediated Trk-dependent Akt/GSK3β signaling, resulting in reduced tau phosphorylation, and decrease of BACE1 through an activated Akt/GSK3β signaling, resulting in reduced Aβ production in the brains. Additionally, human NSCs transplantation attenuated expression of inflammatory mediators, resulting in decreased microgliosis and astrogliosis. In particular, human NSCs induced deactivation of microglia through cell-to-cell contact and secretion of anti-inflammatory factors in vitro. Lastly, human NSCs transplantation facilitated neurotrophins-mediated synaptic plasticity and anti-apoptotic function. Therefore, these results suggest human NSC transplantation could mitigate Alzheimer-like pathology via multiple mechanisms and rescue cognitive deficits of AD.

T-1491

MODULATION OF MYOCARDIAL INJURY BY HUMAN PLACENTA-DERIVED MULTIPOTENT CELLS (PDMCS) VIA REDUCING CARDIOMYOCYTE APOPTOSIS THROUGH SECRETED PARACRINE FACTORS

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Ischemia heart disease (IHD) is a cardiac disease caused by decreased oxygen supply to the myocardium. This increasingly prevalent disease is most commonly caused by myocardial ischemia (MI) due to atherosclerosis, in which thickening and hardening of the coronary arteries result from deposition of lipids in vessel walls. In recent years, stem cell therapy has been proposed as a novel therapy for IHD. Human placenta-derived multipotent stem cells (PDMCs) are multipotent cells expressing bone marrow mesenchymal stem cells (BMMSCs) and embryonic stem cell markers, and can differentiate into lineages of all three germ layers. Moreover, PDMCs are easily isolated and expanded ex vivo, and harbor strong immunomodulatory properties. We therefore were interested in exploring whether PDMCs can be therapeutic for IHD and the mechanisms involved for development of clinical strategies. We have previously demonstrated

that PDMCs harbor significant pro-angiogenic paracrine effects, which contribute to therapeutic results in small and large animal models. Recently, we have found that injection of PDMCs in a pig model of acute MI could significantly enhance cardiac function through decreasing cardiomyocyte apoptosis as well, as demonstrated by histological evidence. We therefore were interested in investigating the specific factors involved in these beneficial effects. In an in vitro model of cardiomyocyte injury mediated by TNF-α, we found that a number of PDMC-secreted factors reduced the level of apoptosis by decreasing annexin V positive cardiomyocytes as well as decreasing the levels of cleaved caspase-3. Moreover, reactive oxygen species (ROS) production was also decreased in ischemic cardiomyocytes with concomitant increases in the expression levels of the antioxidant enzymes catalase and CuZnSOD. These anti-apoptosis paracrine effects of PDMCs can be further enhanced with manipulated interactions with specific extracellular matrix proteins through integrins expressed on PDMCs. Our current findings therefore demonstrate that PDMCs can modulate ischemic cardiac injury through suppression of cardiomyocyte apoptosis. These findings along with our previous data showing pro-angiogenic effects of PDMCs implicate the therapeutic applicability of these versatile stem cells for IHD.

T-1492

MITOCHONDRIAL TRANSFER OF IPSC-DERIVED MESENCHYMAL STEM CELLS REJUVENATES DAMAGED CARDIOMYOCYTE AGAINST ANTHRACYCLINE-INDUCED CARDIOMYOPATHY

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Mitochondrial damages contribute to cardiomyocytes loss in anthracycline-induced cardiomyopathy (AIC). Transplantation of induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) significantly reduced cardiomyocyte death with detection of abundant mitochondrial components of iPSC-MSCs remained in mice of AIC. Co-culture of iPSC-MSCs with neonatal mice cardiomyocytes (NMCs) revealed a direct mitochondrial transfer of iPSC-MSCs to NMCs, preserved bioenergetics and rejuvenated damaged NMCs against doxorubicin induced exhaustion of mitochondrial respiration function. In contrast, mitochondrial transfer from the same iPSC-MSC with mitochondrial defects largely lost cardiomyocyte protection against doxorubicin insults. The key paracrine factors IGF-1 ,VEGF, HGF , NO were not changed between normal and mitochondrial defected iPSC-MSCs. Additionally,the mitochondrial transfer of iPSC-MSCs to NMCs was associated with pro-inflammatory cytokine TNF-α/TNFαip2-induced formation of tunneling nanotubes (TNT). TNT formation of MSCs was abrogated by TNFαip2siRNA intervention and cardiomyocyte rejuvenation by iPSC-MSCs was decreased. Furthermore, TNFαip2 expression was regulated through TNF-α/NF-κB signaling pathway to modulate TNT formation. Conclusion, functional mitochondrial transfer of iPSC-MSCs plays a critical role to rejuvenate damaged cardiomyocytes against doxorubicin-induced mitochondria damage. TNF-α regulated TNT formation can bridge mitochondrial transfer of MSCs to attenuate doxorubicin-induced cardiomyocyte injuries.

T-1493

DETERMINING FACTORS RESPONSIBLE FOR THE REGENERATIVE CAPACITY OF THE STROMAL VASCULAR FRACTION

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We hypothesize that the difference between SVF from diabetic rats and healthy rats is reflected in the regenerative capacity of the SVF. Aim: We aim to optimize the stromal vascular fraction (SVF) based correction of erectile dysfunction (ED) in a cavernous nerve crush injury rat model. To that end, we investigate the consequences of the diabetic state of the SVF-donor rat on the quality and regenerative capacity of the SVF. Furthermore, we aim to determine candidate proteins crucial for the regenerative mechanism by examining proteomic differences between the two groups. ED is a frequent complication of diabetes. Stem cell therapy is a promising future treatment for ED and the strategy involves the use of adipose-derived stem cells within the SVF. The SVF is obtained from the patient's own adipose tissue and reintroduced through penile injection, thereby making the procedure perfectly ethical and immunologically safe. Little is known about the mechanism by which the SVF performs its regenerative effect and studies regarding the impact of the SVF-donor's health status and the regenerative capacity of SVF, are scarce. In vitro studies are conducted to characterize the potential differences between SVF cells from diabetic and healthy rats. Additionally, the rat model for ED, involving the cavernous nerve crush injury, is used to determine the in vivo regenerative capacity of the two SVF populations. Finally, proteomic differences that may affect the regenerative capacity of the SVF will be evaluated in the ED-rat model. Thus, candidate proteins crucial for the regeneration mechanism may be determined. Stem cell therapy with SVF holds great potential as a treatment of diabetic ED. However before this option for treatment can be a clinical reality, further knowledge regarding the mechanism and SVF quality is crucial in order to optimize regenerative effects and to outline the prognosis of the treatment.

T-1494

CELL CYCLE REACTIVATION OF DORMANT COCHLEAR STEM/PROGENITOR CELLS IN NEONATAL FUCCI MICE BY SMALL MOLECULE COMPOUNDS

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Due to the lack of intrinsic regenerative capacity of the mammalian auditory epithelium, sensory hair cell loss results in permanent hearing deficit. Despite its post-mitotic state, a population of tissue resident stem/progenitor cells has been recently described as expressing the R-Spondin receptor Lgr5 and the transcription factor Sox2, two well known stem cell markers in other tissues. In order to identify regulators of cell cycle re-entry of postmitotic cells we have established otic sphere assays and organotypic cultures from FUCCI reporter animals. Here, cell cycle progression can be promptly and dynamically detected by expression of two fluorescently tagged probes:

Geminin-AG, labeling the S/G2/M phases by green fluorescence and Cdt-1-KO2, labeling G0/G1 cells with red fluorescence. To assess the maintenance of stemness, we made use of Lgr5-GFP reporter animals or Lgr5-GFP/ Cdt-1-KO2 double transgenics. We show that activation of Wnt signaling by a small molecule inhibitors targeting GSK3, (CHIR99021), led to an increase in cell cycle re-entry of Lgr5/ sox2 cells. In otic sphere assays, CHIR99021 led to a significant increase in the percentage of Lgr5-GFP positive cells (from 0.9+/-0.2% to 10.9+/-3.1%) as well as an increase in primary and secondary sphere formation, sphere size and GFP intensity. Moreover, a selective increase in S/G2/M cells in the Lgr5+ population was observed. In Organ of Corti organotypic cultures we detected a significant increase in the fraction of Sox2 proliferating cells upon CHIR99021 treatment, identified as Sox2+/Gem-AG+ (from 0.7+/-0.6% to 11.8%+/- 3.12) or Ki67+/Sox2+ (15.6%+/-3.6). At very low frequencies, novel MyoVIIa+/ Edu+ cells were generated in the organotypic culture upon GSK3i treatment. In conclusion, we show that dormant Lgr5 supporting cells can be triggered to re-enter the cell cycle by selective small molecule compounds in postnatal animals. The combination of stem cell and cycle and reporters utilized provides a robust mean to identify novel regulators of auditory organ regeneration and to clarify the contribution of stem cell activity.

T-1495

GROWTH HORMONE RESTORES THE NEUROGENIC POTENTIAL OF THE AGED SUBVENTRICULAR ZONE

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Decreased neurogenesis occurs in the brain during aging. This change is associated with increased oxidative stress, basal chronic inflammation and a decline in a variety of circulating trophic factors, including growth hormone (GH), which has been described to improve tissue regeneration. However, the effects and mechanisms of GH action in the aged brain are unknown. In this study, we investigate the effects of aging on the subventricular zone (SVZ), one of the main murine neurogenic areas, and the influence of GH on the aged SVZ. Our results show that the number of neurospheres/well formed in cultures of SVZ-derived neural precursor cells (NPCs) from old mice (18-22 month-old) was significantly reduced when compared with those from young mice (3 month-old) (Young: 11.6 ± 0.8; Old: 6.4 ± 0.9, $p<0.001$). When old mice were subcutaneously injected for 10 days with GH (2 μg/g body weight), the number of neurospheres formed in primary cultures of SVZ-derived NPCs increased significantly (vehicle: 8.0 ± 1.1; GH: 22.7 ± 1.1, $p<0.001$). Moreover, in vitro GH supplementation for seven days increased SVZ-derived NPCs expansion (control: 13.6 ± 0.7; GH 10-8M; 19.7 ± 1.6 neurospheres/well, $p<0.001$). We also observed in situ a greater SVZ cell-containing area in GH-treated old mice (vehicle: 1877.8 ± 207; GH: 3120 ± 307.3 μm2, $p<0.01$). Furthermore, GH treatment reduced TNF-α mRNA in the SVZ of old mice, as measured by RT/PCR analysis of RNA isolated by laser-capture microdissection of SVZ in brain tissue sections. SVZ-derived NPC from GH-treated old mice showed a trend to secrete lower levels of TNF-α, as measured by ELISA assay. Finally, we verified an increased frequency of neurons (vehicle: 0.64 ± 0.3; GH: 3.8 ± 1.3%, $p<0.05$) and oligodendrocytes (vehicle: 0.06 ± 0.06; GH: 0.7 ± 0.2%, $p<0.01$) differentiated from SVZ-derived NPCs isolated from old mice treated with GH. No significant change was found in the frequency of astrocytes between groups. These results confirm that aging promotes a decrease in the murine neurogenesis and show that GH is able of rescuing the neurogenic potential of the SVZ by a mechanism that may involve down-regulation of the pro-inflammatory factor TNF-α.

T-1496

OPTOGENETIC CONTROL IN A FUNCTIONAL HUMAN EMBRYONIC STEM CELL-DERIVED NEURO-MUSCULAR CO-CULTURE SYSTEM

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Harnessing the full potential of human pluripotent stem cell (PSC) derived neurons requires the functional interrogation of such populations to analyze connectivity. Methods to control defined neuronal populations were long sought after and have become available with the advent of optogenetics. Despite its transformative role in neuroscience, applications of optogenetics in human stem cell biology have lagged behind. Reasons for this may include i) challenges to stably express transgenes in fully differentiated human PSC-derived neurons, ii) the slow maturation rate of human neurons, iii) a lack of suitable models to test effects on downstream networks or iv) concerns about inefficient synaptogenesis across species boundaries after transplantation. Here we show that optogenetic control can be established in human PSC-derived spinal motorneurons. Matured co-cultures of these motorneurons with human primary myoblast-derived skeletal muscle fibers can be induced to twitch upon light stimulation. Classic electrophysiology as well as calcium imaging in combination with pharmacology are employed to characterize this functional human neuromuscular system. We therefore show for the first time that a human ES cell-derived neuronal population with significant potential for regenerative medicine can functionally connect to its human target tissue. To test, whether this system can be modulated in a way that is relevant to human neuromuscular disease, we incubated these co-cultures with sera from patients suffering from myasthenia gravis (MG). MG is an autoimmune disease elicited by the emergence of autoantibodies against the acetylcholine receptor in the skeletal muscle endplate. We found that key aspects of MG, including reduced neuro-muscular transmission and contractility as well as MG treatment can be recapitulated in the completely in vitro generated functional human neuro-muscular culture. We propose the use of this novel culture system for the dissection of disease processes affecting either side of the neuromuscular junction, using patient specific iPSC derived neurons or muscle.

T-1497

THE VOLUME OF MESENCHYMAL STEM CELLS IN PERIPHERAL BLOOD IN HORSES IS ASSOCIATED WITH PHYSICAL STRESS

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Peripheral blood is one of many possible sources of mesenchymal stem cells (MSCs). However, the number of MSCs in peripheral blood is naturaly very low, but may increase during physiological regeneration process. To determine whether physical exercise increases the amount of precursor-MSCs, in our preliminary study we collected peripheral blood of five warmblood sport horses in three sport phases: at rest, 2 hours and 20 hours after work. The exercise was one hour trotting and galloping with a rider. 10 ml of heparinized peripheral blood was processed on Histopaque and the mononucleare cells were cultured in 89%MEM/10%FBS/1%PenStrep at 37°C/5%CO2. Cultivated MSCs were harvested after three weeks using 0.05-0.25% trypsin-EDTA and analysed (total number, viability and CD44, CD90, CD105). In the first phase MSCs were isolated in vitro only in one horse (0.8x106 cells of CD44+, CD90+, CD105+); in the second phase MSCs were isolated in two horses (0.7x106 and 1.2x106 cells of CD44+, CD90+, CD105+); in the three phase MSCs were isolated in four horses (1.4x106 , 1.6x106 , 1.9x106 , 0.8x106 cells of CD44+, CD90+, CD105+). Conclusion: our preliminary data suggest, that it may be possible to collect MSCs from the peripheral blood of a horse under physiological condition. It also proofs physiological process of regeneration.

T-1498

INFUSED BONE MARROW DERIVED CELLS HAVE TWO CAPACITIES THAT PHAGOCYTOSIS OF DAMAGED CELLS AND REPAIR OF FIBROSIS IN MICE

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Recently, many researchers make the study about the possibility that bone marrow cells were useful for liver 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 CCl4 induced liver cirrhosis mice (JBiochem 2003.Hepatology 2004). In this study, we estimated characterization of infused BMC in liver cirrhosis mice using Electron Microscopy (EM). 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-postive 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, hepatoblastmarker -Liv2, Liv8-CD44, mouse oval cell marker-A6, EpCAM, transcription regulator-maternal of inhibitor of differentiation -Maid). 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. And MMP9 positive cells, Maid 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 few F4/80 positive cells and alphaaSMA negative cell. These cells were located on fiber in hepatic cord and repaired fibrosis. The other group cells were small size (2-5microum) 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. However these cells were few F4/80 positive cells and smaller than Kupffper cell in size. We detected two kind of infused GFP positive BMCs. The small BMCs worked the phagocytized damaged hepatocyte, the other round BMCs repaird liver fibrosis

TECHNOLOGIES FOR STEM CELL RESEARCH

T-1500

A SIMPLE AND ROBUST METHOD FOR ESTABLISHING HOMOGENEOUS MOUSE EPIBLAST STEM CELL LINES BY WNT INHIBITION

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Epiblast stem cells (EpiSCs) are pluripotent stem cells derived from epiblasts of postimplantation mouse embryos, representing a useful model for studying "primed" pluripotent states. Here we devised a simple and robust technique to derive high quality EpiSCs using an inhibitor of WNT secretion. Using this method, EpiSC lines were readily established with high efficiency; whole embryonic portions could be used without separation of epiblast from visceral endoderm. Expression analyses revealed that these EpiSCs maintained a homogeneous, undifferentiated status, yet showed high potential fordifferentiation both in vitro and in teratomas. Unlike EpiSCs derived by the original protocol, new EpiSC lines required continuous treatment with the Wnt inhibitor, suggesting some intrinsic differences from the existing EpiSCs. The homogeneous properties of this new version of EpiSCs should facilitate studies on the establishment and maintenance of a "primed" pluripotent state, and on directed differentiation from the primed state.

T-1501

TRANSCRIPTOME ANALYSIS OF MOTOR NEURON SOMAS AND AXONS DERIVED FROM MOUSE EMBRYONIC STEM CELLS: A NEW APPROACH TO UNDERSTAND ALS USING MICROFLUIDICS

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Motor neurons are highly polarized cells that initiate body movement through interaction with muscles by specialized synapses termed neuromuscular junctions (NMJs). Anatomically motor neurons can be subdivided into distinct compartments - the cell body (soma) and associated dendrites, the axon and the synaptic terminal. Each compartment of the motor neuron performs distinct functions. Motor axons are provided with efficient biochemical machinery, since they fulfill highly demanding metabolic and physiological events that take place at the presynaptic terminal. However, the axonal RNA composition of motor neurons is still largely unknown. Motor axons and NMJs appear to be primary targets in the motor neuron disease amyotrophic lateral sclerosis (ALS), with muscle endplates becoming denervated before onset of central motor neuron loss and axons showing altered biology. Some of the early axonal deficiencies reported to date involve local RNA processing and transport. In our study, we cultured spinal motor neurons derived from mouse embryonic stem cells (mESCs), in a microfluidic device and performed deep-RNA sequencing (RNAseq) on somatodendritic and axonal compartments separately. We used mESCs overexpressing the human mutated (G93A) superoxidase dismutase 1 (SOD1) gene to model ALS. In parallel to the RNAseq analysis, we characterized the motor neuron cultures using electrophysiology. We are validating axonal-restricted gene expression at the protein level using immunocytochemistry. In summary, we believe that our work will give new insights into motor axon organization of in health and ALS.

T-1502

USING EMBRYONIC STEM CELLS TO PROVIDE HUMAN NEURONS FOR DRUG SCREENING

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Despite most neuroprotectants working in animal models of stroke, none have been shown to be successful in the clinic. While stem cell based therapies could be of benefit, an alternative use of stem cells is to create an in vitro screening system in which human embryonic stem cells (hESCs) differentiated into neurons are used to test candidate drugs. Aims: to differentiate hESC lines into neurons, develop a model of ischaemic injury and test potential therapeutic agents. In brief, hESCs were cultured in the presence of Noggin for 14 days to induce neuronal differentiation of neuronal progenitors. These progenitors were then grown in non-adherent plates in the presence of growth factors, EGF and bFGF, where they formed neurospheres. Removal of growth factors from the culture medium at this point allowed the neurospheres to differentiate into mature neurons. Human neurons were then cultured for 11 days prior to the induction of injury. Two injury models were used: Oxygen-glucose deprivation (OGD) and oxidative stress (H2O2) Two potential therapeutic agents (hypothermia and NXY-059) were tested at various concentrations and cell death was quantified using a lactate dehydrogenase assay. Hypothermia to 33oC reduced H2O2 and OGD induced cell death by 53% and 45% respectively at 24h. When hypothermia was induced at different times after injury the neuroprotective effect decreased with time however it was neuroprotective even when administered 6h after H2O2 induced cell death. This was not seen in following OGD induction. NXY-059 had no effect on neuronal cell survival in either of the injury models. These results demonstrate that hESCs have the potential to be a useful model for future drug screening. Identifying neuroprotective agents that work in such human in vitro systems may bridge the gap between animal studies and clinical trials.

T-1503

SECRETOME OF HUMAN WHARTONS JELLY MESENCHYMAL STEM CELLS-CONDITIONED MEDIUM AS ANTICANCER THERAPY

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Human mesenchymal stem cells (hMSCs) are powerful tools for tissue engineering for regenerative medicine. Conditioned medium (CM) is medium harvested from cell culture containing various types of metabolite, protein, extracellular matrix protein, mediators material, which is secreted by cells cultivated in the medium. Human Wharton's jelly mesenchymal stem cell-conditioned medium (hWJMSCs-CM) possesses anticancer, wound healing, cerebral ischemia, brain/spinal cord injury, liverfailure properties. From the previous study hWJMSCs had anticancer property. The secreted-various of CM is affected kinds of cells, different condition, different passage, the number of cells, culture medium, culture condition. The objective of these studt to measure the growth factor and cytokines in WJMSCs-CM cultured in normoxic, hypoxic (2.5%; 5% O_2) condition from early passage (P4) and late passage (P8). The hWJMSCs were isolated from umbilical cord of three women after full-term births. The secretome were measured including IL-1α, IL-6, IL-8, and VEGF that both normoxic and hypoxic tension, early and late passage. hWJMSCs secreted IL-1α in CM 2.75-4.85 pg/ml, IL-6 in CM 1194.46-3463.72 pg/ml, IL-8 in CM 2049.63-6322.30 pg/ml, VEGF in CM 24.96-51.86pg/ml. Oxygen and passage condition affect cytokines and growth factor secretion, hypoxic condition and late passage increase cytokines and growth factor level.

T-1504

THE USE OF A SYNTHETIC HISTONE PEPTIDE-DNA CONJUGATE AS A SPIKE-IN REAGENT TO NORMALIZE CHROMATIN IMMUNOPRECIPTIATION EXPERIMENTS

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Chromatin immunopreciptiation (ChIP) is an analytical method

used to investigate interaction of proteins with specific genomic DNA regions in vivo and provide a better understanding of the mechanisms of gene regulation, DNA replication and DNA repair. Variations in the efficiency of the immunoprecipitation, background signal from carryover in immunoprecipitation, and loss of material during the purification of the ChIP DNA are sources of variability that restricts the use of ChIP as a quantitative tool. We have developed a method to improve the consistency and quantification of ChIP data based on the use of a synthetic peptide-DNA spike in reagent that mimics the behavior of cross-linked chromatin in the immunopreciptiation and recovery reactions. A fixed amount of the synthetic peptide-DNA complex is spiked into the chromatin and co-ChIPed simultaneously with the antibody of interest, and the amount of the recovered synthetic DNA is then used to normalize the results obtained with the antibody of interest. The spike in control can be designed using a peptide specific to the ChIP antibody of interest and acts as an exquisitely sensitive molecular readout for antibody-immunogen affinity. Our data show that use of the spike in control improves the dynamic range of ChIP signal, can strongly reduce the variability between ChIP assay replicates or user to user variation, and is very useful for characterization of antibody specificity. The sequence of the spike-in can also be incorporated into NGS libraries to provide a reference signal and normalize off target effects from antibody dependent ChIP-seq signals.

T-1505

WHOLE-BRAIN VISUALIZATION OF SYNAPTIC INPUT OF MURINE HOST NEURONS ONTO GRAFTED HUMAN STEM CELL-DERIVED NEURONS

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The suitability of neuronal transplants for restoration of neuronal function strongly depends on the ability of the donor cells to engage in synaptic interaction with preexisting host circuits. In this study, we set out to map host neurons which project onto and establish synaptic interaction with grafted human neural stem cell-derived neurons. To that end, long-term self-renewing neuroepithelial stem (lt-NES) cells were transduced with a lentivirus coding for avian leukosis and sarcoma virus subgroup A receptor (TVA), rabies glycoprotein and mRFP1 and transplanted into the striatum and hippocampus of adult unlesioned Rag2-/- mice. Twelve weeks after transplantation grafts were infected with stereotactically injected pseudotyped ΔG rabies encoding GFP. In this system, the modified rabies-virus particles can only infect TVA expressing cells from where they undergo retrograde transsynaptic transport into first order neurons projecting onto the grafted human cells, which can be visualized by virtue of their GFP expression. In order to observe the input neurons in a whole brain preparation, fixed recipient brains were subjected to a novel BABB-based tissue clearing procedure and then analyzed with a custom-made high-resolution light-sheet fluorescent microscope tailored to the acquisition of image stacks from tissue samples up to the size of an entire mouse brain down to a 4 µm resolution. 3D visualization of the afferent connectome of the grafts revealed a remarkable degree of orthotopic input of striatal and hippocampal grafts along the corticostriatal and perforant pathways, respectively. Our data point to a pronounced potential of lt-NES cell-derived neurons to undergo synaptic integration upon transplantation in the unlesioned adult brain. We expect this whole-brain visualization of xenogeneic synaptic connectivity to facilitate the assessment of functional integration of grafted neuronal cell populations and to promote comparative analysis of synaptic integration of disease-derived and control neurons.

T-1506

INTEGRATED PLATFORM FOR DERIVATION AND ENRICHMENT OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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Human induced pluripotent stem cells (hiPSCs) can be used to produce a wide range of functional cells with enormous potential for regenerative medicine. Cardiomyocytes are among the most promising hiPSC-derived cells and can be used for cardiac disease modeling, for drug screening and, possibly, as a cell source to treat damaged hearts. However, technical issues during scaling up and reminiscent pluripotent stem cells after differentiation preclude the use of these cells in the regenerative medicine field. We have optimized a differentiation protocol to robustly generate xeno-free hiPSC-derived cardiomyocytes through the use of small molecules inhibitors to modulate Wnt signaling. hiPSCs efficiently differentiated into cardiomyocytes resulting in 60% to 80% of cTnT positive cells. The differentiation yield was also improved by 10.5 fold after a splitting step and taking advantage of cardiac progenitor cell (CPC) proliferation. Unfortunately, 20% of cells still expressed pluripotency markers, e.g. Oct4. To address this problem, we explored the different glucose and lactate metabolism between cardiomyocytes and hiPSCs as a purification method. Conjugating both approaches not only we have enhanced by 5% the amount of cTnT-positive cells but also effectively reduced the percentage of cells expressing pluripotency markers and, consequently, the risk of teratoma formation. Our results showed that our xeno-free scalable platform can be a reliable method to produce and purify hiPSC-derived cardiomyocytes, increasing the possibilities of using these cells for a wide range of applications.

T-1507

SEPARATING HUMAN PLURIPOTENT CELLS FROM DIFFERENTIATED DERIVATIVES USING A SCALABLE, LABEL FREE APPROACH

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The broad potential of pluripotent human embryonic stem cells to grow and differentiate makes them a promising cell source for regenerative medicine. Fulfilling this promise demands

the development of rapid, scalable and label-free methods to discriminate and separate live cell populations to allow industrial or clinical use of stem cells. Here, we identified differences in cell elastic modulus (CEM) for human embryonic stem cells (hESC) versus mesenchymal progenitor and osteoblast-like derivatives and fibroblasts using atomic force microscopy and data processing algorithms to characterise the stiffness of cell populations. CEMs of undifferentiated pluripotent stem cells were on average 5-10 fold lower than those of fully differentiated cells, information we next exploited to achieve scalable cell separation by developing a label-free separation device based on the principles of tangential cross-flow. Our device features a cellulose micromesh of defined pore size sandwiched between two S-shaped semicircular channels etched in mirror-symmetric blocks of stainless steel. Testing the device's capability to separate a 1:1 mixture of a transgenic hESC line H1 expressing a visible Enhanced Green Fluorescent Protein reporter under control of a pluripotency transcription factor promoter (OCT4) from human dermal fibroblasts showed a throughput of 10^6-10^7 cells per minute and up to 98.8% removal of specific cell types per single pass. To test the device's utility for therapeutic cell production we next collected hESC osteolineage derivatives at various days post-initiation of hESC differentiation and mixed them 1:1 with hESCs for subsequent separation. We observed that the level of enrichment of soft, pluripotent hESCs in the flow-through increased with increasing elastic modulus (i.e., increased stiffness) of the differentiating cells. Our data demonstrate the ability to selectively and scalably enrich for cell populations on the basis of cell biophysical properties, without reliance on cell labeling with high-affinity probes for cell specific antigens. Our findings underpin the development of a novel, automatable solution for high-throughput cell separation to meet the future needs of pluripotent stem cell manufacturing for industrial and clinical applications.

T-1508

QUALITY AND SAFETY EVALUATION OF DENTAL PULP DERIVED STEM CELLS

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Procurement of a stable cell source is an essential requirement in order to fulfill stable supply of regenerative medicine products after commercial launch. However, procurement of allogeneic cell sources in Japan for commercial production is restricted, and it is necessary to rely on cell sources outside Japan. We focused on dental pulp as a new cell source. Pulp is a human-derived tissue isolated from extracted teeth discarded as medical waste. Since tooth extraction from a donor is minimally-invasive, opportunity to procure such cell source is relatively less challenging than other sources. We established a method for culturing cells isolated from dental pulp tissue, stable expansion of the cells, and a method for cryopreservation of the expanded cells. This time, we performed a series of quality and safety tests for the expanded cells and evaluated their potential for cell therapy. As a result of quality tests in in vitro setting, such as cell surface marker test, differentiation ability test into multiple lineage., secreted protein profile test and potency assay assuming immunosuppressive properties of the cells, we found that dental pulp-derived stem cells showed results comparable to bone marrow-derived mesenchymal stem cells as mentioned published paper. Results of performing various virus tests, mycoplasma negative test, and endotoxin test as safety evaluation, dental pulp-derived stem cells met the criteria in all evaluation items. In addition, for in vivo evaluation, assuming intravenous administration of the cells, notable adverse events were not observed in the infusion toxicity test. These results suggested that the cells isolated from dental pulp are useful as a candidate for cell therapy.

T-1509

CELLULAR MICROENVIRONMENT ARRAY FOR CONTROLLING STEM CELL PHENOTYPES

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Cellular microenvironments play critical roles in precise regulation of cellular functions and tissue organization; dysfunctions of tissue microenvironments cause tumor formation, progression, and metastasis. Therefore, understanding the mechanisms by which microenvironments exert their effect facilitates the establishment of accurate and efficient methods to manipulate cellular functions and the development of cell-based therapies, drug development, and cancer diagnosis and therapy. However, our understanding of these microenvironments remains limited because we lack proper tools to precisely perturb environmental factors and systematically investigate cellular phenotypic responses. In this study, we integrated microfluidics and nanofibers to create various artificial cellular environments on a single platform; we call this the Multiplexed Artificial Cellular MicroEnvironment (MACME) array. In this platform, image-based cytometry followed by statistical analysis is used for systematic study of cell behaviour under the influence of multiparametric environmental factors. Combining image-based and statistical analyses enables quantitative interpretation of individual cellular phenotypic responses to environmental cues. We applied our strategy to test how environmental cues affect the functions of self-renewing human pluripotent stem cells (hPSCs). This can potentially be used in drug development, cell-based therapies, tissue engineering, and regenerative medicine, due to unique characteristics, such as unlimited self-renewal and the capability for directed differentiation into most cell types. In this context, we expect our strategy (to identify optimal conditions for the maintenance hPSC self-renewal) to be appropriate for such a challenge, and we elucidated how environments alter cellular phenotypes. Our results indicate that the density and composition of extracellular matrices in combination with cell-cell interactions alter the phenotypes and heterogeneity of hPSCs by modifying TGF-β, NOTCH, and WNT signaling pathways. Furthermore, we confirm that the optimal conditions can be scaled-up for macroscopic cultures. The use of this combinatorial approach provides insights into the underlying chemical and physical mechanisms that govern the fate of stem cells.

T-1510

IDENTIFICATION OF STEMNESS IN HUMAN FETAL CARTILAGE DERIVED CELLS

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Recently, fetal derived stem/progenitor cells in various fetal tissues have been widely used in many studies. In this study, we investigated self-renewal ability of human fetal cartilage-derived cells (hFCs) by examining their clonal colony forming ability along with passages. hFCs were isolated from human fetal cartilage tissue with informed consent. hFCs could be culture expanded for more than 15 passages with high proliferation rates and maintained their chondrogenic differentiation ability high until 10 passages. For colony forming unit assay, hFCs at passages 2 ~ 15 were plated at 10 cells per 60 mm dish in basic growth medium. The number of colonies formed was counted to measure colony frequency. Cells from the colonies of each passage were cultivated until senescence to measure cell proliferation rates and used again for the next round of colony forming assay. We found that the colony forming frequency of hFCs were almost 100% at early passages and maintained at least at 50~60% until passage 15. The cell cultivation derived from one colony was possible until passage 15. In contrast, the colony forming ability of human bone marrow-derived mesenchymal stem cells (MSCs) was relatively low and lost significantly after initial plating. These results shows that hFCs are highly potent cells with long-term repopulating ability until more than 15 passages and could be a promising a novel cell source in cell based therapies and industrial utilization. *This study was supported by a grant of the Korea Ministry of Education, Science and Technology, Republic of Korea (20110019681), and the Brain Korea 21 Foundation of Korea Research Foundation.*

T-1511

DEVELOPMENT OF A TWO PART SYSTEM PROVIDING ENHANCED POST-THAW RECOVERY OF PLURIPOTENT STEM CELLS AND REDUCING CELLULAR STRESS ASSOCIATED WITH A BROAD RANGE OF APPLICATIONS

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Pluripotent stem cells (PSCs) and primary cells are foundational tools for basic research and applied applications including regenerative therapy, drug discovery, and toxicological assessment. While stem cells have a tremendous proliferative capacity, long term culture of these cells has been shown to cause an accumulation of mutations that result in genetic instability, increasing tumorigenicity and thus limiting their usefulness in research and clinical applications. Improved solutions to reduce stress on these cells would provide multiple benefits including enhanced cryopreservation and post-thaw recovery, as well as providing support during manipulation of cells in diverse workflows including high throughput screening and gene editing. By using a combination of small molecule library screens, Design of Experiments (DOE) and mathematical modeling methods we developed optimized solutions for cryopreservation and recovery of PSCs passaged as clumps or single cells. Here we describe the utility of a two part system including a xeno-free cryomedium and animal-origin free recovery supplement that is composed of a pro-survival small molecule coupled with antioxidants and free radical scavengers. SelectScreen® Kinase Profiling of the pro-survival small molecule contained within this cocktail indicates a higher degree of specificity of this ROCK inhibitor over traditional ROCK inhibitors, Y-27632 and Thiazovivin. This system provides >80% direct post-thaw viability of PSCs with >70% cell survival following 24 hours post-plating. The recovery supplement also enables single cell passaging, maintaining normal morphology, pluripotency, and karyotype for up to 30 passages. Additional utility of the recovery supplement includes improved recovery over Y-27632 for PSC cryopreserved in alternative cryomedia, recovery of cells post-electroporation, and recovery of cryopreserved normal primary cells. Taken together, these data demonstrate the robustness and versatility of this system in minimizing cellular stress and maintaining cell health and viability in a broad range of applications.

T-1512

PRODUCTION OF TARGETED GENETIC MODIFICATIONS IN THE RAT USING EMBRYONIC STEM CELL AND GENOME EDITING TECHNOLOGIES

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The rat continues to be the preferred model organism for several important fields of biomedical research, including cardiovascular and metabolic disease. Recent advances in rat embryonic stem cell (rESC) and genome editing technologies hold the promise of producing more powerful models of human disease through the precise modification of the rat genome. While still in its infancy, rESC technology has the potential to vastly expand both the size and the nature of genetic modifications available to researchers. We report here the production in rESC of precisely defined mutations in 5 genes, including several of interest to the biomedical community. Using plasmid or BAC-based vectors, we have produced a variety of mutations, including: complete coding sequence deletion, the insertion of reporter genes controlled by the endogenous promoter, and partial or complete replacement of the coding sequence with the cognate human gene (humanization). Here we describe deletions of 5.4 kbp, and an insertion of 9.0 kbp. Targeting efficiency in our rESC was comparable to mouse ESC targeting. We have achieved germline transmission for two targeted mutations and have begun phenotyping studies, including expression analysis of lacZ reporters. We also demonstrate Prm1-Cre-mediated deletion of our vectors' antibiotic resistance cassette in F1 progeny ("self-deleting cassette); incorporation of this element into our vectors eliminates the need for additional manipulations to remove the antibiotic resistance cassette. We have also explored the use of site-specific nucleases in rESC, specifically the Zinc-finger Nuclease (ZFN) and CRISPR/Cas9 systems. We have enhanced targeting efficiency at multiple loci by the introduction of double-stranded breaks in the gene of interest through the use of both these systems. By including nucleases we have also achieved biallelic targeting at several loci. Combining these two technologies allows us to carry out targeting projects with greater efficiency and versatility and may ultimately lead to the production of new human disease models.

T-1513

HUMAN ADIPOSE TISSUE STEM CELLS DERIVED NANOVESICELS PROTECT PANCREATIC ISLET FROM STZ INDUCED ISLET CELL DEATH

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Recently many studies have focused on extracellular vesicles including exosomes and microvesicles for their therapeutic potential. In this study, we evaluated whether nanovesicles protect pancreatic islet against streptozotocin (STZ)-induced cell death. Human adipose tissue-derived stem cell-derived nanovesicles that mimicked exosomes were isolated by extruding stem cells through micro-filters. These nanovesicles could be visualized by transmission electron microscopy and had round structures enclosed by lipid bilayer consisting of exosome-associated proteins CD81 and CD63. The size of these nanovesicles was measured by DLS and was ~100-nm in diameter. The pancreatic islets were exposed to STZ in the presence or absence of nanovesicles isolated from human adipose tissue-derived stem cells. Pancreatic islets treated with nanovesicles showed higher cell viability compared to non-treated islets and an increased level of insulin secretion. Increased level of IL-6, IL-10 were found in the culture medium when pancreatic islet were treated with nanovesicles. The results indicated that nanovesicels were able to protect pancreatic islets from STZ-induced cell death.

T-1514

TOLERANCE INDUCTION TO HUMAN STEM CELL TRANSPLANTS WITH EXTENSION TO THEIR DIFFERENTIATED PROGENY

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There is increasing interest in transplantation of human pluripotent stem cells for therapeutic purposes. It would benefit future application if one could achieve their long-term acceptance and functional differentiation in allogeneic hosts using minimal immunosuppression. Allogeneic stem cell transplants differ from conventional tissue transplants insofar as not all alloantigens are revealed during tolerance induction. This risks that the immune system tolerized to antigens expressed by progenitors may still remain responsive to antigens expressed later during differentiation. Here we show that brief induction with monoclonal antibody-mediated coreceptor and costimulation blockade enables long-term engraftment and tolerance towards murine ESCs, hESCs, human induced pluripotent stem cells (iPSCs) and hESC-derived progenitors in outbred murine recipients. Tolerance induced to PSC-derived progenitors extends to their differentiated progenies, and sometimes even to different tissues derived from the same donor. Global gene expression profiling identifies clear features in T cells from tolerized grafts that are distinct from those involved in rejection.

T-1515

REAC TECHNOLOGY AND HYALURONAN: A CROSSTALK COUNTERACTING STEM CELL SENESCENCE?

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Hyaluronic acid (HA) plays a crucial role in stem cell pluripotency, and its expression progressively declines along with cell differentiation. The inhibition of HA synthesis induces cells senescence. In the present work we investigated whether HA synthesis may play a role in REAC (Radio electric asymmetric conveyer) ability of hindering cellular senescence, as previously demonstrated by us. Therefore, we induced the cellular senescence in vitro by culturing human adipose-derived mesenchymal stem cells (ADhMSCs) up to the 30th passage, in the absence or presence of 4-methylumbelliferone (4-MU), a potent HA synthesis inhibitor via HAS2 repression. REAC treatment was applied to stem cells, in a long-term (30-passage) culture model of cell aging. When cells were exposed to REAC, the expression of all the investigated genes, including Bmi1, Oct4, Sox2, Nanog and TERT was up regulated, as compared to untreated control cells. The REAC effect was dramatically reduced, albeit not completely suppressed, in the presence of 4-MU. In conclusion our results indicate that the effect of REAC in counteracting stem cell senescence is mainly related to the activity of Has2 and thus to HA synthesis and likely to its intracellular patterning

T-1516

DEVELOPMENT OF A GMP CRYOGENIC COLD CHAIN FOR DELIVERY OF REGENERATIVE MEDICINE THERAPEUTICS

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To cryopreserve cells for clinical therapy products, a consistent and biologically robust cryogenic cold chain must comply with Good Manufacturing Practice (GMP). Cooling devices on the market used in GMP processes, generally employ the use of cryogens and are unsuitable for cleanroom use. This project focuses on one key element: controlled freezing and thawing of large banks of cells of human stem cells. Here we qualify the use of liquid nitrogen-free, Stirling-cycle cooling technology (Via Freeze) controlled rate freezing within GMP environments where control of particulates is an important consideration. In this study, we have compared the recovery and biological status of hESC and hiPSC lines frozen down in the Via Freeze with a passive cooling device (Mr Frosty™) using a standardised protocol currently used within the UK Stem Cell Bank. Cells were tested for cell health (propidium iodide/acridine orange, intracellular glutathione, mitochondrial membrane potential), stem cell characteristics (Tra-1-60, SSEA1, 3 and 4), karyology (G-banding) and markers for self-renewal, attachment and recovery immediately upon thawing as well as 5 passages post-thaw. These results were then used to give a profile of "cell health" and functional characteristics for recovered cultures. Cryopreservation

using the Via Freeze did not significantly alter cell adhesion or other cell health markers, nor were there any changes in expression of self-renewal genes and phenotypic characteristics when compared to pre-freeze controls. We have also investigated the use of an ice nucleating agent to control spontaneous nucleation and performed accelerated temperature cycling studies to assess the effect of this on subsequent cell viability as well as examining post-thaw non-adherent cell populations. In summary, nitrogen-free preservation in the Via Freeze device can therefore effectively maintain pluripotent stem cell viability and functionality with equivalent results to a commonly used passive freezing method. The Via Freeze provides a novel approach for cryopreservation that is suited for use in a cleanroom manufacturing environment.

T-1517

A NOVEL CLINICALLY APPLICABLE METHOD FOR PURIFICATION OF HUMAN PLURIPOTENT STEM CELL DERIVED CELLS USING MICRORNA RESPONSIVE SYNTHETIC MRNAS

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Although various studies on the differentiation of human pluripotent stem cells (hPSCs) have been reported, the resulting differentiated cells are often a mixture of heterogeneous populations. Therefore, the purification of distinct target cells, such as cardiomyocytes, is an important step for safe and controllable cell therapy. However, many types of cells such as cardiomyocytes, hepatocytes and β-cells, have no specific cell surface markers that facilitate their identification, thus hindering the clinical applications of hPSC-derived cells. MicroRNAs (miRNAs) are small non-coding RNA that controls gene expression through translational regulation or mRNA cleavage. We have developed synthetic mRNA technologies that modulate reporter protein expression by sensing miRNA activities (miRNA switch) enable purification of target cells differentiated from hPSCs in a safe and effective manner. For purification of cardiomyocytes, we adopted three miRNA switches (i.e., miR-1-, miR-208a-, and miR-499a-5p-switches), and found that purified cardiomyocytes differentiated from hPSCs with higher efficiency (e.g., up to >98% in five hPSC lines) by cell sorting compared to antibody-based purification methods. In addition, we succeeded in the enrichment of cardiomyocyte derived hPSCs without cell sorting using miR-1- or miR-208-Bim switch which regulates selective cell death. These cardiomyocytes purified by miRNA switches displayed similar gene expression profiles to those purified by other methods, were successfully engrafted in immunodeficient mouse heart, and did not form tumor even when injected into testes of SCID mice. Furthermore, we successfully purified endothelial cells, hepatocytes, and pancreatic β-cell derived from hPSCs using cell type-specific miRNA-switches. Our results demonstrated that miR-switch technology can be used for a variety of purposes including purification in any type of cells and is applicable in the field of stem cell-based regenerative medicine.

T-1518

MAPPING OF THE TRANSCRIPTIONAL ARCHITECTURE IN STEM CELLS WITH SUPER-RESOLVED LOCALIZATION MICROSCOPY

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In embryonic stem cells (ES) pluripotency is maintained by the key transcription factors OCT4, SOX2, KLF4 and NANOG. These factors appear to function as a complex, as they bind to many of the same loci throughout the genome. This suggests that combinatorial control of gene transcription by these factors is fundamental to the pluripotent state. Although differentiation of ES cells to specific cell types occurs over several days in culture, significant changes in chromatin architecture occur even within the first 24 hours of differentiation. These changes include an increase in condensed chromatin, a decrease in heterochromatin protein mobility and a decrease in global transcriptional output. By focusing on early transcriptional events, we hope to gain a deeper understanding of how ES cells maintain pluripotency and initiate cellular differentiation. The process of transcription by RNAPII involves the coordinated action of numerous proteins. We are studying the spatial organization and co-localization of the transcriptional machinery within ES cells with quantitative microscopy, namely, super-resolved localization (SRL) microscopy. SRL microscopy, a class of techniques that operate by driving dense populations of fluorescent molecules through multiple blinking cycles, can resolve structure within a cell as fine as a few 10s of nanometers. Unfortunately, it's technically challenging to study nuclear processes with SRL microscopy due to the large spatial extent and recessed location of the nucleus within the cell. With this in mind, we have developed a clustering analysis for quantifying spatial organization with SRL microscopy in the presence of significant confounding noise. We initially applied this technique to investigate the spatial organization of RNAPII within mouse ES cells compared to differentiated erythroid and neuronal cells. We found that SRL resolves the observed wide-field clusters of RNAPII into much smaller foci. Although we observed that the foci area and density was not different in the three cell types, differentiated erythroid cells displayed more clustering of foci at the nuclear periphery compared to ES cells. We will also present preliminary data on our efforts to map the dynamic pairwise interactions of OCT4, SOX2 and KLF4 during the early stages of cellular differentiation.

T-1519

PROLONGED VIABILITY OF INTRACRANIAL HUMAN NEURAL STEM CELL TRANSPLANTS USING MINICIRCLE DRIVEN BCL-2 EXPRESSION

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Globlastoma multiforme (GBM) is a highly invasive brain tumor that is usually fatal within two years of diagnosis. Surgery and radiation are unable to completely eliminate infiltrative GBM cells, so patients rely on chemotherapy to kill residual tumor. Given the difficulties delivering drugs to these invasive tumors, there is a clinical need for novel, GBM-selective delivery approaches. HB1.F3 neural stem cells (NSCs) inherently display remarkable abilities to distribute selectively

to both invasive edges and hypoxic GBM cores. Our lab has genetically engineered these NSCs to produce enzymes that activate benign pro-drugs within the brain into active chemotherapies directly at GBM foci. Unfortunately, tumor recurrence is still inevitable indicating either incomplete tumor coverage or suboptimal pro-drug conversion by the NSCs after transplantaion. Here we hypothesize that therapeutic efficacy can be improved by increasing NSC survival during the critical 3-4 days needed for tumor tropism and enzyme secretion. Our TUNEL and FACS data confirm that >80% of transplanted NSCs currently undergo apoptosis within the first 24 hours. Using in vitro ATP assays and LIVE/DEAD imaging, we demonstrate that NSCs are particularly susceptible to two different insults (oxidative stress, anoikis) that are present within the tumor microenvironment. We further demonstrate NSC susceptibility to these insults can be mitigated when minicircle technology is used to transiently over-express the anti-apoptotic protein, BCL-2. This strategy should be safe given our transient expression system, and because Bcl-2 is not altering NSC proliferation patterns. Our results demonstrate BCL-2 over-expression does not impair tumor-tropism, viability, or therapeutic enzyme expression in vitro. In vivo studies also confirm that this transient BCL-2 expression system significantly prolongs NSC survival within the intracranial glioma setting, without any evidence of tumorigenicity. Efficacy studies are being initiated, and we predict that the improved NSC viability will translate to improved therapeutic efficacy. Together, this research aims to improve performance of NSC-mediated GBM therapy and thereby improve clinical outcomes for GBM patients.

T-1520

UPSTREAM EXPANSION SOLUTIONS FOR STEM CELLS

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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 stem cell therapeutics progress through clinical testing, current in vitro culture methods in 2D vessels are proving cumbersome to scale. Moreover, the concurrent decreased demands for serum from the recombinant protein and vaccines markets may result in a shortage of serum as clinical cell therapy programs are successful. We have developed an approach for selecting media and microcarriers, using adipose-derived MSCs as a model cell line. Media were screened in 2D culture, followed by small-scale microcarrier evaluation in both static and stirred platforms, and finally in the Mobius® CellReady 3L bioreactor. We identified that the interplay between culture surface and media formulation and harvest solutions can contribute to the success of the expansion and recovery system. Next, an evaluation of animal-free media supplementation and cellular detachment solutions was performed. Human platelet lysate was assessed in comparison to fetal bovine serum while cellular detachment was optimized using animal-free enzymes. Platelet lysate supported growth of MSCs in a variety of expansion paradigms, providing a xeno-free system when combined with animal-free detachment. Because cellular therapeutic manufacturing processes are further complicated by the requirement to separate cells from microcarriers whilst retaining cell yield, viability and target phenotypic and functional characteristics, the importance of acknowledging downstream effects while establishing upstream parameters must be considered.

T-1521

PRECOATED RLAMININ-521 CULTUREWARE FOR LONG-TERM SINGLE-CELL CULTURE OF HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), are conventionally cultured on a layer of feeder cells (such as mouse or human fibroblasts) or on a complex mixture of naturally-derived extracellular matrices (such as Matrigel). These cells are passaged as clumps using manual manipulation. Alternatively, ROCK inhibitor (Y-27632) is required to sustain single-cell passaging. However for hPSC scale-up under cGMP conditions these protocols introduces variability and process complexity. Recently, recombinant Laminin-521 has been shown to support ROCK inhibitor independent, single-cell hPSC culture with a wide variety of defined culture media. Here, we developed rLaminin-521 precoated ready-to-use cultureware and demonstrated long-term culture of hiPSCs in xeno-free and serum-free media using single-cell passaging without ROCK inhibitor. Human iPSCs were cultured on rLaminin-521 cultureware in mTeSR1 and NutriStem media for more than 10 passages. The cells exhibited undifferentiated morphology with a high nucleus-cytoplasm ratio. The cells remained undifferentiated as demonstrated by the expression of OCT 3/4 (>95%), SSEA-4 (>95%) and the absence of SSEA-1 via flow cytometry. After 10 passages, the pluripotency of hiPSCs was shown by differentiation into the three germ layers. The rLaminin-521 cultureware offers the robustness of rLaminin-521 with the convenience of a precoated surface and is ideal for long-term culture and large-scale expansion of hPSCs in xeno-free environments.

T-1522

SPATIAL ORIENTATION OF MSC IN 3D CULTURE SYSTEMS AFFECTS THE SECRETOME AND ENHANCES HEMATOPOIETIC SUPPORT

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In the bone marrow (BM) niche, mesenchymal stromal cells (MSCs), are known to support hematopoietic stem and progenitor cells (HSPCs) both via direct cell-cell interactions and by secretion of soluble factors. The underlying mechanism is still elusive. In order to study the hematopoietic support, we employed both 2D and 3D co-culture systems of ABMSC and FBMSC with cord blood derived HSPCs. The hematopoietic compartment was analysed by flow cytometry and colony assays. 3D cultures positively affected the expansion of HSPCs and provided 2,6 fold more expansion benefit when HSPCs were co-cultured with ABMSCs and 5.5 fold expansion with FBMSCs as stromal layer, compared to 2D. Addition of SCF to the system, only strengthened the hematopoietic -supportive effect of MSCs. Outgrowth of CFUGM and BFU-E colonies was significantly

enhanced in 3D HSPC co-culture with FBMSC. Phenotypic analysis showed that CD34+ cells expanded better in 3D than in 2D, as was previously published by others. In addition, more megakaryocyte progenitors were observed in the 3D-FBMSC situation. Previous data from our lab involving a whole genome wide screen comparing ABMSCs and FBMSCs identified 687 differentially expressed genes from which 16 were Wnt pathway-related. In addition, a preliminary (2D) secretome screen revealed that, ABMSC and FBMSCs differ by 213 distinct proteins, from which 42 overlap with our genome wide array data. Amongst those, we identified proteins associated with Wnt, Notch and IGF signalling and a number of extracellular matrix molecules like collagen and versican. Moreover, blocking the Wnt production in our 2D system bisected the hematopoietic support of ABMSC, while the support of FBMSC was unaffected. We conclude that 3D co-culturing systems mimic the natural niche environment and provide appropriate signalling for hematopoietic support. ABMSCs provide overall better hematopoietic support than FBMSCs which in conventional 2D cultures almost lack hematopoietic-supportive capabilities, but in 3D FBMSC almost resemble ABMSC. Next to spatial orientation of MSCs, the 3D system significantly changed the composition of the MSC secretome,-thus our current analysis of the 3D secretome of both ABMSCs and FBMSCs, will reveal factors responsible for the enhanced hematopoietic support.

T-1523

DEVELOPMENT OF BIO-INSPIRED EXTRACELLULAR MATRIX PEPTIDE-GRAFTED SURFACES FOR FEEDER-FREE CULTURE OF HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) hold a great promise as cell sources for regenerative medicine and drug discovery. In general, hPSCs require animal-derived feeder cells (e.g., mouse embryonic fibroblasts) or animal tissue-derived matrices (e.g., Matrigel) for clonal expansion. However, the use of xenogenic sources limits the applicability of hPSCs due to safety issues of pathogen transmission or immunogenicity, the difficulty in quality control originated from batch-to-batch inconsistencies, and high expense. Therefore, development of chemically defined, xeno-free substrates for hPSC culture is essential to produce clinically relevant hPSCs. Here, we developed biomimetic substrates grafted with integrin-binding extracellular matrix peptides for feeder-free, and xeno-free culture of hPSCs. The peptide-grafted surfaces promoted specific integrin-mediated focal adhesion and cell-cell interactions. The enhanced interaction of cell-cell and cell-matrix led to the promotion of hPSC self-renewal and pluripotency. Accordingly, hPSCs could be maintained in feeder-free conditions more than 3 months. The biomimetic peptide-grafted surfaces were compatible with a variety of passaging methods, commercial media, and cell lines, indicating the versatility of the surfaces for hPSC culture. Interestingly, biomimetic peptide immobilization could support feeder-free growth of hPSCs in a surface material-independent manner. For example, hPSCs could be cultured on the nanopatterned substrates and microfluidic devices made of different polymers. The bio-inspired surface engineering to develop the peptide-grafted substrates in this study provides a chemically defined, xeno-free culture platform for clonal expansion and long-term maintenance of hPSCs, and also enables hPSC culture on a variety of biomedical systems. *This work was supported by a grant (NRF-2013R1A1A2A10061422) from the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (MSIP) and Brain Korea 21 plus (BK21PLUS) program, Republic of Korea. Hyun-Ji Park and Kisuk Yang are fellowship awardee by BK21PLUS program.*

T-1524

COMPREHENSIVE ASSESSMENT OF ARRAY AND SEQUENCING BASED PLATFORMS FOR CNV ANALYSIS IN HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) have a countless number of potential for the clinical application. Genomic stability in hPSCs is major issue to keep stem cells pluripotent. To date, since copy number variation (CNV) instability has profound impact on genomic and epigenetic stability of human stem cells, we assessed CNV of embryonic stem cells (ESCs) and induced pluripotent cells (iPSCs) We analyzed CNV of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (iPSCs) which were reprogrammed by two reprogramming methods, sendai virus and modified mRNA, and during culture time. As CNV estimation platform is technically challenging, we applied hPSCs samples to genomic platforms including arrayCGH and two CNV estimation tools, cn.MOPS and control-FREEC, based on whole genome sequencing (WGS) platform. We identified a total of 379 CNVs, 207 CNV gains and 172 CNV losses in all the platforms applied and all the samples studied. We also identified recurrent CNVs as well as de novo CNVs. Here, we present the comprehensive assessment of genomic platforms and the results of CNV regions to see if it is induced by selective advantage or culture adaptation.

T-1525

CHROMOSOME TRANSPLANTATION: A NOVEL STRATEGY FOR CORRECTING GENOMIC DISORDERS RESISTANT TO CLASSICAL GENE THERAPY APPROACHES

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Genomic disorders resulting from large rearrangements of the

genome remain an important unsolved issue in gene therapy. Conventional gene therapy is unable to repair gross mutations such as duplications or complex genomic rearrangements. We propose a novel genomic therapy approach, "chromosome transplantation", which has the potential of curing this kind of disorder. Chromosome transplantation, defined as the perfect replacement of an endogenous chromosome with a homologous one, has not yet been achieved. The transplantation was achieved by following two steps. In the first, an exogenous normal X chromosome (Hprt+) was transferred via microcell-mediated chromosome transfer (MMCT) into recipient male mouse Hprt defective ESCs. The resulting cells were selected in HAT medium, to identify those where the normal X chromosome has been acquired. In the second step, clones where an endogenous sex chromosome had been lost were identified from the initial pool; these clones became either "transplanted" XY (tXY), resulting from loss of the endogenous X chromosome, or "substituted" XX (sXX), resulting from loss of the endogenous Y chromosome. After replacement, the clones maintained in vitro features of stemness, differentiated toward lineages of the three germ layers and contributed to chimera formation. Genome integrity was confirmed by cytogenetic (conventional karyotype and multicolor FISH) and molecular genome analyses (SNP and CNV). In conclusion, we report here the first case of chromosome transplantation leading to normal diploid cells in which a genetic defect has been rescued. HPRT mutations in humans are responsible for the Lesch Nyhan syndrome, a rare neurological disorder. Furthermore, this approach, with minor modifications, could be used to cure various disorders due to other chromosome aberrations of iPS cells derived from affected patients.

T-1526

LAMININ E8 FRAGMENTS AS A VERSATILE PLATFORM FOR FABRICATION OF COLLAGEN-BASED MATRICES HAVING BASEMENT MEMBRANE-LIKE ACTIVITIES

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The extracellular matrix is an essential part of the microenvironment that secures cell survival and regulates cell proliferation and differentiation. Laminins are the major components of basement membranes and have been shown to sustain a wide variety of stem cells including pluripotent stem cells. In this study, we attempted to endow the cell-adhesive activity of laminins to collagen matrices, thereby fabricating three-dimensional (3D) culture scaffolds with defined composition. Such collagen-based 3D matrices will be an ideal substitute for Matrigel, a basement membrane-like 3D matrix that is widely used in manipulating stem cells in regenerative medicine. We utilized the collagen binding domain (CBD) of fibronectin as a vehicle to confer the collagen-binding activity to laminin E8 fragments (LME8s) that retain full integrin binding activity of intact laminins. Given that LME8s consist of three chains, alphaE8, betaE8, and gammaE8, we attached CBD to the N-termini of individual chains, producing LME8s having one, two, or three CBDs. Although LME8s did not bind to collagen or gelatin, CBD-attached LME8s (CBD-LME8s) exhibited significant collagen-binding activity that was dependent on the number of CBDs attached. CBD-LME8s having two CBDs were more potent than those having one CBD by one order of magnitude. However, CBD-LME8s having three CBDs exhibited the activity similar to those having two CBDs. The failure of trivalent CBD-LME8 to exceed divalent CBD-LME8s may result from the steric hindrance due to the preoccupying CBDs that preclude the subsequent access of the third CBD to collagen matrices. To examine the abilities of CBD-LME8s to support the proliferation of stem cells on collagen matrix, we cultivated human iPS cells on collagen-coated plates preloaded with CBD-LME8s. Although iPS cells did not attach nor grow on collagen-coated plates, they adhered to collagen matrices preloaded with divalent CBD-LME8 and efficiently proliferated to yield large colonies. Importantly, divalent CBD-LME8 supported proliferation of iPS cells even on 3D collagen gels when preloaded. These results demonstrated that divalent CBD-LME8s are promising tools for fabrication of collagen-based 3D matrices having potent cell-adhesive activity of basement membranes.

T-1527

ASSESSMENT OF ALTERNATIVE METHODS FOR EVALUATING CELL VIABILITY OF HUMAN UMBILICAL CORD BLOOD DERIVED MESENCHYMAL STEM CELLS

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Accurate determination of cell number is essential for the quantitative description of biological processes. Trypan Blue Exclusion method is still the gold standard, but it has limitation in representing cell's real state, "healthy cells". Cell attachment and growth assay were performed as a complementary method in many studies. However, they are time consuming and require many steps. In this study, alamar blue and presto blue were tested for their suitability as time-saving alternatives to manual counting method in human umbilical cord blood derived mesenchymal stem cells (hUCB-MSCs). Both reagents can be used to indicate metabolism by changes in their absorbance or fluorescence. We induced hUCB-MSCs to have different viability with various stimulations, then investigated cell attachment, growth and activities of alamar blue and presto blue assay on the cells. Alamar blue assay showed more similar manner in the initial attachment and growth than presto blue assay and trypan blue exclusion method. Also, cell attachment assay and growth analysis need at least 24 hrs and 4 days, respectively whereas alamar blue assay takes less than 4 hrs. High sensitivity and accuracy of alamar blue assay were confirmed in the analysis of other type of experiments. Our data suggests alamar blue is useful method for initial in vitro screening assay based on general metabolic activity of the hUCB-MSCs and have possibility to be a rapid detection method as alternatives to manual counting.

T-1528

NOVEL HUMAN IPSC-DERIVED CELL MODELS FOR DRUG DISCOVERY APPLICATIONS

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Current cells for drug discovery applications are limited by either their availability or their lack of suitability for the specific desired application. Availability is a chronic problem for the ideal cell type, human primary cells. Alternatives to primary cells usually involve

immortalized cell lines or more recently, cells differentiated from stem cells, but neither of these currently provides a cell that matches the adult primary cell phenotype. Here we describe our efforts to resolve these issues through the creation of stem-cell-derived cell models that do closely match their adult primary counterparts, thus providing readily-available cells suitable for drug discovery. We have done this by driving alternative fates in cells using transcription factors delivered as synthetic mRNAs. We first have created induced pluripotent stem cells (iPSCs) which represent an infinitely expandable cell resource; these can be derived from disease/mutant cells to generate iPSCs with specific genetic backgrounds that can be further modified (or created de novo) by CRISPR/Cas9 genome editing. We then show that we can utilize mRNA-based cell reprogramming again, in combination with traditional growth factor and cytokine-based methods, to turn iPSCs into cells with desired characteristics. Furthermore, as we show for both neuronal and adipocyte cell models, differentiation driven by synthetic mRNA delivery of lineage-specific transcription factors converts cells to the desired cell fate in a significantly shorter time than traditional differentiation methods. Finally, we show that genetically-encoded sensors represent a significant advancement over current chemical-based assay models. Genetically-encoded sensors are DNA constructs inserted into the cell genome that use a fluorescent protein to provide specific read-outs of cellular events such as Ca2+-influx, membrane depolarization, or transient protein-protein interactions (e.g., GPCR-signaling or kinase cascades). Genetically-encoded sensors have the distinct advantage of not requiring assay components such as small molecule dyes or antibodies. We show here in a calcium-flux model that we can monitor events in real-time (single-digit millisecond response times) in a live cell, further enhancing the capabilities of these iPSC-derived cell models.

T-1529

DERIVATION OF MESENCHYMAL STEM CELLS FROM HUMAN AND EQUINE FRESH AND CRYOPRESERVED UMBILICAL CORD TISSUE

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Creating a repository of mesenchymal stem cells (MSCs) would increase their availability for clinical application. The aim of this study was to assess the optimal isolation and cryopreservation procedures to facilitate umbilical cord tissue-derived MSC (UT-MSC) banking. The cell yield after isolation of MSCs from fresh and cryopreserved umbilical cord tissues was compared. Umbilical cord tissue was maintained in liquid nitrogen for a prolonged time in different types of cryoprotective media cut into 1-2 mm^3 or 1-1.5 cm^3 pieces. Cells from fresh and cryopreserved tissue were isolated using enzymatic digestion or plastic adhesion methods. Their isolation efficacy, growth kinetics, immunophenotype, and differentiation potential were studied. Our results demonstrated that UT-MSCs could be isolated from both fresh and cryopreserved tissue of equine or human umbilical cord while maintaining all the characteristics of MSCs. The cell yield after 30 days in a culture of human UT-MSCs isolated from approximately 1.0 - 1.5 cm^3 of fresh or frozen tissue was 4.1×10^{11} and 1.5×10^{10}, respectively. The difference in cell yield from fresh or cryopreserved tissue was irrelevant due to both maintaining high proliferative ability and culture longevity.

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T-1530

CRISPR-CAS9 APPROACHES FOR HUMAN CELL REPROGRAMMING

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Clustered regularly interspersed short palindromic repeats (CRISPR) based systems originate from prokaryotic immune response and have recently been modified to suite genome editing and transcriptional control purposes. Commonly utilized CRISPR systems comprise of the CRISPR-associated protein Cas9 and small guide RNAs that guide the Cas9 protein to bind to complementary DNA sequences. Catalytically inactivated Cas9 protein (dCas9) can be fused to transactivation domains to build a transcriptional activator. In this work we have optimized the dCas9 transactivator approach for activating endogenous genes that control pluripotency and mediate cellular reprogramming in human cells. We developed a fast and robust protocol for assembling guide RNAs that, in combination with an improved dCas9 activator, efficiently activated human genes involved in pluripotency and differentiation into various lineages. dCas9 activator-mediated activation of endogenous pluripotency factors combined with conventional episomal reprogramming increased colony formation efficiency 3-fold. Most importantly, the need of transgenic OCT4 in pluripotent reprogramming could be completely replaced with dCas9 activator-mediated OCT4 activation. Additionally, we generated a conditionally destabilized activator version by fusing dCas9 to DHRF-derived destabilization domain, that maintained functionality to activate endogenous genes and thus enabled temporal control by trimethoprim addition. Furthermore, activation of endogenous gene transcription with CRISPR/dCas9 transactivators could be used to activate endodermal lineage specific factors both in primary human cells and in pluripotent cell lines. In summary, we have optimized the dCas9 activator system for efficient activation of endogenous genes both for pluripotency and for differentiation in human cells. This system has been used to replace transgenic OCT4 in human cell reprogramming.

T-1531

HARNESSING MURINE EMBRYONIC STEM CELL TROPHIC FACTORS WITH A BIOENGINEERED PERFUSION-BASED PLATFORM

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Innovative technologies to modulate and deliver cell secreted factors, such as tissue-stimulating morphogens and mitogens produced by pluripotent embryonic stem cells (ESCs), are critical for the advancement of regenerative molecular therapies. Therefore, the objective of this work was to engineer a system for robust and controlled delivery of concentrated ESC trophic factors. A packed bed perfusion system to facilitate concentration and continuous delivery of ESC secreted factors was developed for alginate-

microencapsulated murine ESC aggregates using gas permeable tubing with a bed volume of approximately 0.5 mL containing ~3x10^6 cells. Perfusion culture of the encapsulated ESCs (150 µL/hr of serum-free basal media) for up to 4 days yielded a higher concentration of ESC trophic factors in the perfused media than was obtained in static culture (e.g. 4.8-fold increase in VEGF concentration). Culture of the cells under a higher flow rate (300 µL/hr) led to similar VEGF concentrations but an increased per cell production (1.9-fold increase) and total amount (2.2-fold increase) of VEGF, indicating that swifter removal of secreted factors may stimulate a feedback response to increase secretion. To investigate the biological potency of ESC secreted factors from an upstream packed bed system, bone marrow mesenchymal stromal cell (MSC) proliferation was assessed. We initially observed that co-culturing encapsulated ESCs with MSCs led to a 3.9-fold increase in final MSC number (vs. basal media) after 7 days. To examine continuous delivery of ESC trophic factors from the packed bed, MSCs were cultured in a fed-batch regime with the encapsulated ESCs directly upstream, and a 2.6-fold increase in final MSC number (vs. basal media) after 3 days was observed. Ongoing studies are being performed to examine the influence of highly concentrated and continuously provided ESC trophic factors on MSC phenotype and on mixed bone marrow populations ex vivo. Ultimately, this pluripotent stem cell-bioreactor technology enables direct screening of stem cell paracrine factors on downstream cell populations, with notable advantages over standard co-culture and conditioned media approaches, and represents a novel transplant-free, in vivo delivery method for therapeutic translation of potent stem cell regenerative molecules.

T-1532

MEMBRANE LIPID COMPOSITION AND FLUIDITY AS AN INDICATOR OF PLURIPOTENCY

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While pluripotent stem cells revolutionize biology and enable tremendous progress in medicine, heterogeneity in cellular characteristics of these cells within the population and amongst cell lines complicate experimental design, interpretation, and ultimate utility. Because manipulation-induced cellular stress and spontaneous differentiation are major contributors to this heterogeneity, non-invasive, routine monitoring of live cells during propagation in culture is ideal. Although staining with antibodies that recognize cell surface markers and, more recently, live-compatible alkaline phosphatase (AP) substrates have been employed, these methods potentially activate signaling pathways via interactions with the cells. In order to monitor pluripotency with minimal cellular perturbation, we have exploited differences we, and others, have observed in membrane lipid composition in differentiated and undifferentiated cells and show preliminary results that plasma membrane fluidity can be used as a non-invasive indicator of pluripotency. Staining with live-compatible solvatochromic dyes clearly discriminated stem cells from their differentiated counterparts in control experiments. Subsequently, the assay was used to identified different subsets of induced pluripotent stem cells. Gene expression analyses on clones with differential staining are being employed to correlate membrane fluidity and membrane lipid composition with the expression of pluripotency markers.

T-1533

WNT SIGNALING SMALL-MOLECULE MODULATORS AND THEIR EFFECTS ON CULTURED HUMAN LSCS

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The canonical Wnt signaling pathway plays critical regulatory roles in development and stem cells. Aiming to further understand the stepwise signaling cascade in the Wnt pathway and to develop potential novel pharmaceutical agents that interfere with aberrant Wnt signaling, we have taken a systems biology approach by developing small molecules that modulate the protein-protein interaction at different signaling steps in the Wnt pathway. While Wnt inhibitors have therapeutic potential in anti-cancer therapy, Wnt signaling activators may be useful in maintaining stem cell pluripotency, regulating cell regeneration. For such propose, we investigated the effect of Wnt small molecule modulators on human limbal stem/progenitor cells (LSCs) in vitro. Primary human limbal epithelial cells were isolated with Dispase II and cultured on the 3T3-J2 monolayer with or without (control) small molecules that either activate or inhibit Wnt signaling pathway. LSCs were cultured for 14-21 days, the cell numbers and colony formation efficiency (CFE) were measured, as well as the phenotype of the cultured cells, including cell morphology, expression of the putative stem cell markers at the mRNA and protein level, was analyzed. We found that Wnt activators increased cell growth by 75% and upregulated the expression of ABCG2 in comparison to that of the control. In contrast, the presence of Wnt inhibitors decreased CEF and cell growth. It also resulted in the downregulation of ABCG2, one of the markers of LSCs. However, no significant difference was observed in the expression of other makers, such as Keratin (K) 14, Ki67 and ΔNp63, at the mRNA level in all cultures. Furthermore, our immunocytochemical data shows that neither of the Wnt small molecules significantly affected the percentage of K14+ cells, and the cell morphology was not affected by either of Wnt activators or inhibitors. In conclusions, our preliminary data suggests that modulation of Wnt signaling could increase the efficiency of LSCs growth in vitro without promoting cell differentiation.

T-1534

ENHANCING CRISPR GENE KNOCKIN EFFICIENCY IN HUMAN INDUCED PLURIPOTENT STEM CELLS

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Gene 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 recombination (HDR) remains low, in most cases below 1% without enrichment by drug selection or FACS sorting. At such a low efficiency, application of CRISPR in DNA knockin is highly limited. We hypothesized that molecules (small molecules, microRNA and etc.) that inhibit NHEJ and/or enhance HDR would improve CRISPR-mediated DNA knockin efficiency in iPSCs. In a preliminary work to generate a RyR2-V2475F knock-in rabbit model for arrhythmogenic cardiomyopathies, we tested the effects of two small molecules, compound 1 and 2. Supplementation of Compound 1 at 40 μM resulted in 16.7% knockin efficiency. Supplementation of Compound 2 resulted in 12.5% and 4.2% knockin efficiencies at 7.5 μM and 15 μM, respectively. Most recently, we generated CFTRΔF508 knock-in founder rabbits by using Compound 2 in the system with an in vivo efficiency of 25% (1 knockin out of 4 kits born). Given these results, we were encouraged to test these two compounds in iPSCs to investigate if knockin efficiency could also be improved. We will report the results on DNA knockin efficiency in modifying iPSCs using CRISPR/Cas9 with or without the small molecules. We will also discuss applications of the same molecules in other stem cells such as hematopoietic stem cells and mesenchymal stem cells.

T-1535

AUTOMATED, DISPOSABLE DELIVERY TOOL FOR CELL THERAPY USING THE DA VINCI ROBOTIC SURGICAL SYSTEM

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As the use of stem cells to treat a variety of diseases approaches clinical translation, the mode of cell delivery that ensures the best chance of engraftment is an important consideration. Delivery options for the transfer of cells and stem cells into damaged organs and tissues include the intravenous, systemic route or via direct injection, usually at surgery, either open or minimally invasive, laparoscopic or robotic, using the da Vinci Robotic Surgical System. Here we report our development of an automated cell delivery system embodied by a remote, actuated syringe pump attached to the arm of the da Vinci system that enables the precise and controlled delivery of an infusion of cells at a preprogrammed location and depth within the damaged or diseased areas of organs usually operated upon using the da Vinci Surgical System, including the heart, and organs within the abdominal cavity and pelvis. This device comprises of three components: a drive motor assembly, drive cable/sheath assembly, and a tool-mounted syringe. The drive motor assembly is located behind the sterile barrier along with the other da Vinci hardware and is embodied by a linear stepper motor actuator and its associated electronics. The linear stepper motor pushes a drive cable housed in a flexible sheath following the shaft of the da Vinci arm assembly. The drive cable compresses a capsule of saline solution that contains the suspended cells. The capsule is mounted behind a delivery needle positioned by the da Vinci gripper. Using this instrument, we automated the injection process for delivery of precise amounts of the cell suspension at the command of a surgeon tele-operating the robot. The instrument is designed from commodity hardware, costs less than USD 50, is disposal after use, and has the potential to provide a safe, precise, automated and controlled infusion of cells and stem cells using a minimal access robotic approach to any organ already accessible by the da Vinci Surgical System.

ETHICS AND PUBLIC POLICY; SOCIETY ISSUES; HISTORY OF STEM CELL RESEARCH; EDUCATION AND OUT-REACH

T-1537

FROM REGULATION TO INFORMATION: AN 'INFORMED TRUST' APPROACH TO STEM CELL TOURISM

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That global stem cell tourism has been notoriously difficult to regulate is an accepted fact. Most of the global effort focuses on 'supply side' regulations with few 'demand side' (patient-based) approaches. On the supply side, efforts range from strengthening compliance standards in medical and scientific innovation to applying international pressure on countries with permissive regulations to raise standards as a precondition of collaborations. As a result, countries such as China and India, hotspots for stem cell tourism, have responded with stricter regulations although its weak enforcement, if at all, challenge supply-side regulatory efforts as 'unproven' stem cell treatments continue undeterred as do patient uptake for them. On the other hand, limiting the demand-side (i.e. patient choices) is politically and ethically challenging as it infringes on personal freedoms and raises human rights concerns. Indirect efforts by bioethicists to steer patients away from 'emotional' health choices through demonisation of clinicians offering such treatments as rogue traders 'trading on hope' has had little effect, as stem cell tourism continues to expand. Rather, research into the behaviour of stem cell tourists show that patients increasingly i) draw trust from 'experiential' knowledge of stem cell treatments shared via social networks (mostly web-based) at the same time they reference repositories of'expert' knowledge perceived as impartial e.g. the ISSCR guidelines, and ii) view the lack of agency suggested by bioethical positions like 'trading on hope' as 'paternalistic' and often rejecting expert knowledge in favour of the'experiential.' This paper discusses and draws on the lessons from these networks of public trust at the heart of the stem cell patient movements and conceptualises an approach based on reducing information asymmetries or -'informed trust.' Methodologically, the research uses standard secondary data sources and interviews conducted in India and China to answer the questions of i) what resources patients use (or don't) to build their own trust when choosing stem cell treatments?; ii) what is the quality of the data/resources- is

it impartial, sponsored etc?; and iii) what ethical issues arise in the course of i) and ii).

T-1538

A FIVE-STATION MODEL OF STEM CELL DRIVE DESIGN

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Patients with a variety of blood cancers and metabolic diseases may require a stem cell transplant as part of their treatment. However, 70% of patients do not have a suitable genetic match in their family. Stem cell donor-databases are used to match potential unrelated donors to patients worldwide. Individuals aged 17-35 years can register online or at a stem cell drive where they provide consent and a tissue sample (buccal-swab) for Human Leukocyte Antigen (HLA) allele typing. To date, no guidelines have been published to recommend a process for stem cell donor recruitment at drives. Here, I outline a novel approach to stem cell drive design, which features evidence-based strategies to identify the most-needed stem cell donors and to minimize donor ambivalence and withdrawal from the registry. This model of stem cell drive design includes five stations: pre-screening, informed consent, registration, swabbing, and reconciliation. Registrant confidentiality and privacy is maintained at each station. Registrants are first pre-screened to persuade them to register and ensure donor eligibility. Recruiters at the prescreening station target the most-needed stem cell donors according to the literature: young, healthy, and ethnically-diverse males. Recruiters then educate registrants about the stem cell donation process, and secure informed consent according to the World Marrow Donor Association's suggested procedures for procurement of informed consent (2003). Registrants are subsequently guided through registration, which involves providing their contact/demographic information, completing a health questionnaire, and signing a consent form to join the registry. Following registration, registrants proceed to swabbing, where they swab their cheeks to provide a tissue/DNA sample. Finally, registrants visit reconciliation, where their paperwork is error checked, their understanding of the donation process is assessed to verify informed consent, and their kit processed for shipping. In summary, the five-station approach to stem cell drive design, outlined in this presentation, represents a new model for effective stem cell donor recruitment.

T-1539

SKIP (STEMCELL KNOWLEDGE AND INFORMATION PORTAL): ONE STOP DATABASE FOR RESEARCHERS, COMMERCIAL ENTITIES AND CITIZENS

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In 2014 Japanese government launched three Acts in the field 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 realization 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 (http://www.skip.med.keio.ac.jp/) is administrated by an operating committee ("SKIP Operating Committee") of Keio University as Human Stem Cells Informatization Project, the Ministry of Health, Labour and Welfare. To date, we have had more than 550 cell information without duplication. 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 300 per day and is increasing. Researchers can reach disease iPS cells of their interest from the name of disease or ICD-10 code. Visibility of the database increase and we are trying to add detailed relational information of cells and make the database more fruitful. SKIP is developing to be a powerful tool for researchers to liberate their ideas and promote stem cell sciences involving public and commercial entities.

T-1540

DEVELOPMENT DIRECTION FOR THE STEM CELL INDUSTRY ECOLOGY OF KOREA

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Korea is the first country to allow stem cell therapeutics and is putting forth massive efforts to take the lead in the stem cell industry from a national perspective. For this, the government has recently placed a lot of interest in establishing the stem cell industry ecology and much discussions are being made on establishing the roles of the government, industry, academies, public research institutes, and hospitals to construct a healthy and active stem cell industry ecology. A harmonious industrial ecology can induce efficient role sharing and cooperation among the participants in the industry, and based on the technological capacities through this, it is expected to activate the domestic industry and promote entry into global markets. Accordingly, this study aims at introducing plans for the establishment of the stem cell industry ecology that is being discussed in Korea.

T-1541

IMPROVING THE PROCESS FOR INFORMED CONSENT AND INFORMED ASSENT: EXPLAINING STEM CELL BANKING TO PATIENTS THROUGH ILLUSTRATED, READER-FRIENDLY WRITTEN MATERIALS

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Obtaining informed consent (IC) necessarily involves full disclosure, participant comprehension, and voluntary participation. Ethical issues related to these elements were recognized in the case of Henrietta

Lacks, in which the immortalized cell line "HeLa" was obtained and used for research without IC. Despite better clarity about the related ethical issues since that time, it is not always easy to fulfill the elements of IC. The Japanese government has funded a project for the induced-pluripotent-stem-cell (iPSC) banking of rare diseases to promote regenerative medicine and research, bringing to the forefront IC ethical issues. One aspect of this project is to establish cell lines from patients with rare diseases and store them in a stem cell bank with their clinical data for long periods, enabling researchers and commercial companies all over the world to use them for research. To improve IC and informed assent (IA) processes, our team created an illustrated, reader-friendly pamphlet for adults and two booklets for children, based on the standardized consent form template for the aforementioned project. These tools are expected to be used by the researchers involved in the aforementioned project as supplemental IC and IA tools. Rather than explain the research itself, these tools highlight important information from the research ethics perspective, such as participants' rights; information on the return of results, including genetic analysis and virus infections; and broad consent. Cartoon characters for the pamphlet and booklets were also created to depict the banking process. The information selected for the illustrated booklets on consent for children was based on the educational level of the intended readers: the group before entering elementary school; and the group from seven to around nine or ten years old. Some games were also incorporated into the booklets for the children. The pamphlet for adults contains approximately three pages of information adapted from the 11-page consent form. These products have been well received, as it is generally recognized that well-designed tools such as these could help patients better understand complex explanations of stem cell banking. Our poster presents more information on the benefits of utilizing user-friendly supplemental tools for this purpose.

T-1542

PUBLIC AWARENESS AND OPINION ON CORD BLOOD BANKING: SURVEYING THE POPULATION IN JORDAN

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Stem cell transplants using umbilical cord blood (CB) are an expanding field of medicine and scientific research. These treatments are enabled through the collection and storage of CB in facilities known as cord blood banks. CB banks, which first opened in the United States and Western Europe in the 1990s, have attracted the attention of physicians, researchers, and industry alike. However, CB banks have raised ethical and policy questions, such as profitability and funding sustainability, medical justification, informed consent, misleading information, and quality standards. As the field has developed, more CB banks have been built, including recent projects in the Arab world. For-profit, private CB banks are unsupported by the medical and scientific communities, but they currently outnumber public CB banks worldwide, including in the Arab world. Currently, two CB banks_one private and one public_are being built in Jordan, a regional hub for medical tourism. In January 2014, Jordan passed a regulatory law for stem cell research and CB banking_the first in the region. Despite these developments, nothing is known about public opinion and awareness concerning CB banking in the country. Previous scholarship, such as in the United States, Germany, and Canada, has demonstrated the utility and relevance of surveying about the population's knowledge of and attitudes toward CB banking to address weaknesses in practices and policies. Here we present the first study that examines awareness levels and opinions regarding CB banking in Jordan. Based on data from an anonymous questionnaire taken by approximately 500 women at obstetrician clinics in Jordan, public knowledge is extremely poor. The majority of respondents were strongly in favor of receiving additional information about the subject, especially from their obstetricians, suggesting a need for educational campaigns to decrease the knowledge gap and support the public CB bank. The respondents overwhelmingly supported public over private banking, and favored CB storage in Jordan than in an international location. They also cited religion as a major issue influencing their decision about whether or not to store CB. This study identifies areas of priority for policymakers and physicians regarding CB banking, especially as new facilities open in the country.

T-1543

NARRATIVES OF RESEARCH INTEGRITY AMONG BIOLOGISTS AND PHYSICISTS IN THE UK AND INDIA

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In spring 2014 the stem cell community was rocked again by controversy arising from the fabrication of data in two Nature publications on an alternative treatment to create induced pluripotent stem cells. The field was already scrutinized in 2005, after Hwang fabricated several publications on human somatic cell nuclear transfer. Are fraud and other unethical conduct a part of stem cell and biomedical or are these just isolated cases that received excessive media attention? Diverse groups in science, industry, and the public sphere are engaged in ongoing debates about irreproducible results, conflicts of interest and pressure to publish research that encourage scientific fraud. Such tensions are especially significant for biologists. However, biologists are rarely compared to other disciplines to see if the particular issues they face related to responsible conduct of research are really unique. In this poster we will examine: how do biologists compare to physicists in the way they perceive the meaning of research integrity and misconduct and under what conditions do biologists think they are obliged to act when research misconduct has occurred. We analyzed data from more than 200 interviews with scientists in the UK and India. We found that physicists and biologists rarely encountered what they saw as traditional ethics violation: fraud, fabrication and plagiarism. However, scientists often cite numerous lesser violations including issues with authorship, reviewer confidentiality and honesty, and irresponsible conduct of supervisors. Although the two groups of scientists identified similar issues, physicists, in contrast to biologists, tended to see ethical issues as irrelevant to them. Similar ethical issues arose in the UK and India, although UK scientists focused more on pressures to publish while Indian scientists addressed institutional issues including accountability and excessive bureaucracy. And under certain conditions, both Indian and UK biologists and physicists utilize religious and spiritual frameworks to think through ethical approaches to science.

T-1544

THE "EAGLE-I" PROJECT: AN OPEN-ACCESS PLATFORM FOR IPS CELL INFORMATION SHARING AND DISCOVERY

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The eagle-i project of Harvard Medical School and the New York Stem Cell Foundation (NYSCF) have collaborated to organize and openly distribute information about existing iPS cells. The effort has recently expanded to include information about iPSC lines from WiCell, RUCDR, and Coriell. Working with these groups has allowed us to enter and curate information on several hundred different iPSC lines and over a thousand different primary cell cultures from more than 300 different, well-characterized human subjects. As more repositories prepare to receive and distribute several thousand more lines generated by different initiatives, including those funded by NHLBI and CIRM, we anticipate the information pool increasing several fold. To describe the lines, we've developed and refined an ontology that organizes information about stem cells in a way that aligns with the traditional stem cell development pipeline. As such, data records for iPS cells are informatically linked to related data records such as those describing parental/derived cell lines, biospecimens, human subjects, protocols, research labs, etc. Additionally, records can be annotated using constrained vocabularies drawn from existing ontologies. This organization is important for creating data that is interoperable with external systems and reusable by external websites. Finally, we've developed a user-friendly portal that allows researchers to query across resources in the entire eagle-i network to find interesting stem cells or primary cells based on characteristics related to the line itself or to the human subject from which it was derived. All work described here is part of an ongoing NIH-funded effort, and the software, ontology, and the data published on our platform is open-access and freely available for download and/or reuse by the community. Developing standards for describing stem cells is at the core of this effort. The eagle-i project has classically worked with thought leaders to learn how to best organize information about specific resources. We propose here to use the preliminary work we've done with NYSCF as a starting point for working with the greater stem cell community to develop data annotation and publishing standards for greater uniformity across informatics platforms.

CANCER CELLS

T-1545

PHENOTYPIC AND FUNCTIONAL ANALYSIS OF BONE MARROW MESENCHYMAL STEM CELLS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Mesenchymal stem cells MSC are main cells found in bone marrow BM microenvironment. They play a major role in hematopoietic stem cell HSC niche. These niches support acute myeloid leukaemia AML cells proliferation and differentiation. Many studies showed the protection of AML cells from chemotherapy induced apoptosis by BM microenvironment. The mechanism of this protection is still unclear. MSC were obtained from the BM of patients with AML and healthy donors (n = 5 per group). Following parameters were used for: cell morphology, cell proliferation test, cell cycle, immunophenotype, differentiation capacity into osteoblastic and adipogenic lineages. Gene expression profile was determined by Q-PCR. 4/5 of AML BM-MSC have heterogenous morphology and 1/5 has homogenous fusiform, fibroblast-like appearance. AML BM-MSC show, significantly ($p<0.05$), low proliferative ability than normal BM-MSC, which induces an increase of doubling time (62,8 - / + 7h and 44,5 - / + 8h for AML and normal BM-MSC, respectively; $p<0,01$). The analysis of cell cycle show that the number of cells in AML BM-MSC cultures in G2/M phase, significantly $p<0.05$, reduced by 50% compared to normal BM-MSC cultures. However, Both of Normal and AML BM-MSC have similar immunophenotype profile (positive: CD90, CD73, CD105, CD166 and CD146; negative: HLA-DR, CD34, CD45). Both of cell types have a potential to differentiate into osteoblastic and adipogenic lineages showed by specific stains. This result was confirmed by the expression levels of Runx2, BMP2 and ALP Alkaline phosphatase (osteoblasts); and PPAR-γ2 (adipocytes). However, expression of these genes in osteoblasts derived from AML BM-MSC remains, significantly $p<0.01$, lower than the expression in osteoblasts obtained in normal BM-MSC differentiated cells. The proliferative capacity of MSC obtained from the BM of patients with AML are limited and have a heterogenous morphology although the expression of phenotypic markers remains unchanged compared to normal BM-MSC. More significantly, the capacity to differentiate into osteoblasts is reduced in AML BM-MSC. This result suggest that, the bone quality obtained by AML BM-MSC is affected. Therefore, there is an influence on the endosteal microenvironment and that could affect the behavior of CD34 HSC in the endosteal niche.

T-1547

REST-REGULATED MOLECULAR CIRCUITRIES IN THE CONTROL OF SELF-RENEWAL AND TUMORIGENIC COMPETENCE OF HUMAN GLIOBLASTOMA STEM CELLS

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REST (Repressor Element 1 Silencing Transcription factor) is a master repressor of neuronal programs in non-neuronal lineages. It has been shown to play a central regulatory role of developmental programs and stem cell physiology. Aberrant REST function has been associated with a number of pathological conditions and shown to play an oncogenic role in brain childhood malignancies such as neuroblastoma and medulloblastoma. Our previous studies demonstrated that REST is hihgly expressed in human glioblastoma multiforme (GBM) specimens, being particularly enriched in self-

renewing tumorigenic-competent GBM stem cells. Also, REST knock down in GBM stem cells strongly reduces their self-renewal in vitro and tumor-initiating capacity in vivo. Here we extend these results by showing that REST specifically controls a wide set of miRNAs in GBM stem cells by which he establish a molecular circuitry regulating the self-renewing and tumorigenic competence of stem cell compartment.

T-1548

A BIOBANK OF HUMAN GLIOBLASTOMA CELL CULTURES (HGCC) MODELING TUMOR HETEROGENEITY AND MOLECULAR SUBTYPES, USING STEM CELL CULTURE CONDITIONS

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Glioblastoma (GBM) is the most frequent and malignant primary brain tumor. Despite advances in understanding the molecular mechanisms of GBM, these tumors continue to be fatal. The concept of glioma stem cells (GSCs) has attracted a lot of interest, and a glioma-initiating cell bearing stem cell characteristic has been proposed, with the ability to seed new tumors through the capacity to evade chemotherapy and irradiation. Cancer research, including preclinical tumor models and testing of candidate drugs, needs optimized in vitro models that better reflect the patient's disease, including modeling of the cancer stem cell compartment. We have set up a biobanking effort to culture and characterize a new panel of cells, derived from surgical samples of GBM patients obtained with consent, based on a combination of two validated techniques for culturing GSCs. Here, we report on the establishment, genomic characterization and in vivo validation of 48 patient-derived GBM cell lines cultured under stem cell conditions, and provide evidence that they represent all four molecular GBM subtypes. We refer to this collection as the Human Glioblastoma Cell Culture (HGCC) resource, which is derived from Swedish patients during 2009-2012. In our recently published study, a number of these cell lines were screened to identify cellular processes amendable for development of targeted treatments. A small molecule, Vacquinol-1, induced GBM cell death, but spared normal cells. It displayed excellent in vivo pharmacokinetics and brain exposure, and attenuated GBM progression in two animal models. This is the first example of how the new platform can be used successfully towards novel therapeutic opportunities. The HGCC panel and its characterization data, as well as clinical variables, will be made available as a resource featuring a cell biobank and an online database for accurate modeling of GBM diversity. Our aim is to make HGCC an open source repository that will enable stratified studies of disease mechanisms, thereby facilitating the development of novel treatment modalities.

T-1549

EXPRESSION PATTERNS OF CANCER-TESTIS ANTIGENS OF MAGE FAMILIES IN MOUSE PLURIPOTENT STEM AND TERATOCARCINOMA CELLS

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Embryonic stem (ESCs) and embryonic germ cells (EGCs) are pluripotent while embryonic teratocarcinoma cells (ECCs) are their malignant counterparts. It has been shown that prolonged cultivation of pluripotent stem cells may lead to the accumulation of genetic and epigenetic alterations in the genome and increased risk of oncogenic transformation. Therefore, an important task is to find markers for the identification of transformed cells with the oncogenic potential in culturing populations of undifferentiated and differentiating pluripotent stem cells. Cancer-testis antigens (CTA) have specific expression patterns in cancer and normal adult somatic and germ cells, as well as in different embryonic cells. We suppose that changes in CTA profiles may be considered as marker for transformed and abnormally differentiated cells. In order to understand possible role of CTA expression in pluripotent stem and teratocatcinoma cells the gene expression profiles of Mage-a, Mage-b, Mage-d, Mage-e and Mage-l families were studied in undifferentiated and differentiating upon retinoic acid stimulation mouse ESCs, EGCs and ECCs. Quantitative real time PCR analysis showed that expression patterns of the Mage family genes Mage-a1,2,3,4,5,6,7,8, Mage-b1,3,4,5, Mage-d1,2, Mage-e1,2 and Mage-l2 were similar in undifferentiated ESCs, EGCs and ECCs. However, the expression levels of Mage-a2 and Mage-a6 were significantly higher in the undifferentiated nullipotent ECCs F9 than in pluripotent ESCs and EGCs. On the other hand, expression patterns of Mage genes studied were very similar in the ESCs, EGCs and both ECC lines differentiating after retinoic acid exposure. Correlations in expression levels of Mage genes and pluripotent and lineage markers, as well as C-myc and E-ras were also studied. Positive correlations of gene expression levels of Mage-a4 and were found for cells studied. We assume that Mage-a2 and Mage-a6 expressed at higher levels in nullipotent teratocarcinoma cells may be considered as markers for malignant pluripotent cells with abnormal differentiation potential.

T-1550

THE ONCOFETAL ARCHITECTURAL TRANSCRIPTION FACTOR HMGA2 REGULATES THE ATF4 - SERINE BIOSYNTHESIS PATHWAY AXIS AND REPROGRAMS CANCER METABOLISM

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The architectural transcription factor High Mobility Group A2 (HMGA2) is a central regulator of stem cell self-renewal, cell differentiation and proliferation. Deletion of HMGA2 results in pygmy mice with a markedly reduced number of adipocytes while mice that over-express HMGA2 are gigantic and prone to mesenchymal derived benign tumor lipoma, indicating that HMGA2

plays a role in generation of adipocytes from mesenchymal stem cells. HMGA2 is highly expressed during embryogenesis, whereas it is undetectable or expressed at low levels in normal adult tissue. However, HMGA2 is aberrantly re-expressed in the most aggressive subclasses of different cancer types, classifying this gene as a fetal oncogene. It has been demonstrated that HMGA2 is preferentially expressed in cells with breast cancer stem like cells (CSC) properties. Here, HMGA2 is associated with retaining the cells in an undifferentiated state and silencing of HMGA2 allows reduction of proliferation and differentiation of the CSCs. However, the molecular mechanisms are not fully elucidated. In this work we characterized the molecular pathways induced in an aggressive, triple negative breast cancer cell line MDAMB231 after treatment with HMGA2 siRNA. Global transcriptome profiling indicated that reduced HMGA2 levels induced a coordinated down-regulation of all the enzymes in the serine biosynthesis pathway, and also reduced the expression of ATF4, a known regulator of the serine biosynthesis pathway. Moreover, a reduced level of HMGA2 shifts the cells energy metabolism from glycolysis to oxidative phosphorylation.

T-1551

IMPLICATIONS OF THE NEUROGENIC NICHE ON BRAIN TUMOR PROGRESSION: THE SLIT ROBO AXIS ON THE MIGRATION OF HUMAN FETAL NEURAL PROGENITOR CELLS AND BRAIN TUMOR INITIATING CELLS

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Glioblastoma (GBM) cell migration in the brain parenchyma is one of the cardinal features that lead to the short survival of GBM patients. Some of the mechanisms that regulate the migration of cancer cells resemble normal brain development. Brain Tumor Initiating Cells (BTICs) share the properties of self renewal and multipotency with non-cancerous Neural Progenitor Cells (NPCs), with the added ability of tumor initiation. Studying the mechanisms that affect NPCs and BTICs migration will help us develop new therapeutic approaches for neurodegenerative medicine and cancer treatment, respectively. In rodents, the Slit-Robo ligand-receptor system helps in guiding neuroblasts from the subventricular zone (SVZ) to the olfactory bulb. This system also affects the migration of glioma and medulloblastoma cancer cell lines. Here we hypothesize that Slit proteins have a chemorepellant effect on the migration of human-derived fetal NPCs (hfNPCs) and BTICs. Primary cultures were established from intraoperative tissue and maintained in non-differentiating conditions in the presence of EGF, bFGF (20ng/ml each). We demonstrate that the human fetal SVZ expresses Robo1 and Robo2 and this expression is maintained in vitro by hfNPCs. Robo1 and Robo2 expression is also present in BTICs in vitro as demonstrated by immunocytochemistry and western blot. The stimulation of hfNPCs and BTICs with slit2 exerted a chemorepellant effect, as determined by transwell migration and timelapse chemotactic assays. This effect was accompanied by an increase in cell speed. To determine the role of Robo1 in this response, we knocked down the expression of this receptor using shRNA-carrying lentiviral particles. The effects of Slit on hfNPCs and BTICs migration were significantly decreased in Robo1-KD cells, indicating that the expression of this receptor is necessary for the chemorepellant response. Intracellularly, we demonstrate that the activity of the Rho-GTPases, Rac and CDC42, significantly decrease upon Slit stimulation. Together, these results indicate an evolutionarily-maintained role of Slit proteins on the migration of hfNPCs that could have great implications during brain development. Furthermore, this mechanism affects the migration of human GBM BTICs, suggesting a role of this system in the malignancy of brain tumors.

T-1552

UNDERSTANDING HOW TUMOR CELL CLONES COMMUNICATE IN GENETICALLY HETEROGENEOUS GLIOMAS

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Glioblastoma is the most frequent and aggressive brain tumor in adults. Intratumoral heterogeneity is a hallmark of glioblastomas and has been suggested to be a contributing factor to their aggressiveness and low response to treatment. Gene expression based analysis classifies glioblastomas into four subclasses: proneural, neural, classical and mesenchymal. A recent publication showed that individual glioblastomas may contain cells that display gene expression pattern characteristic of these four subclasses in a heterogeneous manner. Further, it has been shown that genetically different tumor cell subclones in glioblastoma may have profound effects on overall tumor growth. Glioma cells with mutated EGFR secrete IL-6 and LIF, which support the growth of surrounding tumor cells with wild type EGFR. We aim to identify further ways that heterogeneous tumor cell populations affect each other's growth. For this we have used the U343 system that consists of cell clones derived from a single glioblastoma tumor: U343MG, U343MGa, U343MGa 31L and U343MGa Cl2:6. U343MG express FN1 and have a mesenchymal morphology, whereas the other clones express GFAP and display astroglial characteristics. We have identified that the U343MG have an invasive capacity and express further mesenchymal markers. We have performed co-culture and conditioned media experiments and found that the clones affect each other's growth via secreted factors. We are performing Secretome Protein Enrichment with Click Sugars (SPECT) in combination with functional genomic experiments to find the genes responsible for this interclonal signaling. Further, the cell clone that will appear at the lowest fraction when cultured with all the other clones have a lower sensitivity towards temozolomide and may thus illustrate an example of how tumor recurrence may occur after drug treatment. These experiments may prove an effective way to study ongoing cell-to-cell communication in the heterogeneous tumors and tumor recurrence after chemotherapy due to tumor heterogeneity.

T-1553

AUTOCRINE PROGASTRIN SECRETION PROMOTES COLON CANCER STEM CELL SURVIVAL AND SELF-RENEWAL

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Subpopulations of cancer stem cells (CSCs) are thought to drive tumor progression and post-treatment recurrence in multiple solid tumors. Here, using human cell lines and cells isolated from tumor biopsies, we show that progastrin expression and secretion was enriched in colon CSCs and promoted their self-renewal. Progastrin also promoted the survival of CSCs with high Aldehyde Dehydrogenase (ALDHhigh) activity, and siRNA or antibody-mediated progastrin inhibition altered the homeostatic proportions of these cells within heterogeneous colorectal cancer (CRC) cell populations. Progastrin down-regulation in human CRC cell xenografts robustly decreased tumor-initiating frequency and impaired ALDHhigh cell tumor-initiating potential. Finally progastrin down-regulation inhibited the high glycolytic activity characterizing ALDHhigh CSCs, switching their mitochondrial activity towards oxidative phosphorylation and thereby decreasing their self-renewing ability. Our results demonstrate that progastrin secretion by colorectal CSCs drives a feed-forward auto/paracrine loop that promotes their survival, self-renewal and tumor-initiating abilities and controls their metabolic profile.

T-1554

A NEW ISOFORM OF HISTONE VARIANT H3.3 IN PEDIATRIC GLIOMAS

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Over 80% of pediatric gliomas, including pediatric diffuse intrinsic pontine glioma (DIPG) correlate with somatic mutations in the *H3F3A* gene, which encodes histone H3 variant H3.3. H3.3 mutations include H3.3K27M and G34R/V, with 70% of DIPG harboring the K27M mutation. ES cell-derived neuronal progenitors (NPCs) expressing H3.3K27M undergo transformation and display an epigenetic signature resembling that of H3.3K27M-DIPG tumors. We have characterized the level of expression of several histone post-translational modifications (hPTMs) in H3.3K27M-DIPG cell lines. We find that global H3K27me3 is reduced, in line with previous reports. We also identify a previously uncharacterized isoform of H3.3. Expression of an epitope-tagged H3.3K27M construct in HeLa cells and non-DIPG NPCs leads to detection of the new isoform. Quantitative microscopy shows that this isoform is incorporated into chromatin, albeit with a kinetics slower than the main H3.3 isoform. Additionally, expression of H3.3 truncated of its N-terminal tail (H3.3[core]) results in long-term residency in PML nuclear bodies and in chromatin loading defects in slowly-dividing mesenchymal stem cells, presumably due to altered interaction with chaperones and/or defective PTMs. Chaperone routing at the level of PML nuclear bodies together with PTMs of the N-terminal tail of H3.3 constitute key regulating steps of H3.3 loading on chromatin, and may have large-scale implications on gene regulation and tumorigenic properties in DIPG tumor cells.

T-1555

MOLECULAR AND CELLULAR CHARACTERIZATION OF INDUCED CANCER STEM CELL-LIKE CELLS

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Cancer stem cells (CSCs) reside at the apices of hierarchies, and have been directly implicated in the maintenance and progression of various types of human cancer. Recently, it was reported that a population of a primary tumor and the oncogenes-expressing primary fibroblasts can reconstitute the original tumor on xenotransplantation. Here, we show that the immortalized human epithelial cell lines were converted to CSC-like cells using our new in vitro culture system. The CSCs induced through an epithelial-mesenchymal transition (EMT) program resulted in the acquisition of mesenchymal traits, the expression of stem cell markers, such as CD133, CD15, c-Myc and TERT. In addition, we compared the gene expression profiles between the induced CSCs and cancer cells by microarray experiments. These results showed the immortalized epithelial cells were successfully converted to CSCs, suggesting that a certain pre-cancerous cell in microenvironments may be changed to CSCs in vivo. *Supported by Ministry of Science, ICT and Future Planning (2012M3A9C6050131 and 20100020349) and the Ministry of Health and Welfare (A110606 and HI14C3477) Grants*

T-1556

MIR-302 AND MIR-145 IN ESOPHAGEAL CANCER

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MicroRNAs are involved in regulating key cellular processes, and their mis-regulated expression has been linked to various pathological conditions including cancers. Cluster of miR-302-367 is exclusively expressed in embryonic stem and carcinoma cells, promoting "stemness" and "reprogramming" states of the cells. In contrast, miR-145 is mostly regarded as a tumor suppressor, where it regulates cellular functions such as cell division, differentiation, and apoptosis. By suppressing the main pluripotency and self-renewing factors (OCT4, SOX2, Myc and KLF4), miR-145 silences the self-renewal program in ESCs. So, the main aim of the current study was to find a potential link between the expression level of hsa-miR-302b and hsa-miR-145 with tumor vs. non-tumor as well as high-grade vs. low-grade states of the esophageal tissue samples. A total number of 40 formalin-fixed, paraffin-embedded (FFPE) samples of esophageal squamous-cell carcinoma (ESCC) were obtained and the tumor and marginal non-tumor areas were delineated and punched off by an expert pathologist. Real-time reverse transcription polymerase chain reaction (RT-PCR) assays were performed using specific LNA-

primers and SYBR Green master mix. The expression level of miR-302b failed to show any significant difference neither between tumor and their non-tumor counterparts, nor among tumors with different grades of malignancies (P>0.05). In contrast, miR-145 was significantly down-regulated in all grades of tumor samples (P=0.001). However, its expression level could not discriminate between different grades of malignancy (P>0.05). In conclusion, our data revealed a significant down-regulation of miR-145 in ESCC tissue samples. This result is in accordance with our previously data. We discovered a significant up-regulation of OCT4A, SOX2 in tumor samples, compared with the non-tumor tissues in fresh frozen tissue samples of ESCC (P=0.001). Furthermore, based on ROC curve analysis data (AUC=0.74, P= 0.001) and considering the high stability and ease of detection of miR-145 in FFPE samples, it seems that evaluating the expression of miR-145 in tumor tissues, and probably serum or other body fluids of patients, has a potential usefulness as a reliable molecular biomarker for diagnosis of ESCC.

T-1557

SOX5, -6 AND -21 ACT TO SUPPRESS TUMOR FORMATION IN A GLIOMA MOUSE MODEL

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Sox5, Sox6 and Sox21 (Sox5/6/21) normally act to promote neural stem cells to exit cycle and differentiate. In cultured human glioblastoma cells, Sox5/6/21 promote cell cycle exit and prevent tumor generation in NOD-SCID mice. Conversely, the ability of the oncogenes H-Ras and AKT to induce glioma in mice is greatly enhanced upon loss of Sox5/6/21. The proliferative advantage seen both in vivo and in vitro, could be explained by high levels of Cyclins and phosphorylated Rb together with low levels of p53, p21 and p27. In accordance with this, p53 or P27 reduced the tumor size in our glioma model.

T-1558

NEURONAL ACTIVITY-REGULATED SECRETION OF NEUROLIGIN3 PROMOTES GLIOMA GROWTH

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Active neurons exert a mitogenic effect on normal neural precursor and oligodendroglial precursor cells, the putative cellular origins for high-grade glioma (HGG). We demonstrate that active neurons similarly promote HGG proliferation and growth in vivo using optogenetic control of cortical neuronal activity in a patient-derived pediatric glioblastoma orthotopic xenograft model. Activity-regulated mitogen(s) are secreted, as the conditioned medium from optogenetically stimulated cortical slices promoted proliferation of pediatric and adult patient-derived HGG cultures. The synaptic protein neuroligin-3 (NLGN3) was identified as the leading candidate mitogen; soluble NLGN3 was sufficient and necessary to promote robust HGG cell proliferation. NLGN3 induced PI3K-mTOR pathway activity and feed-forward expression of NLGN3 in glioma cells, providing mechanistic insight into its surprising role as a mitogen. NLGN3 expression levels in human HGG negatively correlated with patient overall survival. These findings indicate the important role of active neurons in the brain tumor microenvironment and identify secreted neuroligin-3 as an unexpected mechanism promoting neuronal activity-regulated cancer growth.

T-1559

APPLICATION OF IPSC TECHNOLOGY TO CANCER STEM CELL RESEARCH: GENERATION OF INDUCED CANCER STEM CELLS FROM COLON CANCER CELLS BY INTRODUCING OCT3/4, SOX2 AND KLF4

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Cancer stem cells (CSCs) are considered to be responsible for the dismal prognosis of cancer patients. To develop new treatments targeting CSCs, it is important to elucidate the molecular mechanisms underlying the acquisition of CSC properties in CSCs. However, these are still unclear, because CSCs are a rare population of cells in cancer tissue, and the rarity of the CSCs makes it difficult to identify and collect them. Thus generating CSCs in vitro from cancer cells and investigating their characteristics is considered to be a useful method for overcoming this problem. This study aimed to generate induced CSCs (iCSCs) from colon cancer cells by introducing defined factors. We retrovirally introduced a small set of transcription factors: OCT3/4, SOX2 and KLF4 (OSK) into human colon cancer cells, followed by culture with 10%FBS-DMEM, not human embryonic stem cell medium. We then evaluated the CSC properties in the cells. A subfraction of the colon cancer cells transduced with OSK factors showed significantly enhanced CSC properties in terms of the marker gene expression, sphere formation, chemoresistance and tumorigenicity. Moreover, we established a novel technology to collect the iCSCs based on the differences in the degree of the dye-effluxing activity enhancement. In microarray analysis, the collected iCSCs showed unique gene expression pattern, compared to control cells. The xenografts derived from our iCSCs were not teratomas. Notably, in contrast to the tumors from the parental cancer cells, the iCSC-based tumors recapitulated actual human colon cancer tissues in terms of their immunohistological findings, which showed colonic lineage differentiation. In addition, our iCSCs showed self-renewal capacity in serial transplantation experiments. We were able to generate colon iCSCs from colon cancer cells by forced expression of OSK, and collect the iCSCs. The iCSCs-based cells and tissues were similar to actual human colon cancer tissue. By overcoming the sampling limitations of primary human CSCs and by dynamic observation of the CSC development, this method will enable us to elucidate the molecular mechanisms involved in the development and maintenance of CSCs, and will help to establish new therapies and diagnostic technology targeting CSCs.

T-1560

HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS INHIBIT MELANOMA GROWTH IN VITRO AND IN VIVO.

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The effects of adipose tissue-derived mesenchymal stem cells (AT-MSCs) on the growth of human malignancies, including melanoma, are controversial and the underlying mechanisms are not yet-well understood. The aim of the present study was to investigate the in vitro and in vivo anti-tumor effects of human AT-MSCs on human melanoma. The inhibitory effect of AT-MSC-conditioned medium (AT-MSC-CM) on the growth of A375SM and A375P (human melanoma) cells was evaluated using a cell viability assay. Cell-cycle arrest and apoptosis in melanoma cells were investigated by flow cytometry and western blot analysis. To evaluate the in vivo anti-tumor effect of AT-MSCs, CM-DiI-labeled AT-MSCs were circumtumorally injected in tumor-bearing athymic mice and tumor size was measured. AT-MSC-CM inhibited melanoma growth by altering cell-cycle distribution and inducing apoptosis in vitro. AT-MSCs suppressed tumor growth in tumor-bearing athymic mice and fluorescence analysis showed that AT-MSCs migrated efficiently to tumor tissues. AT-MSCs inhibit the growth of melanoma suggesting promise as a novel therapeutic agent for melanoma.

T-1561

CHEMORESISTANT LEUKEMIC STEM CELL EXPANSION IS INHIBITED BY TARGETING WNT/PI3K SELF-RENEWAL SIGNALING COOPERATION

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Cancers typically contain chemoresistant cells that are responsible for treatment failure, but the nature of these cells and how to target them remains enigmatic. Since the Wnt/β-catenin pathway synergizes with the PI3K/Akt pathway to provide the essential components of self-renewal, i.e. proliferation while preventing both differentiation and apoptosis in stem cells, these pathways can be hijacked by tumorigenic cells using oncogenic self-renewal to maintain and regenerate cancers. We tested whether inhibition of this cooperation could selectively eliminate leukemic stem cells (LSCs). Since Akt activates β-catenin by phosphorylation at serine 552 and thus represents a molecular link mediating the synergistic activity of these pathways, we sought to specifically target pS552-β-catenin. Unexpectedly, high-throughput screening (HTS) and subsequent validation assays found that doxorubicin (DXR) inhibits pS552-β-catenin with minimal effects on total β-catenin. DXR exhibits the broadest spectrum of anti-cancer activity known and has been employed as a standard chemotherapeutic agent for decades, but severe side-effects limit its use. We found that while standard chemotherapeutic treatment reduced the bulk leukemic blast cells as expected, it also induced pS552-β-catenin specifically in LSCs and stimulated LSC expansion. However, employing DXR as a targeted therapy rather than a broadly cytotoxic agent by using very low and sustained doses, reduced pS552-β-catenin levels in LSCs, prevented LSC expansion, essentially eliminated LSC tumorigenic activity, and was accompanied by recovery of hematopoietic stem and progenitor cells (HSPCs). Our findings uncover a dichotomous response between bulk leukemic cells and LSCs to standard and targeted therapies and reveal how chemoresistant LSCs can be targeted separately not only from the bulk of the tumor but also from HSPCs. Notably, we show that binary targeting of chemoresistant LSCs by inhibition of pS552-β-catenin dependent oncogenic self-renewal and bulk leukemic blasts by cytotoxic chemotherapy is necessary for optimal survival.

T-1562

GENERATING INDUCED CANCER STEM CELLS FROM HUMAN COLON CANCER CELLS USING A TRANSIENT EXPRESSION SYSTEM FOR DEIFNED FACTORS

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Cancer stem cells (CSCs) are regarded as a promising therapeutic target. However, little is known about the molecular mechanisms underlying the acquisition and maintenance of CSC properties in CSCs. We could generate induced colon CSCs (iCCSCs) from SW480 cells (a colon cancer cell line) by the forced expression of OCT3/4, SOX2 and KLF4 (OSK) using retroviral vectors, which is a continuous expression system for OSK. The iCCSCs showed a high dye efflux ability, and we thus were able to collect the iCCSCs by flow cytometer. In that report, however, whether the continuous expression of the exogenous OSK is needed for the maintenance of the CSC properties in the iCCSCs could not be clarified. To address the issue, it is necessary to establish iCCSCs using a transient expression system for OSK, thereby allowing us to control the exogenous OSK expressions in the iCCSCs. This study aimed to establish colon cancer cells that transduced a transient expression system for OSK, while also investigating whether CSC properties could be induced in SW480 cells using this system. To set up such a transient expression system, we lentivirally transfected TET-O-FUW-OSK and FUW-M2rtTA vectors into SW480 cells. The transduced cells (T-OSK-SW480) were then cultured in 10%-DMEM with or without Doxycycline (Dox) (2 μg/ml). We analyzed these cells in terms of their CSC properties, especially regarding their dye efflux and organoid formation abilities. RT-PCR showed that the T-OSK-SW480 cells expressed the mRNA of transduced OSK depending on the presence of Dox. In the presence of Dox, the T-OSK-SW480 cells exhibited a morphology that was similar to that observed in our previous reported iCCSCs. Both the dye efflux ability and organoid formation were found to increase in the T-OSK-SW480 cells in the presence of Dox, compared the control cells. These results suggest that T-OSK-SW480 cells acquired some CSC properties in the presence of Dox. In future studies, we will perform further experiments and also investigate whether a continuous presence of the exogenous OSK is needed for T-OSK-SW480 cells to maintain their CSC properties. This method will therefore help us to understand and investigate the molecular mechanisms underlying the maintenance of CSC properties in our iCCSCs, leading to find novel therapeutic targets of CSCs.

T-1563

ONCOGENE EXPRESSION STABILIZES CANCER CELL IDENTITY, REVEALED BY CANCER CELL REPROGRAMMING

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Reprogramming is accompanies by dynamic changes of epigenetic modifications and is therefore considered to be a useful tool to induce global epigenetic alteration in cancer genome. It is well known that the efficiency of cancer cell reprogramming is considerably low compared with non-cancer somatic cells. It suggests that transcriptional network of cancer cell is robustly sustained and plays a critical role to maintain cancer cell identity. To reveal what is stabilized cancer cell identity, we utilized cancer cell reprogramming by the forced expression of reprogramming factors (Oct3/4, Sox2, Klf4 and Myc). In this study, we tried to reprogram mouse clear cell sarcoma (CCS) cell line which harbours doxycycline (Dox) -inducible EWS/ATF1 oncogene. We found a negative correlation between reprogramming efficiency and oncogene expression level. Transcriptional suppression of the EWS/ATF1 resulted in the successful generation of Nanog-positive iPSCs from the CCS cells, which are competent to participate in the development of chimeric mice, whereas sustained expression of EWS/ATF1 lead to abrogated reprogramming of the CCS cells. We hypothesize that oncogene expression robustly maintains the transcriptional network of cancer cells and leads to stable epigenetic regulations. Based on this hypothesis, we are currently trying to elucidate the effect of oncogene expression on epigenetic state in cancer cells to understand the mechanism for stable maintenance of cancer cell identity.

T-1564

THE LIN28/LET7 PATHWAY REPROGRAMMING TO STEM LIKE STATE IN ORAL CANCERS BY CONTROLLING EXPRESSION OF OCT4 AND SOX2

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Lin28 is one of the key factors for cellular reprogramming to generate induced pluripotent stem cells (iPSCs), and has been shown to play a critical role in tumorigenicity through suppressing Let-7, the most well reported tumor suppressive micro RNA and is known as a downstream negative effector of Lin28. However, it is unclear whether Lin28 play critical role in inducing cancer stem cell (CSC) properties in oral cancer. In this study, we demonstrated the expression of Lin28, Oct4, and Sox2 are up-regulated in high grade oral cancer and CSC-like CD44+ALDH1+ oral cancer cells, while Let7 is down regulated, comparing to the low grade tumor and non-CSC CD44-ALDH1- cells. Overexpression of Lin28 or knockdown of Let7 in non-CSC cells significantly increased the ability of sphere formation and tumor initiation in vivo, as well as enhanced the expression of endogenous Oct4 and Sox2. In CSC-like cells, knockdown of Lin28 by RNA silencing significantly suppressed the CSC-like capability and mRNA expression levels of Oct4 and Sox2. Furthermore, the knockdown of Let7 effectively reversed the suppressed CSC activity and Oct4/Sox2 expression in non-CSC or CSC-like -sh-Lin28 cells. Notably, Bioinformatics analysis and reporter assay identified ARID3B and HMGA2 as direct targets of Let7. We further showed direct binding of ARID3B and HMGA2 on the promoter regions of Oct4 and Sox2, respectively, by chromatin immunoprecipitated assay. Co-knockdown of Oct4 and Sox2 suppressed the tumorigenicity of CSC-like cells and non-CSC cells with Lin28-knockdown. Clinically, oral cancer patients with a Lin28highOrid3bhighHMGA2highOct4highSox2highLet7low phenotype had a worse prognosis and correlates with more frequent tumor recurrence incident. Most importantly, therapeutic delivery of Lin28 shRNA or Let7 in oral CSCs or recurrent oral cancer cells effectively reduced its lethality and prolonged the survival time in orthotropic-transplanted mice. This study reported a Lin28/Let7 pathway-dependent regulation of stemness genes, Oct4 and Sox2, and tumor-initiation properties in oral cancer though ARID3B and HMGA2.

T-1565

CHARACTERIZATION OF PATIENT BREAST CANCER STEM CELLS ACCORDING TO THEIR EMT PHENOTYPE AND METASTATIC POTENTIAL

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Breast cancer is the second most common cancer in the world and the most frequent cancer among women. Studies suggest tumor initiation, growth and metastasis in breast cancer are driven by so-called cancer stem cells (CSCs). We have established an isolation method to extract breast cancer stem cells from patient tumor tissue and to cultivate them in vitro. We mechanically and enzymatically disaggregate the tumor tissue and seed the cells in a 3-dimensional (3D) culture system under hypoxic conditions. After sphere formation in 3D leading to enrichment of CSCS, the cells are expanded and analyzed in 2D as well. Using this approach, we established nine different BCSC lines. All of them initially retain tumor heterogeneity in the dish and contain subpopulations exhibiting myoepithelial and luminal characteristics as analyzed by their expression of cytokeratins 5/14 and 8/18, respectively. The cells are able to form colonies from single cells in 2D and 3D. Furthermore, the CSC ability of self-renewal was confirmed by engrafting cultured cells into the mammary fat pad of NOD/SCID mice in limiting dilutions. There, all nine cell lines are able to form tumors that recapitulate the original patient´s tumor. Weinberg and others have identified the epithelial-mesenchymal transition (EMT) as a unique trait and prerequisite of CSC identity. We have previously shown that CSCs exist in both states, epithelial and mesenchymal, and we have identified an epithelial CSC state as a precursor to the mesenchymal fate. Epithelial CSCs form mostly solid 3D colonies and can undergo an EMT to become migratory and invasive. We will use these features to predict aggressiveness and metastatic behavior of a given patient tumor in vitro. In future experiments our cell lines will be analyzed in accordance to their epithelial or mesenchymal status as well as their ability to undergo EMT. An EMT marker profile of the cells will be established and their migratory and invasive behavior will be evaluated in gap closure assays and Boyden chamber assays. Additionally, metastasis formation in vivo will be analyzed. Taken together, our cell lines provide a promising tool to study CSCs and EMT and will possibly allow us to predict disease progression in the future.

T-1566

DEVELOPMENT OF A SCREENING PLATFORM TO IDENTIFY DRUGS THAT REPROGRAM PANCREATIC CANCER CELLS

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Patients with Pancreatic Ductal Adenocarcinoma (PDA) have a 73% chance of dying within their first year of diagnosis, thus making it one of the most deadly cancers known. PDA's poor prognosis warrants a desperate need for further research, understanding, and therapies. Previous studies have shown that PDA arises from digestive enzyme producing acinar cells due to a Kras mutation. The cells undergo acinar-ductal metaplasia and become extremely proliferative. Furthermore, our lab has shown that in PDA, expression of basic helix-loop-helix (bHLH) transcription factors is lost, while their inhibitor Id3 is over-expressed. Together, the data suggest that bHLH signaling is highly dysregulated in PDA, leading us to investigate whether restoring bHLH activity would return the cancer cells back into their quiescent acinar state. Remarkably, we discovered this to be true in multiple pancreatic cancer cell lines. By inducing bHLH activity, we were able to reprogram the cells into a quiescent acinar fate. This was characterized by the expression of acinar digestive enzymes including trypsin and cell cycle inhibitors including P21. Having found a genetic basis for reprogramming pancreatic cancer cell fate, we endeavored to translate these findings for clinical utility. Therefore, we developed a novel high-throughput screening assay consisting of multimerized bHLH binding domains driving luciferase for testing the ability of small molecules to induce bHLH expression. An initial drug screen on a library of kinases showed that the drug Triciribine (TCN) is a weak inducer of bHLH activity. As a result, we are testing a number of TCN analogs to determine if changes in functional groups can induce more significant bHLH expression. To conclude, we have identified a pathway that controls pancreatic cancer cell growth and cell fate. Current efforts are aimed at developing a drug that would modulate this pathway and exhibit promise as a potential therapy.

T-1567

IN-VIVO QUANTITATIVE LEUKEMIC CELL CYCLE IMAGING USING INFRARED REPORTERS

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Leukemic stem cells (LSCs) are known to be quiescent and have the ability to self-renew throughout the body. Despite the importance of tracking cancer stem cells in live animals, *in-vivo* cell cycle kinetics have not been reported and the behavior of LSCs in the body is not fully understood. Recently, we identified two Infrared Fluorescent Proteins (IFPs) that absorb and emit wavelengths high enough to allow penetration through tissue. The first protein, smuRFP, was generated by isolating Allophycocyanin from red algae and undergone accelerated evolution in E. coli. The second protein, Infrared Fluorescent Protein 2 (IFP2), was derived from bacterial phytochrome and was engineered for enhanced brightness. These proteins were tagged with cell cycle regulated proteins from the fluorescence ubiquitination cell cycle indicator (FUCCI) system. smuRFP is an Infrared Fluorescent Protein that is fused to hCdt1 and is expressed in G1 phase of the cell cycle. IFP2 is tagged to hGeminin and is present in the S/G2/M phase of the cell cycle. This allows us to visually quantify cell cycle progression *in-vivo*. A human cancer cell line (SKNO-1 acute myeloid leukemia) was transduced with lentivirus to express IFP reporters. These cells received puromycin selection post transduction. Stable SKNO-1 cell line expressing smuRF were generated and will undergo a second transduction to express IFP2. Our goal is to create an SKNO-1 stable cell line expressing both IFP reporters to quantify cell cycle kinetics in real time. The transduced SKNO-1 cell line will be injected into the mice for further quantification of cell cycle progression *in-vivo*. This will help us understand leukemic stem cell quiescence, migration and tumor formation within the animal model.

T-1568

RETAINED CAPACITY TO EXPRESS CHD5 DETERMINES THE ABILITY FOR NEURONAL DIFFERENTIATION IN NEUROBLASTOMA

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The chromatin remodeler CHD5 has been proposed to be a key tumor suppressor at 1p36, a locus frequently exhibiting loss of heterozygosity (LOH) in tumors of neural origin, such as neuroblastoma. Our previous work revealed that CHD5 facilitates repression of Polycomb target genes, activation of neuronal genes and is required for terminal neuronal differentiation in the developing neocortex. One consequence of CHD5 depletion is an accumulation of undifferentiated progenitors. Here we demonstrate that retained capacity to express CHD5 determines the competence of human neuroblastoma cells to differentiate in response to retinoic acid treatment both in vitro and in a xenograft mouse model. We furthermore show that in the more malignant neuroblastoma cells, exhibiting 1p36 LOH, the capacity to express CHD5 from the remaining allele can be restored through demethylation agents. This treatment restores the tumor cells' potential for terminal neuronal differentiation in response to retinoic acid treatment, paving the way for chemotherapeutic intervention. Thus, loss of CHD5 in pediatric neuroblastoma couples its role as a tumor suppressor to its essential endogenous function in the generation of post-mitotic differentiated neurons from neural progenitors.

FRIDAY, 26 JUNE, 2015

Poster Presentations

18:00-19:00 ODD numbered posters presented
19:00-20:00 EVEN numbered posters presented

PRE-CLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL CELLS

F-1001

OBTAINING MESENCHYMAL CELLS FROM UMBILICAL CORD BLOOD CRYOPRESERVED CULTURES WITH LYSATE PLATELET RICH PLASMA

Avila Portillo, Luz Mabel, Bonilla, Marco M., Reyes, Leidy, Franco, Derly J., Avila Garcia, Jenniffer Priscila J., Riveros, Angela G., Carmona, Jose Vicente

Regenerative Medicine Stem Lab, Bogota, Colombia

There are gaps in the knowledge around obtaining mesenchymal cells (MSC) from units of cord blood (UCB), as well as the optimization of the cultivation for the expansion of MSC from cryopreserved UCB. The object of this work was to isolate and expand cryopreserved MSC cultivated with lysate platelet-rich plasma (LPRP). An experimental laboratory study was conducted. After signing the informed consent, cord blood (CB) was collected from maternal service gynecology patients in the Central Military Hospital from January 2012 to October 2014. It was then processed, cryopreserved for three months and then thawed. Cell viability was then verified pre- and post-freezing. Once thawed the CB was cultured in a DMEM LG medium supplemented with LPRP, flow cytometry for CD34, CD45, CD73, CD90 and CD105 was performed, and an osteogenic differentiation was induced in the adherent cells. Finally a statistical analysis of mean difference was applied in order to test the determinants of growth. 21 CB samples were collected; the average pre-frozen viability was 98.6% with D.E. 2.99, with a decrease of 26.37% after thawing. MSC growth was achieved in 57.14%. When comparing the count of nucleated cells, the viability of pre-freezing and post-thawing versus the obtainment of MSC from UCB, there was no significant difference. This study found 57.14% of adherent cells obtained from cryopreserved UCB, cultured with DMEM-LG+LPRP.

F-1002

TELOMERASE OVEREXPRESSION IN HUMAN MESENCHYMAL STEM CELLS OFFERS PROTECTION AGAINST OXIDATIVE DNA DAMAGE ACCUMULATION

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Adult stem cells and especially human mesenchymal stem cells (hMSCs) are currently used in several cell-based therapies. Their use in such therapies requires in vitro culture, during which however, they quickly reach replicative senescence. Replicative senescence has been linked to macromolecular damage and especially reactive oxygen species (ROS)-induced damage. The most frequently occurring oxidized DNA lesion is 8-oxo-2-deoxyguanosine (8-oxo-dG) the incorporation of which to the DNA can cause double strand breaks (DSB), characteristic features of cellular senescence. It has been shown that overexpressing of telomerase reverse transcriptase (TERT) counteracts telomere shortening and prevents replicative senescence. Moreover, there is recent evidence for telomere length independent functions of telomerase, which appear to promote cellular resistance against oxidative stress. Here, we studied the senescence as well as the response to external acute oxidative stress of hMSCs from adipose tissue. We also generated hMSCs overexpressing the telomerase catalytic subunit (hTERT). Control and hTERT overexpressing hMSCs were cultivated until they reached senescence and they were exposed to H2O2 at several stages (early passages, middle passages, and senescence reaching passages). DNA damage was assessed by confocal microscopy, utilizing an antibody for 8-oxo-dG as well as antibodies against the gamma-H2AX (γH2AX) histone and p53-binding protein 1 (53BP1), both of which are found on DSBs foci. We demonstrated that as cells senesce accumulate DNA damage and that senescent cells are unable to repair the damage induced by exogenous oxidative stress. Additionally, we showed that hMSCs overexpressing hTERT show no difference as compared with the control cells in the amount of damage they accumulate due to the external oxidative exposure. However, senescence reaching hTERT overexpressing cells show significantly lower percentages of oxidative DNA lesions and DSB as compared to control cells of the same passage. This means that hTERT offers a significant protection against the DNA damage that accumulates in the nuclei of the cells during normal cellular ageing. These results offer novel insights regarding the limitations of ex vivo amplified cell preparations for therapeutic applications.

F-1003

PROFOUND ACTIONS OF AN AGONIST OF GROWTH HORMONE RELEASING HORMONE ON ANGIOGENIC THERAPY BY MESENCHYMAL STEM CELLS

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Clinical trials of mesenchymal stem cell (MSC) therapy have shown promise for the treatment of cardiovascular disease including heart failure and critical limb ischemia. However poor cell survival and engraftment into host tissues limits efficacy. Growth hormone-releasing hormone (GHRH) regulates growth and development through pleiotropic actions on multiple target cell and tissue types. Here we studied the effect of the GHRH agonist, JI-34, on MSC survival and angiogenic therapy in a mouse model of critical limb ischemia. Treatment of MSCs with JI-34 improved MSC viability and mobility and markedly enhanced endothelial tube formation in vitro. These effects were paralleled by increased phosphorylation and nuclear translocation of STAT3. In vivo, JI-34 pre-treatment enhanced the engraftment of MSCs into ischemic hindlimb muscles and augmented reperfusion and limb salvage compared with untreated MSCs. Significantly more vasculature and proliferating CD31+ and CD34+ cells were detected in ischemic muscles that received MSCs

treated with JI-34. Our studies demonstrate a novel role for JI-34 to markedly improve therapeutic angiogenesis in hindlimb ischemia by increasing the viability and mobility of MSCs. Our findings support a role for GHRH agonists in preconditioning of stem cells, prior to transplantation, to enhance cell survival, and homing, and related therapeutic activity.

F-1004

IS THE POTENCY OF ALL MESENCHYMAL STEM CELLS AFFECTED BY CRYOPRESERVATION?

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It has become accepted that the cryopreservation of MSCs impairs their therapeutic properties. Though some recent studies are beginning to refute this theory (at least in GMP-grade cells), this could have serious implications for the future of cellular therapies. We and others have found previously, that not all MSCs behave the same. Indeed, cells from different sources may have different phenotypes and, therefore, different therapeutic benefits. Since the published cryopreservation studies have only assessed bone marrow derived MSCs, we hypothesised that MSCs from other sources may be affected differently by cryopreservation. In this work, we compared cultured and freeze-thawed umbilical cord tissue derived cells (UCX®) for their phenotype, including their in vitro and in vivo immunomodulatory and pro-angiogenic properties. Cell phenotype was not affected by cryopreservation in terms of surface expression of MSC markers. In order to study the impact of cryopreservation in the immunomodulatory properties of UCX®, we tested the cells for their ability to inhibit T cell activation in vitro and found no significant differences between cultured and freeze-thawed cells. In addition, in an in vivo model of adjuvant induced arthritis (AIA), cultured and freeze-thawed cells were equally able to reduce the arthritic index and paw volume over a period of 64 days. The ability of cultured and freeze-thawed UCX® to form tubes was compared in an in vitro matrigel angiogenesis assay and no significantly differences were found. To further assess the effect of cryopreservation in perfusion recovery, a mouse model of hindlimb ischemia was used. After ischemia induction, cultured and freeze-thawed UCX® were injected in the ischemic muscle and laser Doppler analysis was performed. Similar levels of blood flow were observed in both experimental conditions, at day 21 post-ischemia. Interestingly, these values were significantly higher when compared to the control group, suggesting that UCX cells induce capillary or collateral development after ischemia. Taken together, our results show that unlike what has been described for BM-MSCs (RD grade), the immunomodulatory and angiogenic potential of umbilical cord tissue derived MSCs (UCX®) is not impaired following cryopreservation.

F-1005

BONE MARROW CONCENTRATE AND PLATELET RICH PLASMA DIFFER IN CELL DISTRIBUTION AND INTERLEUKIN 1 RECEPTOR ANTAGONIST CONCENTRATION

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Bone marrow concentrate (BMC) and platelet rich plasma (PRP) are biologics used extensively in sports medicine, but differences in their cellular and molecular composition has not been determined. This study compared the cellular distribution, anabolic and catabolic cytokine concentrations in BMC and PRP from the same patient cohort. The results of this study will provide important clinical recommendations when choosing regenerative medicine approaches to treat musculoskeletal injuries. Patients (19) undergoing orthopedic surgery were enrolled. Bone marrow aspirate (BMA) from the iliac crest was processed to generate BMC using a commercial system. Blood was obtained to make PRP utilizing the same system as for BMC. Bone marrow samples were cultured to measure colony forming units and flow cytometry was performed to confirm that cultured cells were mesenchymal stem cells (MSCs). Cellular and platelet concentrations were assessed for all samples. Catabolic cytokines and growth factors important for musculoskeletal tissue regeneration were measured using multiplex ELISA. Colony forming units were significantly increased ($p<0.0001$) in BMC (41.4 ± 27.4) compared to BMA (7.8 ± 12.3). Flow cytometry markers were consistent with MSCs (CD73, CD271 positive; CD45, CD34 negative). Platelet counts were not significantly different between BMC and PRP ($p=0.46$). Platelet-derived TGFβ1 and PDGF were not significantly different between BMC and PRP ($p=0.05$). IL-1ra in BMA (4,510 pg/mL ± 2,994) was twice previously reported values for autologous conditioned serum (ACS). In BMC, IL-1ra (13,432 pg/mL ± 8,588) was at least an order of magnitude greater than ACS. The ratio of IL-1ra:IL-1β (range 493:1 - 17,568:1) in all BMC samples was above the 100:1 ratio reported to inhibit the catabolic cytokine IL-1β. Cellular and cytokine composition are significantly different between BMC and PRP. Concentrations of PDGF and TGF-β1 were equivalent, but BMC had significantly greater concentrations of monocytes and IL-1ra. Bone marrow concentrate and platelet rich plasma have distinct molecular compositions with BMC offering mesenchymal stem cells and increased concentrations of IL-1ra compared to PRP.

F-1006

PRECONDITIONING AND REJUVENATION OF MESENCHYMAL STEM CELLS USING SERUM OBTAINED AT THE ACUTE PHASE OF STROKE

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Presenting appropriate stimuli to cells may promote a transient adaptive response ("preconditioning") so that injury resulting from subsequent exposure to a harmful stimulus is reduced. Anoxic preconditioning of stem cells has been tested for the promotion of cell survival after transplantation, mostly in ischemic myocardium. We investigated the characteristics of mesenchymal stem cells (MSCs) preconditioned by growth in ischemic serum derived from a stroke model. Rat bone marrow MSCs were grown in normal rat serum, stroke model rat serum, or fetal bovine serum (FBS). We then evaluated proliferation rates and changes in cell cycle, survival in ischemic brain conditions, levels of expression of trophic factors, and late phase senescence. Rat MSCs culture-expanded either with ischemic or normal serum exhibit higher cell numbers and higher proliferation indices than MSCs cultured with FBS. Cell cycle analysis showed that the proliferating phase of the cell cycle was increased in the former compared to the latter. Compared to MSCs grown in normal serum or FBS, MSCs cultured with ischemic serum highly expressed trophic factors. In addition, MSCs culture-expanded with ischemic serum displayed superior survival under ischemic brain conditions, as well as delayed senescence. Culture expansion using ischemic serum obtained at the acute phase of stroke could constitute a novel preconditioning method that is feasible and effective for neurorestoration of stroke. Based on the results of this study, the STARTING-2 trial (a prospective, randomized clinical trial evaluating the efficacy of intravenous autologous MSCs culture-expanded with autologous ischemic serum) is currently ongoing.

F-1007

MICROVESICLES DERIVED FROM HUMAN MESENCHYMAL STEM CELLS (HMSCS) TREATED WITH BRAIN EXTRACT REDUCE ISCHEMIC STROKE DAMAGE IN A RAT MIDDLE CEREBRAL ARTERY OCCLUSION MODEL

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Studies have shown that transplantation of mesenchymal stem cells has been shown to improve functional outcome in rat model of ischemic stroke. Subsequent studies suggested that paracrine factors of hMSCs can replace the beneficial effects of hMSCs in these models. We investigated whether microvesicles (MVs) of human mesenchymal stem cells (hMSCs) pretereated with rat brain extract contribute to the attenuation of ischemic brain injury. After culturing hMSCs with MV-depleted cell-free fraction of normal rat brain extracts for 48 hr, conditioned medium was collected and cell-free MVs generated from hMSCs were isolated. Single intra-arterial injection of MVs (0.2mg/kg) was administered into Sprague-Dawley rats 48 hr after permanent middle cerebral artery occlusion (pMCAo). The functional outcome was collectively evaluated by open field, torso twist, prehensile posture and beam balance tests. Compared with those of the PBS-treated controls, the behavioral deficits and cerebral infarction of rats after pMCAo were significantly attenuated by MV treatment. MV reduced microglial cell and astrocyte activation and enhanced endogenous neurogenesis at early stage of pMCAo. To obtain mechanistic insights of the therapeutic effects of MVs, we analyzed proteomic analysis. Other than common proteins associated with vesicle transport and MV biogenesis, we could identified proteins associated with anti-inflammation, angiogenesis, neurogenesis and apoptosis. Our results demonstrate that the intra-arterial administration of MVs from hMSCs prereated with brain extracts may ameliorate ischemic brain injury by modulation of reduced inflammation and enhanced neurogenesis resulting in functional improvement which mimic to the therapeutic effect as hMSCs at early stage of pMCAo. Thus, MVs from hMSCs could be a feasible application of stem cell-based, noninvasive therapy for treating stroke. *Study supported by the Bio Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT and Future Planning, Korea, No.2012M3A9B4028639.*

F-1008

ACCELERATED WOUND HEALING EFFECTS OF VITAMIN C ON MESENCHYMAL STEM CELLS UNDER HYPERGLYCEMIC CONDITION VIA ANGIOGENESIS

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Prolonged hyperglycemia in diabetes is responsible for clinically delayed and impaired wound healing as it severely disrupts wound microenvironment, sometimes leading to several infections and amputations. Current therapeutic approaches for wound care fail to address the pathologic conditions, including poor perfusion and persistent inflammation in diabetic wound microenvironment, rendering such treatments ineffective. Mesenchymal stem cells (MSCs) have been shown in recent reports to promote normal wound healing. However, as a single therapy, MSCs showed variably beneficial effects in diabetic wound. Here we report the synergistic effect of oral vitamin C on MSC therapy, resulting in an accelerated diabetic wound healing. In our study, MSCs cultured under normoglycemic condition were found to upregulate VEGF-α mRNA in the presence of TGF-β1. The expression of *VEGF*-α mRNA in response to TGF-β1 was significantly lower in MSCs under hyperglycemia; however, with vitamin C treatment, reduced upregulation of *VEGF*-α observed in high glucose condition in the presence of TGF-β1 reached the level equivalent to the normal glucose counterpart. To evaluate functional effects using a tubular formation assay, an angiogenic model in vitro, a conditioned medium from MSCs under hyperglycemia exhibited an impaired angiogenic capability compared to conditioned medium from normoglycemia. In a diabetic nude mouse model, an oral vitamin C in adjunct to MSC therapy resulted in accelerated wound healing with increased capillary density. Here, we report a synergistic effect of oral vitamin C on MSC therapy in accelerating diabetic wound healing. The beneficial effects of vitamin C could be partly due to an increased angiogenic paracrine secretion of MSCs under hyperglycemic condition.

F-1009

MSCs MODULATE TISSUE REPAIR RESPONSES AFTER LOCAL INJECTION WITHIN THE SCARRED VOCAL FOLD

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Introduction: Damage to the vocal folds (VFs), often induced by surgery, trauma or radiotherapy frequently leads to scar formation. This fibrous scar tissue damages the vibrating layers of the VFs leading to decreased viscoelastic properties and dysphonia. Currently there is no effective treatment in preventing or resolving this scar tissue. Human bone marrow mesenchymal stem cells (hMSCs) are immunosuppressive and have been used as a successful cell-based immunotherapy. The aim of this study is to establish whether local injection of hMSCs can modulate the early inflammatory response within the scarred VF and promote the wound healing process. Methods: Scarring was surgically induced by bilateral resection of the lamina propria (LP) within the VFs of New Zealand white rabbits (n=20). hMSCs (characterized as per ISCT guidelines) were immediately injected into the scarred area (100,000 cells in 0.1ml human AB plasma) of 10 animals (total 20 VFs). The remaining animals served as controls. Animals were sacrificed at day 2 and day 4 (n=5/group at each time point). VFs were removed and separated from the larynges with one VF taken for histology, fluorescent in situ hybridization (FISH) for surviving injected hMSCS, and immunohistochemistry (IHC) of the macrophage phenotype (M2 CD163 and RAM11 as a generic macrophage marker). The other VF was taken for QPCR. Results: Injected hMSCs were found to have engrafted into the VFs, using FISH at both time points. VFs, where hMSCs were injected, demonstrated increased levels of CD163+ anti-inflammatory M2 macrophages within the LP and muscle, at both time points compared to control animals. QPCR confirmed the induction of an anti-inflammatory effect by the hMSCs, with reduced expression of pro-inflammatory cytokines such as interleukin (IL)1 α/β, IL6 and tumour necrosis factor (TNF) α. Conclusion: This study demonstrates the ability of hMSCs to modulate the early wound healing response through the recruitment and differentiation of monocytes from the vasculature and skewing of tissue resident macrophages to an anti-inflammatory phenotype. These findings suggest that local injection of hMSCs may offer a novel cell-based therapeutic for the treatment of scarred VFs.

F-1010

MITOCHONDRIAL TRANSFER FROM HUMAN PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS TO EPITHELIAL CELLS PROTECTS AGAINST OVA-INDUCED ASTHMA

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Our previous studies have show that human pluripotent stem cell-derived mesenchymal stem cells (iPSCs-MSCs) prevent allergic airway inflammation (AAI) in mice via intravenous administration. There is emerging evidence that stem cells can restitute damaged cells via mitochondrial transfer. Here we showed that iPSCs-MSCs significantly alleviated AAI and mitochondrial dysfunction of epithelia by intratracheal injection. We used three different methods to demonstrate that iPSCs-MSCs transfered mitochondria to damaged epithelial cells in vitro. Using mGFP-iPSCs-MSCs, whose mitochondria were specificly transfected with mGFP, and the technology of Live Cell Imaging, we observed the formation of gap junctional channels (GJCs) and mitochondrial transfer, which finally protected the damage of mitochondria of epithelial cells. We also observed the mitochondrial transfer from iPSCs-MSCs to epithelial cells in the lung of mice and demonstrated that overexpression of Connexin 43, a pivotal gap junction channel protein, in iPSCs-MSCs leaded to the enhanced mitochondrial transfer and greater therapeutic efficacy in a mouse model of OVA-induced asthma. These data suggest that iPSCs-MSCs protected against AAI by rejuvenating epithelial bioenergetics through Cx43-dependent mitochondrial transfer.

F-1011

HUMAN PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS MODULATE THE DIFFERENTIATION AND MATURATION OF MONOCYTE-DRIVED DENDRITIC CELLS

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Induced pluripotent cells (iPSC) are able to be induecd into mesenchymal stem cells (iPSC-MSCs), thus represent a novel and limitless source for MSCs-based therapies. However, little is known about the immunoregulatory property of iPSC-MSCs on Dendritic cells (DCs). DCs are the most potent professional antigen-presenting cells in initiating immune response. Considering the crucial role of DCs in controlling immune response, we investigated the immunomodulatory effects of iPSC-MSCs on differentiation, maturation and functions of DCs. Human CD14+ monocytes were purified from peripheral blood mononuclear cells (PBMCs) using the MACS Monocyte Isolation Kit. Immature DCs (iDCs) were obtained via culturing CD14+ monocytes in the presence of IL-4 and GM-CSF for 5 days. The iDCs were further cultured with LPS for 2 days to obstain mature DCs (mDCs). To study the effect of iPSC-MSCs on DC differentiation, CD14+ monocytes were cultured directly with iPSC-MSCs for 5 days at 1:10 ratio. To study the effects of iPSC-MSCs on DC maturation, iDCs were co-cultured with iPSC-MSCs for 2 days before maturation. The influence of iPSC-MSCs on differentiation, maturation and functions of DCs was assessed for DC phenotypes and endocytosic ability under flow cytometry, and induced cytokines in supernatants identified using enzyme-linked immunosorbent assay (ELISA). We found that the expression of CD1a increased and CD14 expression decreased after CD14+ monocytes with the treatment of GM-CSF and IL-4. After additional LPS treatment, the expressions of CD54, CD80, CD86, CD83 and HLA-DR were highly up-regulated. Human iPSC-MSCs significantly inhibited DC differentiation by decreasing the expression of CD1a and increasing the expression of CD14. Notably, iPSC-MSCs did not affect the acquirement of mature markers of DCs after LPS stimulation, including CD40,CD54, CD80, CD86, CD83 and HLA-DR, but induced the production of IL-10 and decreased the production of IL-12p70 by DCs. Moreover, high endocytosic functions of mDCs after treatment with iPSC-MSCs were also observed. Our data showed that human iPSC-MSCs modulated the differentiation and maturation of monocyte-drived dendritic cells.

F-1012

IMPROVEMENT OF HEART FUNCTION BY BONE MARROW MESENCHYMAL CELLS IN AN EXPERIMENTAL MODEL OF CARDIOTOXICITY ASSOCIATED WITH RADIATION THERAPY

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The therapeutic options for patients with cancer now include increasingly complex combinations of medications, radiation therapy (RT), and surgical intervention. Many of these treatments have important potential adverse cardiac effects and are likely to have significant effects on patient outcomes. Cell therapy appears to be promising for the treatment of chronic and degenerative diseases, including cardiomyopathy induced by RT, as the current therapeutic options are insufficient. Aims: Evaluate the potential of bone marrow mesenchymal cells (BMMCs) in radioinduced cardiac damage Female Wistar rats, 3 months old (Ethics Committee 054/14), were divided into 2 groups, non-treated irradiated group (IR n=15) and irradiated and BMMC treated (IRT n=10). Echocardiography was performed to evaluate heart function. After euthanasia, 3 months post treatment; the left ventricle was removed and prepared for RT-qPCR (VEGF and Pro Collagen I) and histological (picrosirius) analysis. In both groups, 45 days after irradiation, ejection fraction (EF) was in the normal range for these animals (> 70%). However, the BMMC treated group had EF (83.1% ± 2.6) while the non-treated IR group showed a significant reduction (76.1% ± 2.6) in relation to the treated group. In addition, we observed an increase in VEGF gene expression and a decrease in Pro Collagen I in IRT when compared to IR group. We also observed by histology that the collagen deposition was reduced in IRT (10.26% ± 0.83) when compared to IR group (25.29% ± 0.96). Treatment with BMMCs was able to prevent ejection fraction reduction and collagen deposition in irradiated animals. The increase of VEGF and the decrease of pro collagen I gene expression might explain, at least in part, the cell therapy benefits.

F-1013

COMBINATION CELL THERAPY FOR SPINAL CORD INJURY IN RATS

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Spinal cord injury is one of the most important CNS disease which leads to disability in young ages and there is no definite treatment for solving this issue. Due to regeneration ability of stem cells, they could be used as a new method to diminish the consequences of spinal cord injury. In this study we have designed a protocol for combination cell therapy. 105 male Sprague-Dawley rats were randomly selected and divided in 7 groups (n=15). 1st Group: Control group. 2nd Group: Sham-operated group. 3rd Group: MSCs 3 hours after injury. 4th Group: NSCs 48 hours after injury. 5th Group: sorted neurons and oligodendrocytes 4 days after injury. 6th Group: MSCs 3 hours after injury and neural stem cells (NSCs) after 48 hours. 7th Group: MSCs after 48 hours, NSCs after 48 hours, sorted neurons and oligodendrocytes after 4 days (Combination cell therapy). Isolation Mesenchymal stem cells (MSCs): The MSCs isolated from rats femur by flashing complete culture media (DMEM, 10 % FBS and 1 % pen/strep). The isolated stem cells were differentiated to osteocyte and adipocyte. The MSCs were injected through tail vein and they were labeled with PKH-26GL for in vivo tracking. NSCs isolation: The NSCs were isolated from spinal cord of 14 day-rat embryo and they were cultured in NSA- media (DMEM/F12, 2% B27, bFGF 10 ng/ ml, EGF 20 ng/ml) after 5 days the isolated cells formed some spheres called neurospheres. Neurons and Oligodendrocytes Sorting: The NSCs were differentiated by adding 5 % FBS to NSA-media. The neurons and oligodendrocytes were sorted using anti-PSA-NCAM and anti-O4 antibody. Astrocytes were depleted with Anti-GLAST anti-body. BBB scale evaluation: The clinical examination of the different groups reveals the most functional recovery in group that received combination cell therapy. (P value = 0.03). In vivo imaging: In vivo imaging represents migration of MSCs near spinal cord injury and the site of injury which means that they were attracted to injury zone. HandE staining: HandE staining was shows that 7th group has the least lesion volume. (P value = 0.01). In conclusion, the combination cell therapy may provide the best functional recovery for spinal cord injury and it may because by different phase cell transplantation.

F-1014

MICROARRAY ANALYSIS OF GENE EXPRESSION IN LOW SERUM CULTURED ADIPOSE DERIVED STROMAL CELL

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We established human adipose tissue-derived stromal cells (hASCs) cultured in low (2%) serum (hLASCs), which have great potential of clinical application for inflammatory diseases. We recently documented that hLASCs significantly attenuated folic acid induced acute renal damage than did hASCs cultured in high (20%) serum (hHASCs). In this study, microarray analysis was performed to compare the gene expression profile between hLASCs and hHASCs. Human abdominal subcutaneous adipose tissue was obtained from patients underwent liposuction. Cells were cultured under the two conditions; a low serum culture medium containing 2% fetal bovine serum (FBS) and a high serum culture medium containing 20% FBS. Total RNA was isolated from hLASCs and hHASCs. We performed cDNA microarray analysis and confirmed several gene expressions by real-time PCR. 312 genes were significantly (False Dicovery Rate:FDR < 0.05) up-regulated (Fold Change > 5.0) and 211 genes were down-regulated in hLASCs. Microarray analysis revealed several differentially expressed genes (CXCR7, SOD3, TLR3). Data were validated by real-time PCR, confirming the differential expression profiles of CXCR7 (Fold change 26.54), SOD3 (Fold change 4.79) and TLR3 (Fold Change 2.22) in hLASCs. Our data suggest hLASCs may be characterized by cell migration, intense oxidative stress and immune response through toll-like receptor activation, which determine therapeutic potential of hASC.

F-1015

THE COMBINED MAMMALIAN ARTIFICIAL CHROMOSOME STEM CELL (cMACSTEM) TECHNOLOGY AND ITS APPLICATIONS

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Mammalian artificial chromosomes (MACs) are non-integrating, autonomously replicating chromosome-based vectors that may carry a vast amount of genetic material. MACs ensure potentially prolonged, safe, and regulated transgene expression and are attractive genetic vectors for "gene replacement" or for controlling differentiation pathways in target cells. Engineered MAC technology was born in 1991 with the discovery of Satellite-DNA-based artificial chromosomes (SATACs). SATACs can be made by induced de novo chromosome formation in cells of different mammalian and plant species. These artificially generated chromosomes are composed of predictable DNA sequences, and they contain defined genetic material. SATACs have also passed a number of hurdles crucial to their further development as gene therapy vectors, which includes large-scale purification, transfer of purified artificial chromosomes into different cells and embryos, generation of transgenic animals and germline transmission with purified SATACs, and the tissue-specific expression of a therapeutic gene in the milk of transgenic animals. SATACs were used in a cell therapy protocol where a mouse model of Krabbe's disease was treated with the cMACSTEM method. Treatment resulted in an up to five-times longer lifespan for mutant mice. For the cMACSTEM technologies, both pluripotent and multipotent self-renewing cell types could be used as attractive universal host cells. SATAC-modified stem cells have great potential to address a wide spectrum of diseases. Among these, the most prominent are the therapies of lethal genetic disorders where the constitutive overexpression of single or multiple transgenes is necessary, and cancer therapies. We present here the latest achievements with the cMACSTEM system. We used the method to develop therapeutic approaches for genetic disorders like X-SCID and SMA. We also present our initial results about our breast cancer therapy program. Our future goal is to develop personalized therapies for genetic disorders and cancers.

F-1016

REGENERATIVE EFFECTS OF TRANSPLANTING VERTEBRAL BODY-DERIVED MESENCHYMAL STEM CELLS TO THE DEGENERATE INTERVERTEBRAL DISC IN RABBIT

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Our objectives were to assess the possibility using vertebral body-derived mesenchymal stem cells (VBd-MSCs) for the intervertebral disc (IVD) regeneration. Human VBd-MSCs were transplanted in a rabbit model of disc degeneration. Changes in disc height, according to plain radiography, T2-weighted signal intensity on MR imaging, histology, sulfated glycosaminoglycan (sGAG)/DNA, and associated gene expression levels, were evaluated among healthy controls without surgery, sham-operated animals in which only disc degeneration was induced, and VBd-MSC-transplanted animals for a 16-week period. Disc regeneration was also confirmed at the gene expression level using quantitative real-time polymerase chain reaction (qRT-PCR). Sixteen weeks after cell transplantation, the VBd-MSC-transplanted groups retained disc height and signal intensity, but the sham-operated groups progressively lost disc height. Macroscopic and histological evaluations confirmed relatively preserved nucleus with circular annulus structure in the VBd-MSC-transplanted groups compared to indistinct structure seen in sham-operated groups. Immunohistochemistry staining in the VBd-MSC-transplanted groups revealed definite recovery of the lamellar pattern and increased cellularity with more dense extracellular matrix (ECM) compared with more the sham-operated groups. These results suggest that the transplantation of VBd-MSCs into injured IVDs could be an effective treatment for degenerate intervertebral disc disease. *This research was supported by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT and Future Planning (2012M3A9C6049862).*

F-1017

THERAPEUTIC POTENTIAL OF TOPICAL MESENCHYMAL STEM CELLS FOR CEREBRAL ISCHEMIA INJURY

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Previous studies have shown that mesenchymal stem cells (MSCs) have the neuroprotection effects and promote functional recovery in the model of middle cerebral artery occlusion (MCAO). In these studies, MSCs were administrated via systemic infusion. Due to the blood-brain barrier and pulmonary trapping, engraftment rate of the infused MSC into the brain parenchyma was very low. In this study, we investigated neuroprotection effects of MSCs which were transplanted to the brain by means of topical application. 0.4 x 106 MSCs derived from the adipose tissue of transgenic green fluorescent protein (GFP)-Spague-Dawley (SD) rats were applied to the external surface of cerebral cortex of wild-type SD rats (N=10) 1 day after MCAO. The MSCs were then held in position by a thin layer of fibrin glue. In the control group (N=10), no treatment was given. 7 days after topical application, GFP +ve cells were found in the penumbra of the infarct. These cells expressed markers of Nestin, NeuN and GFAP. There was significantly less neuronal death in both infarct and penumbral area in animals treated with topical MSCs. MRI showed the infarct volume was smaller. Functional outcome was also significantly improved as determined by motor-rod test, water maze and gait analysis. No GFP-MSCs were found in the other somatic organs in the test and control animals. In conclusion, topical application provides a direct delivery of MSCs to ischemic brain and facilitates the recovery of neurologic motor function from MCAO.

F-1018

PLACENTA DERIVED MESENCHYMAL STROMAL CELLS EXPANDED IN HUMAN SERUM DISPLAY HIGHER PROLIFERATION AND ENHANCED IMMUNOMODULATORY EFFECT

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Evidence exists indicating that mesenchymal stromal cells (MSCs) are promising candidates for novel cell therapeutic applications. One major obstacle for their clinical use is the biosafety of fetal bovine serum (FBS), which is a crucial part of all media currently used for the culture of MSCs. Although some recent studies recommended substituting FBS with human serum (HS) for the expansion of MSCs for clinical use, the functional capacity of the expanded cells has only been partially explored. In addition, limited experience indicates that HS may replace FBS in some but not all culture systems. However, relatively little is known about using HS instead of FBS for isolation and expansion of placenta derived MSCs (PL-MSCs). Therefore, this study aimed to comprehensively compare the exploit of HS and FBS as a supplement in terms of their impact on PL-MSCs expansion. The quality and functionality of PL-MSCs including cell surface marker expression, adipogenic and osteogenic differentiation, and immunosuppressive capacity were studied. The results demonstrated that PL-MSCs cultured in DMEM supplemented with 10% HS have similar characteristics to PL-MSCs cultured in DMEM supplemented with 10% FBS. In addition, PL-MSCs cultured in DMEM supplemented with 10% HS have greater expansion potential than PL-MSCs cultured in DMEM supplemented with 10% FBS. Interestingly, PL-MSCs cultured in DMEM supplemented with 10% HS have higher immunosuppressive effect than PL-MSCs cultured in DMEM supplemented with 10% FBS. The results obtained from this study may imply some application in the use of HS for the expansion of PL- MSCs to be used as immunomodulator in future applications.

F-1019

NANOSTRUCTURED EPICARDIAL PATCHES FOR FUNCTIONAL ALLEVIATION OF LEFT VENTRICLE IN INFARCTED MYOCARDIUM: PORCINE MODEL STUDY

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Myocardial Infarction (MI) leads to death of cardiomyocytes followed by Left Ventricular (LV) remodeling leading to chronic cardiac dysfunction. Numerous clinical trials have pointed out that the impediment posed by dynamic cytokine rich myocardial microenvironment that prevents the locally administered cells from homing at the site of injury resulting very low efficiency. We hypothesized that biodegradable nanostructured epicardial patches would provide a multimodal platform for controlled release of repair factors while promoting in situ retention of therapeutic cells for improved LV functioning. Nanotopographically controlled core-shell biopolymer system of poly(L-lactic acid)-co -poly(ϵ -caprolactone)/ collagen nanofibers containing chemokine SDF-1 (PCS+) were fabricated using electrospinning that displayed porous, uniform nanostructures with diameter less than 500nm. In vitro analysis confirmed the capacity of PCS+ in mimicking the highly sensitized nanoscale structural and biochemical cues in cardiac extracellular matrix (ECM) while exhibiting controlled release of SDF-1 and potential to recruit both cardiac cells as well as mesenchymal stem cells (MSCs). An MI Porcine model (female domestic Pigs) was established by the ligation of left circumflex coronary artery (LCX) and using non-absorbable sutures, to create 1-2 cm infarct on LV. The nanofiber scaffolds with (PCS+) and without SDF-1 (PCS-) were implanted on the epicardium of the infarcted region on the LV of the heart. After transplantation, post 4 weeks PCS+ 15% improvement in the fractional shortening without the formation of scar tissue along with integration of nanofiber into the myocardium. PCS+ promoted formation of new tissue mass that's expressed cardiac marker proteins alpha actinin and connexin 43 indicating formation of gap junctions with successful retention of injected MSCs along with formation of new blood vessels. In this study we, for the first time demonstrate the safety, efficacy and feasibility of chemotactic nanofiber based epicardial patch application onto infarcted myocardium highlighting the positive impact of biocomposite nanostructured biomaterial strategies and its potential to serve as a method for the repair, restoration and regeneration of the infarcted myocardium.

F-1020

A COMPARATIVE STUDY OF THE THERAPEUTIC EFFICACY OF MULTIPOTENT ADULT PROGENITOR CELLS AND MESENCHYMAL STEM CELLS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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We have previously demonstrated in vitro that bone marrow derived multipotent adult progenitor cells (MAPC) have a greater propensity for neuro ectodermal differentiation than mesenchymal stem cells (MSC). Hence we undertook this study to compare the therapeutic efficacy of MAPC and MSC in experimental autoimmune encephalitis (EAE). MAPC and MSC were cultured from the bone marrow of 3 to 4 weeks old C57BL/6 mice and EAE induced in 12 weeks old mice. Experimental studies were performed in the following groups of 6 mice each- 1: Healthy control; 2: Disease control; MAPC treated; (iv) MSC treated. A clinical scoring of the neurological deficit was done daily. All groups were sacrificed 38 days post injection and the brain, spinal cord and spleen of all the mice were harvested and studied for histopathological changes, demyelination, inflammatory cell infiltrate and the following neural markers - GFAP (glial cell), NG2 (oligodendrocyte precursor), PDGF-R (oligodendrocyte), NF200 (neuro-filament), Gap43 (axonal) and Nestin (neural precursor). An in vitro evaluation of the immunomodulatory properties of both the cell types was also performed. Both MAPC and MSC treated groups had a significantly less severe EAE in comparison to the saline treated group ($p<0.05$), with a greater suppression in the MAPC group in comparison to the MSC group ($p<0.05$). Infiltration by T cells and

macrophages was significantly lesser in the MAPC and MSC treated groups in comparison to saline group (p<0.05), though there was no difference between the MAPC and MSC groups. Homing of PKH26 labeled MAPC and MSC to the inflamed areas was seen. Co-localization of PKH26 with GFAP, NG2, NF 200, Gap43, PDGFR, and Nestin, without any evidence of multinucleation, indicated a trans- differentiation of transplanted MAPC and MSC into various neural cells rather than fusion with resident cells. Gene expression and protein analysis of these markers showed a significantly higher fold expression in the MAPC group in comparison to the MSC group (p<0.05). MAPC and MSC exhibited equivalent in vitro immunomodulatory properties. Thus rats treated with MAPC had significantly greater suppression of EAE and more potent neuro regeneration, in comparison to the MSC treated rats. Thus MAPC may be a potential cell type superior to MSC for the treatment of EAE/ multiple sclerosis.

F-1021

BRIDGING THE MOA AND THE POC FOR CLINICAL APPLICATION IN CASE STUDY

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To assign the cells as candidates for cell-based medicinal products for a disease, the mode of action (MOA) and the proof of concept (POC) should be shown. In this presentation with case study, we will focused on MOA and POC, and bridge these two concepts, in which adipose tissue-derived multi-lineage progenitor cells (ADMPCs) would be developed as cell-based medicinal products for liver cirrhosis. To treat the patients, the pathogenesis and pathophysiology of the disease should be reflected onto the MOA. Liver fibrosis is characterized by excessive accumulation of extracellular matrix with inflammatory status in situ, therefore, anti-inflammatory cytokines and fibrinolytic enzyme secretion is anticipated as MOA. After showing the appropriate MOA, the MOA and the POC should be bridges. The key issues are what kind of animal models should be selected. In the presenting case, MOA is that the cells act as vehicle for the delivery of anti-inflammatory cytokines and MMPs. Tetra carbon chloride (CCl4)-chronic induction evolved radicals, followed by inflammation, resulted in fibrosis of the parenchyma. So, the expected mode could be applicable in this animal models. To acquire the POC after bridging to MOA, the appropriate route of administration (ROA) should be concerned. Mice could be used in the animal-POC-study because systemic administration via tail vein is friendly for researchers. In the case study, the POC of the developing cell-based products by the improvement of liver fibrosis and function by systemic administration of ADMPC. Before and after POC acquisition, it should be discussed whether the animal models could reflect the clinical pathophysiology, to show the limitation of those animal models. In conclusion, 1) MOA should be planned from the pathophysiology of the target diseases, 2) the POC-study should be designed to bridge to the MOA, and 3) the applicable limitation should be concerned in clinical use for the patients of the disease.

F-1022

IMMUNOGENIC RESPONSE TO ALLOGENIC MESENCHYMAL STEM CELL TRANSPLANT IN IMMUNOCOMPETENT MICE

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The immunomodulatory properties of mesenchymal stem cells (MSCs) have been the focus of several studies over the past few years due to their therapeutic potential. Many in vitro studies have shown that these cells can be hypoimmunogenic, indicating the possibility of using them in allogenic transplants without any harmful effects. MSCs also have the ability of exerting their biological function through both paracrine and endocrine pathways, enhancing the liberation of anti-inflammatory cytokines, such as IL-4 and IL-10, as well as inhibiting the secretion of pro-inflammatory cytokines like IFN-γ and TNF-α. However, recent in vivo studies show that these cells can generate an immune response when transplanted into an immunocompetent host, with the formation of memory T cells. This indicates that MSCs may not actually be immune privileged. The main goal of this study has been to evaluate the role of activated and non-activated MSCs when transplanted to the renal subcapsular space of allogenic mice. Adipose-derived mesenchymal stem cells were isolated from C57Bl/6-GFP transgenic mice. The cells were characterized by adipogenic and osteogenic differentiation assays and immunophenotyping was performed by flow cytometry. The animals were divided in three different groups for the transplant. Male C57Bl/6 mice were transplanted with 2x105 C57Bl/6-GFP cells. In the allogenic groups, male BALB/c mice were transplanted with 2x105 C57Bl/6-GFP cells or 2x105 C57Bl/6-GFP cells pre-activated by incubation with 20 ng/mL IFN-γ and 30 ng/mL TNF-α for 20h before transplantation. Histological and immunofluorescence analysis of graft-bearing kidneys showed that no GFP+ cells were found in any allogenic transplant after 28 days. At 7 and 14 days, the transplant sites showed a massive proliferation of inflammatory cells, which vanished after 28 days, indicating complete rejection of the transplanted cells. The syngeneic transplant was not rejected, showing GFP+ cells through the whole period of study. Cytokine quantification in the blood plasma showed no difference between any of the groups. An in vitro lymphocyte proliferation assay was performed, which indicates that MSCs alone can induce the activation of allogenic T lymphocytes. This could explain their rejection in vivo.

F-1023

AUTOLOGOUS BONE MARROW CONCENTRATE FOR THE TREATMENT OF DISCOGENIC LOW BACK PAIN LOW BACK PAIN: AN IRB CONTROLLED PROSPECTIVE STUDY WITH MINIMUM 2 YEAR FOLLOW UP

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The clinical outcome of fusion surgery for discogenic low back pain average a 35% to 43% improvement in pain and a 12.5% reoperation rate at 2 year follow up. The use of autologous BMC may provide a non-surgical option for treating discogenic low back pain. PURPOSE: This is the first report to assess the safety and efficacy of an intradiscal injection of BMC for treating discogenic low back pain with minimum 2 year follow up. Methods/Materials: Twenty six patients with a diagnosis of discogenic low back pain

were injected at one (13 patients) or two disc levels (13 patients). The average patient age was 37.7 years (ranging from 18-61). MSC cell counts were obtained in 20 of 26 patients using CFU-F assays, and the average CFU-F/ml patient BMC was 2713. Patients with less than 2000 CFU-F/ml had diminished clinical efficacy. All patients had pre-injection MRI's with Pfirrmann grade 4-7 discs. Two year follow up was obtained in 100% of patients. All patients were surgical candidates with a pre-injection, ODI (56.5%) and VAS (80.1/100). Bone marrow concentrate was collected from the patients iliac crest, concentrated by centrifugation (ART-21 system), and then re-injected into the symptomatic disc(s) utilizing fluoroscopic guidance. All patients were treated under IV sedation in a 45 min procedure. Average ODI / VAS scores improved from pre-procedure scores of 56.5% / 80.1 to: 22.8 / 29.2 at 3 months, 24.4 / 26.3 at 6 months, 25 / 33.2 at 12 months 16.5% and VAS to 20.2 out of 100 at 24 months (p≤0.0001 at all time points compared to initial scores). Twenty patients had MRI scans 12 months after injection with 8 improving one or more Pfirrmann grade (assessed by a blinded independent reviewer). There were no adverse events. Only 5 of 26 patients elected to proceed with surgery. Only one of these had improvement with surgery. Conclusions: This study demonstrates the safety and efficacy of autologous BMC injection as an alternative to surgery through 2 yr. follow up. These preliminary results suggest patients should fail autologous BMC injection prior to fusion/artificial disc replacement surgeries for discogenic low back pain.

F-1024

CULTURING HUMAN ISLETS WITH PRECONDITIONED MEDIA FROM HUMAN ADIPOSE DERIVED STEM CELLS REDUCES APOPTOSIS AND IMPROVES ISLET FUNCTION

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The transplantation of human islets has the promise to cure diabetes type 1. However, the treatment is limited by early graft loss. Mesenchymal stem cells have been shown to have islet protective properties. Similar cells can be isolated from various tissues, including adipose tissue yielding adipose tissue derived stem cells (ASC). Incubating ASCs in hypoxia (1% O2) has been shown to increase secretion of several cytokines, including VEGF and IL-10. We investigated whether condition media (CM) from ASCs cultured under hypoxic conditions could have beneficial effects on human islets compared to condition media from ASCs culture under normoxic (21% O2) conditions or to unconditioned control media. Human ASCs were incubated in ASC media consisting of MEM-alpha supplemented with 10% FBS and exposed to hypoxia (1% O2) or normoxia (21% O2) for 48 hrs. The two different conditioned media were harvest and mixed with equal amounts of human islet media consist of CMRL 1066 supplemented with 2,5% human serum. Equal numbers of human islets were then incubated for 48 hrs in mixed media containing either hypoxia CM, normoxia CM or unconditioned control media. Human islets viability was measured by Cell death ELISA (Roche), and functionality was evaluated by measuring the glucose stimulated insulin secretion (GSIS). Human islets cultured with hypoxia CM show a significant reduction in apoptosis as measured by Cell death ELISA (n=8, p<0.0029) vs. control islets incubated with unconditioned media. We also found improved GSIS in human islets cultured in hypoxia CM vs. control islets incubated in the unconditioned media (n=7, p=<0,036). Although we observed similar tendency in islets cultured in the normoxia CM group when compared to islets incubated the unconditioned group, these failed to reach significance (cell death p=0,194, GSIS p=0,145). The difference between islets incubated with hypoxic and normoxic CM did not reach significance. Human ASC cultured in hypoxia releases factors that significantly reduce apoptosis and improve function of human islets.

F-1025

INTRAPERITONEAL TRANSPLANTATION OF AMNIOTIC FLUID STEM CELLS COULD RESCUE THE EXPERIMENTAL COLITIS

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The colitis or inflammatory bowel diseases (IBD) is increasingly prevalent in recent years due to diet changing and manufactured food up-taking. The stem cell may offer an alternative mean to cure IBD or colitis to solve the problems from failed optimal outcome of traditional IBD treatment. With the property of giving rise to three germ layers, expressing pluripotent markers, anti-inflammatory and repairing trait, amniotic fluid derived stem cells (AFSCs) are believed to hold the promise in regenerative medicine. Our group had demonstrated AFSCs could be applied onto several diseases including myocardia infarction and liver fibrosis. In this study, we used pig amniotic fluid derived stem cells (pAFSCs) to treat the experimental model of colitis in mice. Acute colitis was induced in 8 week-old wild type mice by administering 2% dextran sulfate sodium. Then we transplanted 3 million pig Ds-red-harboring amniotic fluid stem cells to the mice with colitis via intraperitoneally injection. The results demonstrated the stem cells transplanted groups having longer colon length and longer survival compared to untreated groups (colitis mice). In histology study, untreated groups showed significant more inflammatory cells in the lamina propria with crypt damages. The cytokines TNF-α and IFNγ were both found significantly decreased in the stem cell transplanted group. We concluded pAFSCs might have benefits for gastrointestinal disorders. The pAFSCs showed the ability of inhibition the shortening of colon after induction of colitis, decreasing the inflammatory area with epithelial mesenchymal transformation. Amniotic fluid stem cells could ameliorate experimental colitis in mice and these results might be an potential treatment for the IBD or colitis in the future.

F-1026

MANUFACTURE OF CLINICAL-GRADE HUMAN CLONAL MESENCHYMAL STEM CELL PRODUCTS FROM SINGLE COLONY FORMING UNIT-DERIVED COLONIES BASED ON THE SUBFRACTIONATION CULTURING METHOD

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Stem cell products derived from mesenchymal stem cells (MSCs) have been widely used in clinical trials, and a few products have been already commercialized. However, the therapeutic effects of clinical-grade MSCs are still controversial owing to mixed results from recent clinical trials. A potential solution to overcome this hurdle may be to use clonal stem cells as the starting cell material to increase the homogeneity of the final stem cell products. We have previously developed an alternative isolation and culture protocol for establishing a population of clonal MSCs (cMSCs) from single colony forming unit (CFU)-derived colonies. In this study, we established a good manufacturing practice (GMP)-compatible procedure for the clinical-grade production of human bone marrow-derived cMSCs based on the subfractionation culturing method (SCM). We optimized the culture procedures to expand and obtain a clonal population of final MSC products from single CFU-derived colonies in a GMP facility. The characterization results of the final cMSC products met our preset criteria of morphology, cell surface antigen expression, differentiation potential, and suppression of lymphocytic proliferation. Animal toxicity tests were performed in a certified good laboratory practice (GLP) facility, and showed no toxicity or tumor formation in vivo. These tests include single injection toxicity, multiple injection toxicity, biodistribution analysis, and tumorigenecity tests in vivo. No chromosomal abnormalities were detected by in situ karyotyping using oligo-FISH, providing evidence of genetic stability of the clinical-grade cMSC products. The manufacture and quality control results indicated that our GMP methodology could produce sufficient clonal population of MSC products from a small amount of bone marrow aspirate to treat a number of patients.

F-1027

LESS INVASIVE LIVER REGENERATION THERAPY FOR LIVER CIRRHOSIS PATIENTS USING CULTURED AUTOLOGOUS BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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From our basic studies, we reported that bone marrow cells (BMCs) infused via a peripheral vein efficiently repopulate the cirrhotic liver and produce collagenases, resulting in reduced liver fibrosis, and a significant increase in survival. We also confirmed that frequent BMC infusion contributes to suppression of tumor initiation. Based on these results, we started "autologous bone marrow cell infusion therapy" for liver cirrhosis patients as liver regeneration therapy using non-cultured autologous whole BMCs, and subsequently reported its safety and efficacy. However, this therapy involves BM aspiration under general anesthesia. Therefore, we have developed a less invasive therapy that uses cultured autologous BM-derived mesenchymal stem cells (BMSCs) aspirated under local anesthesia. We started using this therapy after regulatory approval of the application "Policies for clinical research using human stem cells" in Japan (ClinicalTrials.gov; No. NCT02327832). Here, we reveal the underlying mechanisms of cultured human (h) BMSCs on liver cirrhosis. We systemically infused hBMSCs into thioacetamide-induced NOD-SCID cirrhotic mice. Liver fibrosis and antioxidant effects were assessed with Sirius red staining and a kinetic study of inhibition of diammonium salt oxidation. We also screened the profile of microRNAs in hBMSC-infused livers using a miRNA array. We evaluated the protective effects of hBMSCs or hBMSC-derived exosomes on thioacetamide-induced oxidant conditions by measuring cellular reactive oxygen species (ROS). Mice treated with hBMSCs showed reduced liver fibrosis ($p<0.05$), higher serum antioxidant activity ($p<0.001$), and significantly higher amounts of hepatic miR-200a-3p which targeted Keap1 mRNA. Human hepatocytes co-cultured with not only hBMSCs, but also their exosomes, showed reduced ROS levels ($p<0.001$). Moreover, hepatocytes in which the miR-200a-3p target site was blocked showed significantly higher levels of ROS, despite supplementation with BMSC-derived exosomes. These results strongly indicate that the infusion of cultured BMSC-secreting exosomes contributes to reducing hepatic injury and fibrosis through stabilizing redox homeostasis for liver cirrhosis patients.

F-1028

MESENCHYMAL STEM CELLS SUPPRESSE IMMUNE REACTION TRIGGERED BY BMP2

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Human periodontal ligament stem cells (PDLSCs) and bone marrow stem cells from maxilla (BMMXs) are considered as mesenchymal stem cells (MSCs) identified in human periodontal ligament from extracted third molar teeth and bone marrow from maxilla. The characteristics of MSCs are a self-renewal capacity and multi-lineage differentiation potential. Bone morphogenetic protein 2 (BMP2), osteo-inductive growth factors, have a critical function to develop the bone and cartilage. However, clinical applications in orthopaedics and dentistry show the adverse effects, such as low level of osteogenesis and high level of inflammation, associated with high-dose BMP2. In our study, we focus the effect of BMP2 on the immune system and bone formation, and the immunosuppressive properties of MSCs on inflammation. Herein, the effects on the immune system were investigated using human monocytic cell line THP-1. At the high concentrations of BMP2, over 500ng/ml, the proliferation of THP-1 is decreased. In addition, high-doses of BMP2 on THP-1 in vitro induce the expressions of inflammatory cytokines, such as IL-8, RANTES and ICAM1. While co-culturing with PDLSCs, the expression patterns of IL-8, RANTES, ICAM-1 are decreased compared to THP-1 only. On the other hand, BMMXs co-culturing THP-1 show the decreases of

IL-8 and RANTES and the increases of ICAM1. Also, both PDLSCs and BMMXs with THP-1 induce IL-6, which is not expressed in only THP-1, PDLSCs, and BMMXs. RANTES, functioning of the migration of leukocytes in immune and inflammatory responses, and ICAM1, intercellular adhesion molecule-1, have a function to generate triggering of immune reaction in high concentrations of BMP2. The accumulation of immune cells in close proximity to MSCs lowers the proliferation and bone formation activity of MSCs. In conclusion, high concentrations of BMP2 could be a problem triggering inflammation clinically and MSCs engraftment with BMP2 is a new therapeutic treatment to suppress the immune cell activity.

F-1029

COMPARISON OF TELOMERE LENGTH AND TELOMERASE ACTIVITY OF GESTATIONAL TISSUE-DERIVED MESENCHYMAL STEM CELLS AND BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) have an ability to migrate to injured/ischemic tissues where they enhance tissue repair by modulating immune response and inducing neovascularization. Due to their unique and versatile properties, MSCs have been regarded as a potential source for various clinical applications. Currently, the standard source of MSCs for most applications is bone marrow. However, the isolation of MSCs from bone marrow (BM-MSCs) requires an invasive procedure and their number has been reported to decline with age. Therefore, the isolation of MSCs from alternative sources, such as gestational tissues, which can be easily obtained in large quantity with non-invasive procedure might be more suitable. Although the results from our previous study showed that MSCs from gestational tissues have higher proliferative capacity and could be expanded in culture for a longer period of time compared with BM-MSCs, the mechanism underlying those differences is still unknown. The present study aims to study the mechanism underlying the differences in proliferative capacity between BM-MSCs and gestational tissue-derived MSCs by comparing their telomere length and telomerase activity. Passage 2, 4, 6, 8 and 10 of cultured MSCs derived from umbilical cord (UC-MSCs), Wharton's jelly (WJ-MSCs), placenta (PL-MSCs), chorion (CH-MSCs) and BM-MSCs were harvested to study their telomere length and telomerase activity using telomere-specific qPCR and TRAPeze Kit RT Telomerase Detection Kit, respectively. The results showed that the telomere length of UC-MSCs, WJ-MSCs, PL-MSCs, and CH-MSCs were longer than those of BM-MSCs from the same passages. However, there was no difference in telomerase activity among the various MSC sources. Although the telomerase activities of all MSC sources were slightly higher than those of peripheral blood mononuclear cells which served as negative control, they were much lower than those of human embryonic stem cells and human induced pluripotent stem cells. In conclusion, our study demonstrated that the gestational tissue-derived MSCs have longer telomere and could be expanded in culture for a longer period of time compared with BM-MSCs. These MSCs might be more appropriate for various clinical applications in the future.

F-1030

TRANSPLANTATION OF TISSUE-ENGINEERED BONE FOR TREATMENT OF COMBAT CASUALTIES

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The gold standard in cure of bone defects is autologous bone grafting. Combat bone defects have in most cases considerable sizes and their treatment with auto-bone graft is difficult owing to limited donor tissue availability. Based on positive results obtained during our phase I/IIa clinical trial in treatment of bone defects after high-energy civil trauma, we have the aim to reconstitute combat critical-sized bone defects with tissue-engineered bone (TEB) as medical innovative stem-cell based interventions. Nine victims of military battles in Eastern Ukraine had critical bone defects (ranged 5-12 cm) of various localization: clavicle - 1, humerus - 3, femur - 1, tibia - 2, calcaneum - 2. Between them, six were blast injuries and three - bullet wounds. TEB is composed of scaffold (acellular allogenic bone in form of blocks or chips in a fibrin gel) and autologous cultured bone marrow mesenchymal stem cells (MSCs) and periosteal progenitor cells (PPCs) in ratio 3:1. Bone fragments were fixed using external (Ilizarov device) or internal (plate or intramedullar) constructions. MSCs were obtained by plating whole bone marrow (5 ml) followed by their culturing in αMEM with 10% platelet lysate under 5% O_2. PPCs were obtained from periosteum fragments of fibula and expanded in DMEM/F12 with 10% human serum and 10 ng/ml FGF-2 under 5% O_2. Quality control of seeded TEB was done by staining with FDA/PI. Bone tissue formation was checked every 1.5 month by X-ray examination. For TEB creation we used 10^8 cells avg. The required MSC amount was obtained at first passage and conformed to minimal ISCT criteria (three-linear differentiation, $CD73^+CD90^+CD105^+CD45^-$ phenotype) and cells were slightly positive for alkaline phosphatase. The PPCs had $CD73^+CD90^+CD105^+CD45^-$ phenotype, differentiated into osteoblasts and chondrocytes (but not into adipocytes) and were strongly positive for alkaline phosphatase. Quality control of TEB revealed a uniform seeding with live cells. Results of primary X-ray examination (1.5 month) showed the integration of TEB with recipient bone and graft bone density was similar to host bone density. The primary data of clinical application of TEB indicate a feasibility of critical-sized bone defect restoration in combat casualties.

F-1031

HUMAN ADIPOSE DERIVED MESENCHYMAL STEM CELLS IN CELL THERAPY: SAFETY AND FEASIBILITY IN COMPASSIONATE CLINICAL APPLICATIONS

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Based on the capacity of adipose-derived stem cells (ASC) to secrete in vitro VEGF/FGF/SDF in hypoxia and to promote in vivo angiogenesis/osteogenesis in preclinical relevant models, this study aims to assess the safety/efficacy of ASC-derived cell therapies in compassionate clinical indications. Three autologous ASC products were proposed to 27 patients without any success of conventional therapies:(i)a scaffold-free osteogenic 3D graft for the treatment of bone non-union (congenital pseudarthrosis/intercalary bone allograft implantation after tumour resection);(ii)a biological dressing for dermal reconstruction of non-healing chronic wounds (radionecrosis,drepanoytosis,vasculitis) and (iii)a cellular suspension to enrich fat autologous graft for a tongue with severe postradiation dysphagia. The safety was studied by the quality control of the final product (ASC purity, genetic stability (FISH/karyotype), microbiological/endotoxin contamination) and the evaluation of adverse events after transplantation. The feasibility was assessed by the ability to reproducibly obtain the final ASC-based product with specific characterization, the respect of the timing between ASC procurement and the surgical intervention, the capacity to produce enough material to treat the lesion, the surgical handling of the graft and the ability to manufacture the graft in line with Belgian hospital exemption procedures. No adverse event occurred at the site of adipose tissue biopsy. For 20 patients (7 patients were not grafted due to non-optimal final graft or spontaneous healing), in-process controls (on cellular samples collected prior to graft delivery) found no microbiological/mycoplasma/endotoxin contamination, optimal ASC purity and genetic safety. Therefore, each graft fulfilled the release criteria for implantation. Each type of graft was reproducibly obtained in function of their respective clinical applications without significant delay for implantation and the surgical handling was always in phase with the surgical procedure. No serious adverse events were noted up of 51 months. We demonstrated that autologous ASC transplantation can be considered as a safe and feasible therapy for specific clinical indications in respect with ASC properties and physiopathology of the disease.

F-1032

ENGINEERING MESENCHYMAL STROMAL CELLS (MSCs) TO BE MORE IMMUNOEVASIVE BY ALTERING CELL CULTURE CONDITIONS

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We demonstrate that by minimally altering culture conditions for adult human bone marrow (BM) mesenchymal stromal cells (MSCs) to serum-free, cytokine-supplemented, non-adherent conditions, we can change the expression of adhesion molecules and human leucocyte antigens (HLA-ABC). This change is not accompanied by any other phenotypic changes; cells maintain the expression of minimal MSC cell surface antigens (CD90 (99.9%), CD105 (98%), CD73 (99.9%), CD14 (0.2%), HLA-DR (0.1%), CD34 (0.1%) and retain their ability to undergo trilineage differentiation. However, these cells show a decrease in HLA-ABC expression (d7: 47%, vs 99.9% d14: 10.4% vs 95.9%, and d21: 3.8% vs 90%), vascular cell adhesion protein-1 (VCAM-1) expression (d7: 14.6% vs 95.2%, d14: 1.4% vs 88% , and d21: 0.1% vs 80%) and Intercellular adhesion molecule-1 (ICAM-1) expression (d7: 13% vs 78.2%, d14: 0.5% vs 70%, and d21: 2.6% vs 62.3%). A standard 51Cr cytotoxicity assay showed that MSCs cultured in suspension, under serum-free, cytokine-supplemented conditions had reduced susceptibility to cytotoxicity from NK-92, a permanent allogeneic NK cell line under clinical investigation for treating hematopoietic malignancies (9.08% ± 1.86% killing vs. 59.17% ± 3.66% killing at a 40:1 NK-92:MSC ratio). Cytotoxicity at lower effector-to-target ratios (20:1, 10:1, and 5:1) was effectively 0% (SD <2%) for MSCs grown in altered conditions, compared to approximately 30% (SD <3%) for traditional, adherent-grown MSCs. In vivo experiments in an acute inflammatory (lipopolysaccharide (LPS) paw edema) murine model are ongoing to examine effects on modified homing and anti-inflammatory properties when MSCs are grown in altered, suspension cultures vs. traditional cultures. We conclude that by subtly changing culture conditions we can alter adhesion molecules and HLA-ABC expression, which in turn affects immunoevasion, homing and migration of MSCs, equipping the cells for potentially more potent therapeutic effects in treating immune-related disorders.

F-1033

ADIPOSE DERIVATE STEM CELL THERAPY ENHANCE ANGIOGENESIS IN OLD AND ATHEROGENIC MICE MODEL OF HINDLIMB ISCHEMIA

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Aging is a major factor for the increase of atherosclerosis in the world population. Cellular therapy for regenerative medicine is very attractive but many questions are not defined. In this study we investigated the improvement in the hind limb circulation using human adipose derived stem cells (ADSCs) into the ischemic muscular tissue in old atherogenic mices (C57BL06 ApoE-/-) 30 weeks old. Thirty-one male mice under unilateral hind limb ischemia were divided into 3 groups that received: cultured ADSCs (ADSCc), fresh ADSCs (ADSCf), or PBS (CTL) by intramuscular injection (50 µL containing 5x106 cells). Perfusion analysis was done by Image Laser Doppler at 0, 7, 14 and 21 days after ischemic induction, when the animals were sacrificed. Vascular endothelial growth factor (VEGF), metalloprotease-2 (MMP-2) and metalloprotease-9 (MMP-9) were quantified in gastrocnemius muscle by immunohistochemistry. Perfusion did not show statistical difference in the CTL group during treatment, while a significant increase was observed in ADSCc and ADSCf groups during treatment (day 0 x day 21: p=0.01 and p=0.02 respectively). The comparison of the groups demonstrated that treatment with ADSCc was associated with the best results when compared to the CTL group after 21 days (p=0.01). The ADSCf group did not show significative difference when compared to CTL group. The semiquantitavive analysis of MMP-2, MMP-9 and VEGF present more concentration in the ADSCc and ADSCf than CTL groups. These results suggest an increase in the vasculogenesis and angiogenesis with treatment with ASDCc.

MESENCHYMAL STEM CELL DIFFERENTIATION

F-1034

THE INVOLVEMENT OF THE EXTRACELLULAR MATRIX PROTEIN POLYDOM IN THE REGULATION OF MOUSE BONE MARROW MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) from bone marrows have the ability to self-renew and differentiate into various types of cells such as adipocytes, osteocytes and chondrocytes. It remains unclear how stemness, growth, survival and differentiation of MSCs are regulated. This regulation is thought to be mediated by extracellular environment involving growth factors, cytokines, extracellular matrix proteins and direct contact with neighboring cells. Here, we focused on the extracellular matrix protein polydom, which has a huge multidomain structure. Polydom is expressed by a population of MSCs. Moreover, it has been reported that polydom is localized to the surface of some bone marrow MSCs, participating in the regulation of cell adhesion. We postulated that polydom has the ability to regulate the function of MSCs. To test this hypothesis, we employed mice lacking polydom. However, it is difficult to prepare MSCs from polydom knockout mice, since the mice die soon after birth. Therefore, we used mouse embryonic fibroblasts (MEFs), because MEFs have been reported to resemble MSCs, showing the capability to differentiate to adipocytes, osteocytes and chondrocytes. MEFs from polydom knockout mice showed low efficiencies of colony formation and differentiation into adipocytes and osteocytes, suggesting the potential role of polydom in the regulation of MSCs. To address this possibility, we generated polydom conditional knockout mice, in which the gene disruption is inducible by tamoxifen through CreER system. In polydom conditional knockout MSCs from bone marrows of 10-week old mice, abilities of colony formation and differentiation into adipocytes and osteocytes were decreased in comparison with control MSCs, like the case of MEFs. Furthermore, the conditional knockout of polydom led to the reduction in the growth of MSCs. These results indicate that polydom is a regulator of MSCs from bone marrows.

F-1035

KERATINOCYTE CO-CULTURE WITH STEM CELLS FROM HUMAN AMNIOTIC MEMBRANE INDUCES KERATINOCYTE DIFFERENTIATION

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Previous studies have shown that stem cells derived from human amniotic membrane (HAMSCs) showed no commitment to differentiate into keratinocytes. HAMSCs were isolated from male neonates. Based on the hypothesis of tissue regeneration, where differentiated cells could be derived from stem cells, a keratinocyte co-culture model was prepared. The keratinocytes and HAMSCs were both cultured in Keratinocyte Serum Free Medium in a Transwell® Clear Polyester Membrane Insert 6-well culture plate. Initially, the stem cells were cultured on the bottom of the dish and then, an insert (pore size 0.4 µm) was placed into the plate. Later, the keratinocytes were cultured into the membrane of the insert. Morphological changes of the stem cells were observed. The HAMSCs were harvested at day 1 day 3, day 7, day 10, day 14, and day 21 for RNA extraction. The quantitative analysis of the RNA samples were obtained by reading the optical density using Eppendorf BiophotometerPlus while qualitative analysis, by agarose gel electrophoresis for intact 28S and 18S ribosomal sub-units. The RNA was then subjected to RT-PCR using One-Step RT-PCR with primers for stem cell and keratinocytes markers; *Nestin, Nanog, KRT5, KRT14, FLG, INV* and *AQP3*. Housekeeping gene, β-*actin* was used as the normalizer for relative expression levels of each gene. The RT-PCR products were separated by 1% agarose gel electrophoresis stained with Syber® Safe DNA gel stain. Results showed that there were morphological cell changes of co-cultures of HAMSCs starting from day 7 onwards. Expressions of keratinocyte gene markers were also observed from day 7 onwards. However, stem cell gene marker expressions were consistent throughout the days of treatment. The control samples showed no differentiation. The findings suggest that keratinocyte co-culture has the potential to induce keratinocyte differentiation of the HAMSCs.

F-1036

CIGARETTE SMOKE REDUCES VIABILITY, DIFFERENTIATION CAPACITY AND MIGRATORY ABILITIES OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN VITRO

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Cigarette smoking is a major risk factor for the leading morbidity and mortality causes including cardiovascular diseases, COPD, rheumatoid arthritis etc. Exposure to cigarette smoke (CS) leads to unregulated inflammation, tissue destructions, induced autoimmunity and decreased regenerative capabilities within the affected organs and organ systems. Stem cell-based therapies are among the most promising therapeutic options for several diseases. Understanding the impact of cigarette smoke CS on stem cell function would be valuable for further therapeutic strategies and preventive measures in disease managing. We have assessed an impact of cigarette smoke extract (CSE) on bone marrow-derived mesenchymal stem cells (BM-MSC) in vitro. Commercially available CSE was applied on BM-MSC and cell viability, level of apoptosis, MMP's expression, migratory traits and differentiation capabilities were investigated. CSE exposure has induced a decrease in viability as detected by WST-8 metabolism and LDH leakage assays. In agreement, early signs of apoptosis

and reduced migration have been detected in CSE-exposed cells. However, expression of MMP-2/-9/-14, utilized in mobilization and invasion was found up-regulated. Differentiation capabilities were also impaired. Our results suggest that CSE exposure impairs BM-MSC responses necessary to act on the stage of tissue injury and might lead to the exacerbation of the damage. Our findings contribute to the understanding of the regenerative abilities of mesenchymal stem cells and their susceptibility to noxious substances. This might help shaping the development of stem cells-based therapy approaches in the future.

F-1037

REPROGRAMMING ADULT MESENCHYMAL STEM CELLS INTO FUNCTIONAL HEMATOPOIETIC STEM CELLS USED FOR THE INDIVIDUAL TREATMENT OF APLASTIC ANEMIA

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Although hematopoietic stem cell (HSC) transplants are routinely used to treat patients with blood diseases, challenges remain, including obtaining enough HSCs to ensure optimal engraftment, and avoiding immune rejection and other complications associated with allogeneic grafts. Novel approaches to generate clinical-grade HSCs are therefore needed to overcome limitations of current approaches. We report here that about 90% of 2-3 X108 human AD-MSCs that are CD44+,CD29+, CD105+, CD73+, CD166+, CD133-, CD45-, CD38-, CD34- could rapidly transdifferentiate into hematopoietic stem cells (CD49f+/CD133+/CD34+) in four days and their descending blood cells in vitro, after transfected with two small RNAs in the presence of special cytokines. The sRNAs were high-effectively delivered into MSCs by a novel peptide means. These adipose-derived HSCs (AD-HSCs) could form different types of hematopoietic colonies as nature-occurring CB-HSCs did. Upon the primary and secondary transplantation into sublethally irradiated NOD/SCID mice, these MSC-HSCs engrafted for long term and differentiated into all hematopoietic lineages such as erythrocytes, lymphocytes, myelocytes and thrombocyte. Furthermore, we demonstrated the first evidence that the rapid transdetermination of MSCs was induced by transfection of sRNA-EID1 through activation of CBP/p300 related transcriptional factors. More excitedly, these MSC-derived HSCs can reconstitute hematopoietic function in eight patients with severe aplastic anemia. At a median follow-up of 381 days (Ranging from 126 to 718), Of them, five patients got complete remission, becoming transfusion-independent and achieved hematopoietic reconstitution after the transplant. Two patients achieved partial and short remission, whereas only one recipient relapsed and died about seven months after transplantation because of tuberculosis infection. Conclusion: our findings identify the sRNA-EID1 that dictates a directed transdifferentiation of MSCs toward HSCs, create a new source of HSCs used for the individual treatment of aplastic anemia and cancers, and avoid complex bone marrow match and many complications post-transplantation.

F-1038

PROTEOMIC PROFILING OF ADIPOGENIC DIFFERNTIATION OF HUMAN MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSC) are multipotent, self-renewing cells with the potential to differentiate in vitro into cells of the mesodermal lineage such as adipocytes. They are thus the target of a multitude of approaches aiming to develop and enhance cell based therapy and tissue engineering. MSC are used further as a model to study developmental processes. In this project we profiled the differentiation of MSC into adipocytes over the course of 3 weeks using quantitative high resolution mass spectrometry-based proteomic analysis combined with stable isotope dimethyl labeling to in-depth characterize the differentiation pathway. Prior to proteomic analysis, human bone marrow- derived MSC from three independent donors were differentiated using commercial adipogenic medium, following the differentiation by classic Oil red staining.

F-1039

ACCELERATING THE YELLOW TO RED BONE MARROW TRANSITION

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Worldwide, more than 50.000 bone marrow transplantations are performed annually, although the mortality rate still is close to 50% within the first three years after allogeneic transplantation. Forty percent of these fatalities relate to the patients being severely immune compromised during the post-ablation period, before the graft has fully reconstituted the hematopoietic system. Reducing the time of engraftment is therefore critical to increasing the chance of survival in these patients. Preventing adipocyte formation, the most abundant cell type in the human bone marrow, in the post-transplant period has been demonstrated to accelerate hematopoietic stem cell (HSC) engraftment and subsequent hematopoietic recovery in mice. We are interested in understanding the kinetics and molecular mechanisms of the highly plastic transition between yellow (adipocytic) and red (hematopoietic) bone marrow. Bone marrow adipocyte formation was measured in vivo after HSC transplantation and correlated with the kinetics of hematopoietic recovery. After HSC transplantation adipocytes infiltrate the bone marrow reaching maximum expansion after 10 to 15 days. Bone marrow hematopoiesis is recovered after around 25 days, which is consistent with the exit of severe neutro- and thrombocytopenia on day 30 and 35, respectively, and recovery of pre-transplant cell blood counts by day 45. In order to uncover molecular mediators of the yellow to red transition, we have developed a high-throughput 2D in vitro Mesenchymal Stromal Cell (MSC) culture system. Using a label-free technique, Digital Holographic Microscopy, we quantified adipocytic differentiation based on real-time lipid accumulation and screened the Prestwick library of FDA-approved drugs and natural compounds for inhibitors of adipogenesis. Enhancement of hematopoiesis by these anti-adipogenic candidates is then tested

via a novel 3D in vitro HSC/MSC co-culture system using the bone marrow-derived line OP9 and primary murine hematopoietic stem and progenitor cells simultaneously. All current clinical approaches to enhance hematopoiesis target the HSC itself. Here we propose targeting bone marrow adipogenesis as an alternative strategy to accelerate the yellow to red bone marrow transition thereby improving post transplant survival.

F-1040

MIR 410 INHIBITION FACILITATES AN INDUCTION OF RETINAL PIGMENT EPITHELIUM FROM UMBILICAL CORD BLOOD DERIVED MESENCHYMAL STEM CELLS

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The retinal pigment epithelium (RPE) is one of the major component of the eye. This highly specialized cell type facilitate the maintenance of the visual system. Because their loss induces an irreversible visual impairment, RPE generation techniques has recently investigated as a potential therapeutic approach for RPE degeneration. A microRNA-based technique is a new strategy for producing RPE cells from adult stem cell sources. Previously, we identified that antisence microRNA-410 (anti-miR-410) induces the RPE differentiation from amniotic epithelial stem cells. In this study, we investigated the RPE differentiation from umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) via treating anti-miR-410. We identified miR-410 as a RPE-relevant microRNA in UCB-MSCs among 21 putative human RPE-depleted microRNAs. Inhibition of miR-410 induces overexpression of immature and mature RPE-specific factors, including OTX2, RPE65, Bestrophin and EMMPRIN. These RPE-induced cells were able to phagocytize microbeads. Our microRNA-based strategy demonstrated the proof of principle for RPE differentiation in UCB-MSCs by anti-miR-410 treatment, without the use of additional factors or exogenous transduction. These findings suggest that miR-410 inhibition can be a useful tool for directed cell differentiation and an attractive method for allogeneic cell therapy in human retinal degenerative diseases.

F-1041

RAT PROXIMAL FEMUR MSCS READILY DIFFERENTIATE INTO ADIPOCYTES, WITH LIPID ACCUMULATION REGULATED BY DIFFERENT PATHWAYS FROM THOSE OF SUBCUTANEOUS ADIPOSE-DERIVED STEM CELLS

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Osteoporosis may be caused, at least in part, by aberrant differentiation of bone mesenchymal stem cells away from an osteoblastic phenotype towards adipogenesis. This depletes the pool of osteoblasts that maintains bone integrity. We therefore examined the propensity of MSCs from different bone depots to accumulate lipid, as this might indicate which cells contribute to fatty bones seen at the end stage of the disease. Both cortical (hard shaft) bone (cb) and proximal end (pe) (trabecular and hard) bone were denuded of cells by collagenase digestion. Macerated bone was then incubated in culture medium until cells migrated from the fragments after a week. These cells were compared to bone marrow (bm) MSCs by flow cytometry. The single population cbMSCs and bmMSCs exhibited high expression (>95%) of CD90 (naïve mesenchymal marker) and CD106 (bone MSC marker), but very low expression (<3%) of CD45 (naïve haematopoietic marker) and CD26 (fibroblast marker). peMSCs were ≥90% positive for CD90, CD106 and CD26 and 23% positive for CD45, suggesting a substantial difference between the MSCs from different bone depots. Both bmMSCs and cbMSCs differentiated into osteoblasts within 7 days and into adipocytes within 21 days. In contrast, peMSCs differentiated rapidly (≤ 7 days) into adipocytes, yet required 21 days to differentiate into osteoblasts, similar to adipose-derived stem cells (ADSCs). Comparing lipid accumulation between peMSCs and ADSCs by omitting components of the adipogenic differentiation media, it was found that whereas ADSCs have an essential requirement for dexamethasone (Dex, a glucocorticoid) in the media for adipogenesis, deletion of Dex only partially (~50%) inhibited lipid accumulation in peMSCs. Furthermore, the effect of omission of both indomethacin (a PPARg agonist) and Dex was additive only in peMSCs, suggesting that in these cells the regulation of lipid accumulation by Dex may involve a separate pathway from that of PPARg. Lipid accumulation in peMSCs is therefore substantially different from bmMSCs, cbMSCs and ADSCs.

F-1042

HEPATICALLY DIFFERENTIATED HUMAN SHED IN ANIMAL MODEL

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Our goal is to transplant in vitro hepatically differentiated CD117+ stem cells from human exfoliated deciduous teeth (SHED) into rats and to prove that the hepatically differentiated cells reproduces human hepatic tissues including blood vessels and bile ducts in vivo. CD117+ fraction was separated by MACS from SHED cell cultures established previously. To induce hepatic differentiation DMEM was supplemented with ITS-x, embryotrophic factors and hepatocyte growth factor for 5 days, following, oncostatin and dexamethasone were added to the culture medium for another 15 days to promote maturation of hepatically induced cells. Human hepatically-specific markers were examined after differentiation by fluorescence. Urea in culture media was measured by ELISA. Glycogen was visualized by PAS staining. Twelve F344-rats were subjected to 90% liver resection. Hepatically differentiated cells were transplanted in the spleens (1×107 cells/animal) of six of the animals. The livers and spleens were collected 40 days after the transplantation. The hepatic markers in the liver were tested by histochemistry. Expression of human albumin in rats' livers was proven by in situ hybridization. Concentrations of human albumin, α-feto-protein (aFP) and IGF-1 in serum were determined. After in vitro hepatic differentiation around 90% of cells were deemed as hepatic-like; aFP, albumin, IGF-1 and HNF4-α were all shown to be positive by fluorescence. The concentration of urea in the media increased ($p<0.05$). Cell clusters expressing human-specific hepatic markers were found in the rats' livers and spleens by fluorescence. Human specific albumin, aFP and IGF-1 were found in rats' serum. Hepatocyte-like cells differentiated in vitro were transplanted into the rats and they functioned as

human hepatocytes. SHED may therefore be ideal cell source for cell-based therapy of patients requiring liver transplantation.

F-1043

DEVELOPMENTAL ENGINEERING OF CARTILAGE TEMPLATES FOR ENDOCHONDRAL OSSIFICATION USING MOUSE MESENCHYMAL STEM CELLS

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Mesenchymal stromal/stem cells (MSC) are able to give rise to various tissue types upon lineage specific differentiation conditions, including cartilage, which is our main focus of research. In spite of recent progress made in cartilage generation in vitro, the endurance of the cartilage engineered in vivo is still challenging. Part of the problem is due to three aspects: 1) The heterogenous nature of MSC, which harbors a minor and variable fraction of "stem" cells and early progenitors; 2) in vitro expansion of MSC that compromise their pluripotency; 3) Cartilage differentiation protocol. Here, we characterized in detail the freshly isolated adult mouse endosteum-derived MSC Sca-1+ PDGFR-α+ population (PαS) as previously described by Prof. Matsuzaki's group, since these cells proved to be the most robust in tri-lineage differentiation assays. We identified four subsets within PαS MSC based on the combined analysis of CD73 (ecto-5'-nucleotidase) and CD90 (Thy1) markers. These subsets exhibit differential capacities to give rise to various lineages upon triggering differentiation in vitro. We ontogenically determined the first appearance of PαS MSC during embryonic limb development and identified a hierarchical relationship between the four subsets of PαS MSC and their potential to differentiate into the tri-lineages. Furthermore, by applying a protocol based on molecular programs operating in mouse embryonic limb development, we generated cartilage templates from these subsets without undergoing hypertrophy during the time of the study. These templates were assessed in detail for their potential to trigger endochondral ossification in vivo upon subcutaneous engraftment in nude mice. As a result, the MSC subsets harbor a differential capacity to follow endochondral route to form bone, which is able to support host-derived hematopoiesis.

F-1044

MYOD MEDIATES MYOGENIC DIFFERENTIATION POTENTIAL OF EQUINE ADIPOSE-DERIVED STEM CELLS

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Mesenchymal stem cells have multiple lineage differentiation potential like adipocytes, osteocytes, chondrocytes and myocytes. Adipose derived stem cells are easily collected from a lot of adipose tissue also it has similar property of mesenchymal stem cell surfaces marker. Lately, adipose derived stem cells are notable researching for regenerative medicine both in vivo and in vitro. In this study, we induced myogenic differentiation through myogenic determination 1(MyoD) with lentiviral transduced system. First of all, we constructed plasmid of MyoD using Pljm1-GFP plasmids. Also Pljm1-GFP plasmids are used as negative control in this work. Pljm1 vector has puromycin resistance region so we could select well transduced of MyoD and GFP cells. When transduced cells are fully proliferated in the dishes, changed media condition growth media to differentiation media with low concentration serum. After that, the transduced EqASC-MyoD cells were formed myotube like cells by cell fusion. In RT-PCR and immunocytochemistry results, we detected late differentiation stage marker such as troponin I and myosin heavy chain. In conclusion, when MyoD induced at the equine adipose derived stem cells, the cells had myogenic potential and changed media condition to differentiate of muscle, it seems like myotube formation. From these results, adipose stem cells are remarkable resources to clinics and also equine muscle diseases will be overcome through MyoD enhanced equine adipose derived stem cells.

F-1045

EPHB2 REGULATES NANOG EXPRESSION TO MAINTAIN DIFFERENTIATION POTENTIAL OF UMBILICAL CORD BLOOD DERIVED MESENCHYMAL STEM CELLS VIA DNMT1-MEDIATED P21 PROMOTER HYPERMETHYLATION

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Eph/ephrin system as a possible regulator of stem cell functions is involved in cell-cell and cell-microenvironment interactions in the stem cell niche. However, the role of Eph-ephrin in regulating MSCs functions has not been fully understood yet. Therefore, the present study investigated the role of EphB signaling as a regulator of maintaining MSCs functions. We found that EphB2 and ephrinB2 are expressed mainly in umbilical cord blood derived MSCs (UCB-MSCs). Treatment with pre-clustered ephrinB2-Fc (0.5 µg/mL) induced phosphorylation of EphB2 and increased Nanog expression known as one of the pluripotency genes in maintaining MSCs properties, but not Sox2 and Oct4 expression. Pre-clustered ephrinB2-Fc also induced phosphorylation of Akt (Ser 473 and Thr 308)/mammalian target of rapamycin (mTOR), and inhibition of Akt/mTOR attenuated EphB2-ephrinB2 signaling axis-mediated Nanog expression in UCB-MSCs. The functional role of EphB2-ephrinB2 signaling in regulation of UCB-MSCs growth was confirmed with assay for proliferation and population doubling time determination. In addition, differentiation potential of UCB-MSCs into adipogenic, chondrogenic, and osteoblastogenic lineages was reduced by EphB2 silencing. Notably, EphB2-facilitated Nanog expression contributed to increasing Dnmt1 expression in UCB-MSCs and the Dnmt1 induced the hypermethylation of cyclin dependent kinase inhibitor p21 promoter. But silencing EphB2 expression by siRNA increased not only p21 expression level but also the mRNA of genes associated with the developmental markers (Pax6, Gata4, Gata6, Sox17, and FoxA2), germline markers (Stella, Dazl, Vasa, and Scp3), and tissue-specific genes (Nestin, Nkx2.5, and cTn1), causing spontaneous differentiation of UCB-MSCs. These findings identify the EphB2 signaling as a target of enhancing therapeutic benefits in regard to maintaining UCB-MSCs functions. In conclusion, the EphB2-ephrinB2 signaling regulates Nanog expression to support the differentiation potential of UCB-MSCs via Dnmt1-mediated p21 promoter hypermethylation.

F-1046

IMAGE-BASED CELLULAR MORPHOLOGICAL PROFILING FOR SCREENING CHEMICAL COMPOUNDS IN STEM CELL CULTURE

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By the rapid advances in stem cell applications, various types of culture methods/protocols have been developed for specialized cell culture. Small chemical compounds are starting to be screened as candidate molecule to regulate the functions of stem cells as medium additives. To eliminate the risk of unexpected infectious risks and quality instability of animal-derived crude materials as medium additives, such chemical molecules that can be produced and tested with strict quality check has been required strongly for stem cell applications for clinical therapies. Also from the aspect of reducing the cost of biological large molecules as medium additives, such small molecules have a great potential to reduce the cost of products by their stable large-scale production technologies. However, to test the efficacy and functionality of such small molecules on stem cells, the conventional cell assay technologies had been facing a great difficulty. Since stem cell culture requires long in vitro culture, conventional molecular-biological evaluation techniques, such as fluorescent staining techniques, have been found to be limited to assay end-point reactions because of their invasive manner, and lose large amount of information that describes cellular responses during their culture. To acquire more delicate and continuous cellular responses to small molecule additives, we have invented non-labeling image-based morphological analysis technique by combining image processing and bioinformatic algorithms. We here show the profiling effects of our morphological cellular responses to six chemical compounds that are known to inhibit cascades of gene network including ROCK inhibitor for regulation of adipogenic differentiation culture of bone-marrow derived mesenchymal stem cells. By our method, we succeeded in profiling the similar chemical compound effect from early stage of cellular morphologies without any staining consumption.

F-1047

THERAPEUTIC POTENTIAL OF HUMAN AIRWAY STEM CELLS: MICROARRAY ANALYSIS OF MULTILINEAGE DIFFERENTIATION

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Human airway stem cells (human nasal inferior turbinate derived mesenchymal stem cells, hTMSCs) are attractive source of adult stem cells for therapeutic application because they can be easily obtained and cultivated with a highly proliferative capacity. They have been shown to differentiate into other cell types of the mesenchymal lineage and also into non-mesenchymal cells. The ability of hTMSCs to differentiate into chondrocytes, osteocytes, and neural cells makes them potential replacement therapeutic candidates in intractable disease. However, it is not fully clear whether multilineage differentiation (osteogenesis, chondrogenesis, and neurogenesis) of hTMSCs is associated with a specific gene expression pattern. In the present study, we investigated the gene expression pattern of representative transcription factors and marker genes along those three lineages during a particular lineage differentiation of hTMSCs by means of cDNA microarrays. Using high-density oligonucleotide micro-arrays, we obtained the differential gene expression profile among the different differentiated hTMSCs. In addition, a direct comparison of this microarray-generated transcriptome with the published serial analysis of gene expression data suggests that a molecular context of differentiated hTMSCs is more similar to that of differentiated several kinds of adult cells. Altogether, our results will provide a basis for studies on molecular mechanisms controlling core properties of hTMSCs. Taken together, our results here provide an hTMSCs-specific genetic catalog that may facilitate future studies on molecular mechanisms governing core properties of these cells.

F-1048

OSTEOGENESIS OF OSTEOGENIC CELL SHEET FROM CANINE ADIPOSE DERIVED MESENCHYMAL STEM CELLS

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Through years of research, it was proven that the utility of canine adipose-derived mesenchymal stem cells (Ad-MSCs) for bone regeneration have effective therapeutic potentials. The osteogenic cell sheet was reported as a new method for transplantation of cultured canine Ad-MSCs. We studied the osteogenic differentiative ability of osteogenic cell sheet for the appropriate application time for therapeutic use. Canine Ad-MSCs were cultured in proliferation medium, osteogenic differentiated medium, and osteogenic cell sheet medium. We evaluated the cells at 4, 7, 14 and 21 days. Runt-related transcription factor-2 (RUNX-2), alkaline phosphatase (ALP), osteopontin and bone morphogenetic protein-7 (BMP-7) mRNA expressions were evaluated using semi-quantitative polymerase chain reaction. The osteoblastic activity was evaluated through the ALP activity colorimetric assay kit and the ALP direct staining at 4, 7 and 14 days. In osteogenic differentiated cells and osteogenic cell sheet, RUNX-2 mRNA expressions were upregulated at 4 days, ALP mRNA expressions were upregulated at 7 days, osteopontin and BMP-7 mRNA expressions were upregulated at later stage of the culture (14-21 days) compared to undifferentiated cells. Especially osteopontin mRNA level was highly upregulated at 21 days in osteogenic cell sheet. The osteogenic differentiated cells and osteogenic cell sheet show positive ALP staining compared to undifferentiated cells at 4 and 7 days, and osteogenic cell sheet show higher positive ALP staining after 14 days. Our data suggest that osteogenic cell sheet from canine Ad-MSCs maintained in vitro osteogenic potential. The cells cultured in osteogenic cell sheet medium between 14 - 21 days might be available for therapeutic use.

F-1049

SUPPRESSION OF POLYAMINE BIOSYNTHESIS PROMOTES OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS AND STIMULATES BONE FORMATION IN OVARIECTOMIZED RAT MODEL

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Polyamines are a series of low molecular-weight polycations involved in various cellular processes such as proliferation, differentiation, and apoptosis. Correlation between polyamine metabolism and stem cell differentiation has been implicated in several studies. Our previous studies suggest that exogenous polyamines, including putrescine, spermidine, and spermine, as well as their biosynthetic inhibitor, alpha-difluoromethylornithine (DFMO), were capable of promoting osteogenic differentiation while suppressing adipogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs). In this study, we found that the level of intracellular polyamines in hBMSCs was reduced and polyamine biosynthesis was suppressed in the presence of exogenous polyamines as well as DFMO during osteogenic differentiation, suggesting that intracellular polyamine levels may be one of the key factors to regulate the differentiation fate of hBMSCs. In animal studies, we treated 15-week-old ovariectomized Sprague Dawley rats with putrescine, spermidine, spermine, or DFMO for 6 weeks. Seven weeks after treatment, trabecular thickness was increased in the treatment groups compared with the ovariectomized control. Scanning results of rat vertebrae using micro computed tomography (micro CT) also indicate an increase in bone volume in the presence of exogenous polyamines and DFMO. These results support that exogenous polyamines and DFMO play similar roles in directing the differentiation of hBMSCs, and are capable of stimulating bone formation in ovariectomized rats. Polyamines and their derivatives may thus be considered potential lead compounds in the development of new drugs for the treatment of skeletal disorders such as osteopenia and osteoporosis.

F-1050

EFFECTS OF SUBSTRATE STIFFNESS ON CARDIOMYOCYTE DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

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Enormous ongoing researches aim to understand the proliferative and regenerative characteristics of stem cells developed from various sources due to their potential applications to tissue engineering, regenerative medicine, and cell-based therapies. Human mesenchymal stem cells (hMSCs) have become one of the most intensively studied cell types and can differentiate into a variety of mesodermal cell lineages, including adipocytes, chondrocytes, myocytes, and osteoblasts. The extracellular Matrix (ECM) can provide physical and biochemical supports or signals to the surrounding tissues and cells. The stiffness of ECM have been known that can affect cell behaviors, such as adhesion, cell spreading, proliferation, migration, differentiation, etc. However, the mechanism of hMSCs sensing rigidity or geometric cues is still unclear. The aim of this study is to figure out whether changing stiffness of substrates can make different influence on hMSC activities. We cultured hMSCs on flexible polyacrylamide sheets coated with type-I collagen. Varying the final concentration of acrylamide and bisacrylamide regulated the stiffness of polyacrylamide gel from 1 to 31 kPa. Proliferation, differentiation, and traction forces of hMSCs were determined by alarmaBlue® assay, quantitative PCR, and traction force microscopy respectively. Our results showed that hMSCs on stiffer polyacrylamide gels demonstrated better proliferation, morphology, spreading, and migration than those on softer gels. While the overall pattern of traction forces was similar for hMSCs on soft and hard substrates, cells on stiff gels generated significantly stronger traction forces than those on soft gels. In addition, the effect of substrate rigidity on the differentiation of hMSCs into cardiomyocytes was observed by examining the expression of the specific proteins of cardiomyocytes, such as MLC-2a and cTnT. During the differentiating process, the cardiac gene expression increased and the values of 31 kPa group were significantly higher than the values of other groups as well as coverslip group. Taken together, it indicates that substrate rigidity can regulate and affect hMSC behaviors, including cardiac differentiation.

F-1051

APOLIPOPROTEIN A-1 PROMOTES OSTEOGENESIS OF MESENCHYMAL STEM CELLS VIA STAT3, CXCL6, AND CXCL8

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Bone loss caused by osteoporosis leads to severe bone fracture. It is an important public health issue that occurs in 1/4 of the women and /1/8 of men. Current therapies do not prevent 1/3 of bone fractures induced by osteoporosis and some drugs have detrimental side effects like increasing the risk of developing cancer or cardiovascular disease. Thus there is an unmet need for new treatment of osteoporosis. By a high throughput screens with 12380 genes in primary human mesenchymal stem cells (MSCs), we identified Apolipoprotein A-1 (Apo A-I) as a positive regulator of bone formation as revealed by significantly elevated alkaline phosphatase (ALP) activity, which is an early functional marker of bone differentiation. In ovariectomized mice, which deprive the estrogen production, mimics the osteoporosis occurred in postmenopausal women, we found transgenic mice expressing Apo A-I in advance completely prevent osteoporosis through synchronously promoting bone formation and inhibiting bone resorption. Moreover, Apo A-I expression could be upregulated by the treatment of BET bromodomain inhibitor I-BET151 in vivo. Interestingly, I-BET151 can completed cure osteoporosis by concurrently promoting bone formation and inhibiting bone resorption. Of note, Apo A-I enhanced the expression of chemokines CXCL6/GCP-2 and CXCL8/IL-8 via STAT3 activation. A decrease in STAT3 activation or downregulate the expression of CXCL6/CXCL8 by chemicals or shRNA blocked Apo A-I-

mediated osteogenesis. In addition, overexpression of CXCL6 promoted osteogenesis. In freshly isolated MSCs, there is more cells expressed CXCL6 and its corresponding receptor CXCR2 in Apo A-I transgenic mice while compared with wild type mice. Apo A-I has been reported to block tumor formation, treat cardiovascular/Alzheimer diseases, increase insulin sensitivity, and inhibit asthma. Thus, Apo A-I is a promising new agent that may be employed to prevent or treat bone loss diseases with potentially fewer side effects and multiple beneficial effects.

F-1052

OSTEOGENIC DIFFERENTIATION OF ADIPOSE-DERIVED STEM CELLS COMBINED WITH ENDOTHELIAL CELLS IN CALCIUM PHOSPHATE SCAFFOLDS

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Adipose-derived stem cells (ASCs) have shown great potential for bone tissue engineering, particularly when combined with biomaterials such as calcium phosphate or hydroxyapatite. However, more studies are needed to develop the ideal combination of biomaterial/cellular components for bone repair.
Objectives: The aim of this study was to evaluate the role of endothelial cells (ECs) co-culture with ASCs combined to calcium phosphate cement for osteogenesis in vitro. ASCs were isolated from adipose tissue of Lewis rats by collagenase digestion and characterized for surface markers, cell proliferation and differentiation. Cells between passages 4 and 7 were associated with 3D scaffolds of calcium phosphate, with or without ECs of the EOMA line. Cell adherence was evaluated by counting non-adhered cells stained with Giemsa. Proliferation rates of ASCs in 2D and 3D conditions after 3-day incubation was determined using the MTT test. The osteogenic potential of ASCs combined with the calcium phosphate cement, with or without addition of EOMA cells, was determined by real-time RT-PCR analysis of the expression of bone marker genes. Rat ASCs showed morphology, immunophenotype, and proliferation and differentiation potential characteristic of mesenchymal stem cells. Adherence to the calcium phosphate scaffolds was close to 100%, in the two cell concentrations analyzed. ASCs proliferated less when associated with the scaffolds than in conventional 2D conditions. However, expression of bone markers was enhanced by cultivation of ASCs in association with calcium phosphate cement, in normal or osteoinductive media. The addition of endothelial cells to the system increased significantly the expression of bone marker genes, particularly when osteoinductive media was used in the cultures. These results show that the osteogenic potential of adipose-derived stem cells cultivated with calcium phosphate cement is increased by co-cultivation with endothelial cells. This co-culture system is relevant for the implementation of bone tissue engineering processes for clinical applications. This work was supported by MCTI/CNPq/MS Brazil.

F-1053

CHARACTERIZATION OF ENDOMETRIOTIC STEM CELLS ON ENDOMETRIOSIS PATIENTS IDENTIFIES CANCER STEM CELL PHENOTYPE

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Endometriosis associated ovarian cancers (EAOC) is a rare but a possible molecular event occurring among few endometriosis patients. The importance of better understanding of the carcinogenic linkage between this common gynaecologic disorders is undebatable. In the context of its stem cell origin, we hypothesize that endometrial Mesenchymal stem cells (eMSC) as a consequence of altered microenvironment, may undergo de-differentiation into endometrial cancer stem-like cells (CSCs) which might act as an early event of tumor initiation in EAOC patients. We obtained paired Endometrium and Endometriotic cyst biopsies (n=11, during timed surgery), unpaired (n=7) from premenopausal women diagnosed with endometriosis and endometrial biopsy from healthy volunteers (n=17). eMSCs were isolated from both sites using standardized enzymatic method and sorted by flow cytometry for MSC markers CD90, CD73 and CD105. Sorted eMSCs were expanded and utilized for exploring CSC phenotype in subset of stem cells. Firstly, we observed an unique G1/S cycling distribution in some of our patient's eMSCs as previously reported with CSCs, when incorporated with 5-Bromodeoxyuridine into DNA of newly dividing cells. Then, we performed RT-PCR gene expression array using panel of markers reported for CSCs in Endometrial and Ovarian cancer. We found a possible linkage to cancer development by identifying a subset of patient groups who exhibited increased expression of several CSC markers, genes involved in cancer metabolic pathway and epithelial mesenchymal transition. Specifically, these patients also presented a significant enrichment of reprogramming genes when grown as 3D Spheroids. We confirmed the clonal expansion of specific CSC markers expression in these patients using flow cytometry and confocal imaging. This study opens up a new mechanism for understanding the etiology of EAOC and other benign gynecological disorders.

F-1054

DIRECT CONVERSION OF MURINE ADIPOSE STROMAL CELLS INTO ACINAR CELLS USING A CO-CULTURE SYSTEM

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A loss of salivary gland function often occurs after radiation therapy in head and neck tumors, though secretion of saliva by the salivary glands is essential for the health and maintenance of the oral environment. Transplantation of salivary acinar cells (ACs), in part, may overcome the side effects of therapy. Here we directly differentiated mouse adipose-derived stromal cells (ADSCs) into ACs using a co-culture system. Multipotent ADSCs can be easily collected from stromal vascular fractions of adipose tissues. The isolated ADSCs showed positive expression of markers such as integrin beta-1 (CD29), cell surface glycoprotein (CD44), endoglin (CD105), and Nanog. The cells were able to differentiate into

adipocytes, osteoblasts, and neural-like cells after 14 days in culture. ADSCs at passage 2 were co-cultured with mouse ACs in AC culture medium using the double-chamber (co-culture system) to avoid mixing the cell types. The ADSCs in this co-culture system expressed markers of ACs, such as alpha-amylases and aquaporin 5, in both mRNA and protein. ADSCs cultured in AC-conditioned medium also expressed AC markers. Cellular proliferation and senescence analyses demonstrated that cells in the co-culture group showed lower senescence and a higher proliferation rate than the AC-conditioned medium group at Days 14 and 21. The results above imply direct conversion of ADSCs into ACs under the co-culture system; therefore, ADSCs may be a stem cell source for the therapy for salivary gland damage. *Supported by grants from NRF funded by MEST (2006-2004042 and No. 2014050477 through the Oromaxillofacial Dysfunction Research Center for the Elderly at Seoul National University) and MAFRA (111160-4) of Korean Government.*

F-1055

IN VITRO AND IN VIVO STEM CELL CHARACTERISTICS OF HUMAN DENTAL FOLLICLE AND PERIODONTAL LIGAMENT CELLS ISOLATED USING TWO DISTINCT METHODS

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Cells derived from human periodontal ligament (PDL) and dental follicle (DF) are alternative stem cell sources because of their attractive multidifferentiation potentials. However, the desirable isolation method to obtain these cells remains under discussion. In this study, we compared the stem cell properties of the above two cell types isolated using the two most widely used isolation procedures. To investigate the in vitro and in vivo stem cell characteristics of human DF and PDL cells isolated by enzyme digestion (EZ) and outgrowth (OG) procedures. Human DF and PDL cells were collected from the extracted third molars of healthy volunteers (age range, 15–29 years). For collecting the enzyme-digested DF (DF-EZ) and PDL (PDL-EZ) cells, tissues were first treated using a collagenase/dispase solution. Then, 1×10^5 cells were seeded onto 10-cm-diameter cell-culture dishes. For collecting the outgrown DF (DF-OG) and PDL (PDL-OG) cells, the tissues were cut into small pieces, cultured in dishes containing Dulbecco's modified Eagle medium (DMEM/F12) with 15% fetal bovine serum, and then subcultured. Cells at passage 3 or 4 were used for all the experiments. For in vitro characterization, cell proliferation, cell surface markers, gene expressions, and multilineage differentiation capacity were examined. For in vivo experiment, the cells were transplanted into severe combined immunodeficiency (SCID) mice and analyzed by histological and immunohistochemical observations. DF cells exhibited higher proliferative potential than that by PDL cells. Endothelial adhesion and pericyte marker levels were significantly higher in EZ-treated cells than in OG-treated cells ($p < 0.05$). Gene expression for PDL and cementum markers was higher in PDL than in DF cells. Further, EZ-treated cells showed increased rate of differentiation into mature osteogenic and adipogenic lineages than that by OG-treated cells in vitro. Histological analysis showed that all treated cells formed the cementum-like tissue in vivo. In particular, PDL-OG transplants formed cellular cementum-like tissues. Our results suggest that both the cell types are candidates for periodontal tissue and tooth root regeneration. Notably, PDL-OG might be the most competent cells for cementogenesis.

F-1056

EXPRESSION OF TYPE 1 ANGIOTENSIN RECEPTOR DEFINES A SUBPOPULATION OF ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS COMMITTED TO ADIPOSE DIFFERENTIATION

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Recently renin-angiotensin system (RAS) was shown to be involved in the regulation of fat mass. However, mechanisms underlying the effect of RAS on adipogenesis, in particular on adipogenic differentiation of adipose-derived mesenchymal stem cells (ADSCs) remains poorly understood. Previously, we found the expression of angiotensin receptors (AGTRs) on human ADSCs. This study was aimed on functional characterization of cells expressing AGTR1. ADSCs were isolated from subcutaneous fat tissue of healthy young donors (n=5) and analyzed at the next day after isolation as well as upon culturing up to 5 passages. mRNAs encoding AGTR1 and 2 in ADSC were analyzed using real-time PCR. Activity of AGTR1 was analyzed by the ability of its specific agonist to activate Ca2+ mobilization in single cells using Fluo-8 probe. Subpopulations of ADSCs expressing AGTR1 were evaluated by flow cytometry and purified using FACS. ADSC phenotype characterized by flow cytometry was CD90+/ CD73+/ CD105+/ CD45-/ CD31- for all samples and these cells were capable of adipogenic and osteogenic differentiation. We found that these cells contained mRNAs of AGTR1 and AGTR2. Total population of ADSCs contained 2.2 ± 1.06 % of cells expressing AGTR1. Moreover, the proportion of cells carrying this receptor does not change during passages, but at 5th passage we also identified additional population of ADSCs with high expression of AGTR1 (AGTR1bright). ADSC functional analysis with Fluo-8 Ca2+ indicator and specific antagonist showed that activation of AGTR1 on ADSCs induces Ca2+ mobilization. Motility and proliferation of ADSC expressing AGTR1 were 1.3 and 2.5 times lower comparing to AGTR1-negative population. Evaluation of the differentiation potential revealed that AGTR1-expressing subpopulation differentiated into adipocytes more rapidly, which may indicate that these cells are committed to adipose differentiation. Taken together, our data indicate that human ADSCs contain a small subpopulation of cells expressing AGTR1. These are committed to adipogenic differentiation, which might be a target for RAS regulation of adiposity. This study was supported by RSF grant 14-15-00439.

F-1057

PHASE 1 STUDY TO EVALUATE THE SAFETY AND EFFICACY OF INTRAMUSCULAR HUMAN PLACENTA-DERIVED CELLS (PDA-002) IN SUBJECTS WHO HAVE DIABETIC FOOT ULCER WITH PERIPHERAL ARTERY DISEASE

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PDA-002 is a mesenchymal-like cell population derived from normal, full term, human placenta. PDA-002 has demonstrated the ability to secrete angiogenic factors, and improve blood flow in animal ischemia models. For subjects who have DFU with PAD, therapeutic angiogenesis using PDA-002 offers a promising therapeutic option. The primary objective of this study was to assess the safety of PDA-002 in subjects who have DFU with PAD. Subjects were enrolled sequentially into each of 4 dose cohorts (3×10^6, 10×10^6, 30×10^6, and 100×10^6 cells) administered intramuscularly on Day 1 and 8. The study population included subjects with diabetes, PAD (defined as ABI>0.5-≤0.9 or TBI >0.35-≤0.7), and a DFU (Wagner Grade 1 full thickness only or Grade 2) present for at least 1 month despite standard of care. Fifteen subjects were enrolled in the study (n=3 for cohorts 1-3, and n=6 for cohort 4). The mean duration of DFU prior to study enrollment was 60.6 weeks. No subjects met the study stopping rules or the criteria for a dose limiting toxicity. There were no treatment-related SAE's. There was 1 non-treatment related death. Three months efficacy data were available for 15 subjects at the time of abstract submission. Five subjects had complete healing, and 2 subjects had approximately 50% healing of their index ulcer within 3 months. The mean increase in ABI from screening to prior to dosing was 0.003. The mean increase in ABI at 3 months following dosing was 0.16. PDA-002 was safe and well tolerated in subjects who have DFU with PAD. Subjects with chronic DFU that did not previously respond to conventional therapy experienced healing of their ulcers and increases in their ABI after treatment with PDA-002. A placebo-controlled Phase 2 study (NCT02264288) has been initiated to evaluate PDA-002 in subjects who have DFU with PAD.

F-1058

NEW MESENCHYMAL POPULATION OF MURINE BONE MARROW CAN REPAIR THE INJURED SCIATIC NERVE

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Mesenchymal stem cells (MSCs) in bone marrow have been believed to regenerate various mesenchamal tissues, such as bone, cartilage, muscle and adipose. Bone marrow stromal cells (BMSCs) are composed of many kinds of cells including MSCs, and they have been reported to regenerate nerve tissue. To clarify the character of MSCs, it is essential to purify MSCs. However, there was not any established method to isolate them. Recently, we have established a new method to purify bone marrow-derived populations showing a high frequency of osteoprogenitors, thus named them highly purified osteoprogenitors (HipOPs). Although our previous studies demonstrated that HipOPs enriched with MSCs have a high differentiation potential into osteoblasts, chondrocytes and adipocytes compared to BMSCs, their differentiation potential for neural cell lineage was still unclear. In this study, we evaluated the efficacy of HipOPs in promoting neural cell lineage. BMSCs were collected from C57BL/6J mice femurs and tibiae, and HipOPs were sorted from BMSCs by negative selection using magnetic beads technique. BMSCs and HipOPs were cultured in the presence of basic fibroblast growth factor and epidermal growth factor. HipOPs generated frequent large neurospheres compared to BMSCs. The frequencies of neural progenitors in BMSCs and HipOPs were evaluated with limiting dilution assay. The frequency of neural progenitors in HipOPs was 1/402, whereas that of BMSCs was 1/48990 (t=36.91, p<0.001). To examine the repair efficacy of HipOPs for injured nerve system in vivo, we established the evaluation method of repairing efficacy. We cut and removed a 1.5mm section of the left sciatic nerve of Crlj:CD1-Foxn1nu mice because a clean cut nerve repaired partially by itself within a few months. Collagen sponges with 5×106 HipOPs or no cells were put into the 1.5mm inter-nerve gap. Transplanted mice were tested with von Frey filament and practiced behavior observation at 2, 4, 6 and 8 weeks after the operation. The repairing efficacy with the HipOPs transplantation showed much better on the von Frey test (p=0.002) and the behavioral test (p=0.000043) than the control. The HipOPs fraction is a useful resource of neural cell lineage to repair neural injury.

F-1059

THE EFFECT OF MAST CELL DERIVED EXOSOMES ON HUMAN BONE MARROW MESENCHYMAL STEM CELLS MIGRATION

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Mesenchymal stem cells (MSCs) have the ability of self-renewing and multilineage potential, and can migrate to injured or inflamed tissues. However, the mechanisms underlying migration of MSCs still remain poorly understood. Mast cells are important cells regulating inflammation, beyond allergy, and release multiple potent mediators as well as extracellular vesicles such as exosomes. Exosomes are membranous nanovesicles of 30 to 100 nm in size, which are suggested to carry communications between cells. The aim of this study was to determine whether mast cell derived exosomes can influence migration of human bone marrow MSCs. Human bone marrow MSCs were expanded in vitro and used for experiments in early passages. Mast cell exosomes were isolated from culture media using differential centrifugation protocols. In vitro migration and invasion assays toward exosomes were performed with Boyden chamber. In vitro scratch assay was performed in monolayers of MSCs. The expression of matrix metalloproteinase (MMP) was analyzed by gelatin zymography. Gene expression in MSC with and without treatment of exosomes was analyzed by real time PCR. MSCs migrated toward mast cell-derived exosomes in a dose dependent manner, and scratch closure was more complete after mast cell exosomes exposure. The release of MMPs and TGF-beta into the supernatant by MSCs was increased after exosomes exposure compared with untreated cell. Furthermore, the stimulation of mast cell exosomes influences migration related gene expression in MSCs. Mast cell-derived exosomes can induce migration of MSC and induce release of MMPs involved in the invasion process. The TGF-beta production of MSC was up-regulated by treatment of mast cell exosomes, which could be associated with the immune regulatory function of MSC in inflammatory diseases.

F-1060

THERAPEUTIC POTENTIAL OF BONE MARROW-DERIVED, ALLOGENEIC MESENCHYMAL STROMAL CELLS (STEMPEUCEL®) FOR PATIENTS WITH CRITICAL LIMB ISCHEMIA DUE TO BUERGER'S DISEASE

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Thromboangiitis obliterans, is a non-atherosclerotic, segmental inflammatory disease that most commonly affects the small and medium-sized vessels. CLI is a severe form of the disease that results in rest pain and non-healing ulcers and gangrene. Approximately 40% to 50% of "no-option" patients will undergo amputation within 6 - 12 months and approximately 15% will also require contralateral amputation within 2 years. Stempeucel® is manufactured from BMMSC obtained from healthy volunteers and the product is comprised of pooled allogeneic BMMSCs obtained from multiple donors. Stempeucel® express MSC-associated surface markers, differentiate into bone, cartilage and adipose cells, possess potent immunosuppressive activity and secrete various angiogenic factors including VEGF, angiopoietin, IL8 and HGF. Preclinical studies have demonstrated that stempeucel® is non-toxic and non-tumorigenic. Administration of the product ameliorates limb necrosis, promote blood flow and and prevent limb loss in a mouse model of hind limb ischemia. We have recently completed a phase II, clinical trial in patients with Buerger's disease (NCT01484574) with two doses of stempeucel® (1 and 2 million cells/kg body weight). 36 patients each were enrolled in the cell arms and 18 patients in the control arm who received standard protocol of care (SPOC). The cells were injected intramuscularly around the gastrocnemius muscle and around the ulcer. At six months follow up data revealed that both the primary end points (relief of rest pain & healing of ulcers) were significantly better in 2M/kg group as compared to SPOC. Rest pain reduced 0.3 units (SE=0.13) per month, which was statistically significant (p = 0.0193), CI= (-0.55,-0.05) whereas, there was 11% (SE=0.05) significant decrease in ulcer size per month (p=0.0253) CI= (0.80, 0.99). In addition, ABPI (p=0.0132) also show significant improvement in 2M/kg dose group, suggesting improved blood flow in the affected limb. The improvement in quality of life, total walking distance has also been observed in patients in the stempeucel® treatment arms. None of the adverse events was found to be associated with stempeucel® administration. Hence, it is concluded that stempeucel® is safe and provides therapeutic benefits to "no-option" patients suffering from Buerger's disease.

MESENCHYMAL CELL LINEAGE ANALYSIS

F-1061

PLACENTAL DERIVED MULTIPOTENT CELLS POSSESS TROPHOBLAST SPECIFIC FEATURES

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Primary cultures have distinct immunophenotype from their in vivo progenitors. Culture conditions induce expression of markers specific for ontologically different cell types that complicate the establishment of origin of obtained cells. The aim of this study was to investigate placenta-derived multipotent cells (PDMCs) that possess several trophoblast related features. PDMCs were obtained by explants primary culture methods. PDMCs simultaneous expressed both mesenchymal (vimentin (Vim), alpha-smooth muscle actin (a-SMA), ERG, CD90, CD73, CD105) and trophoblast markers (pan-cytokeratin (pCK), cytokeratin 7 (CK7), chorionic gonadotropin (CG), CDX-2, EOMES). Moreover, it was observed the expression of both trophoblastic and nontrophoblastic types of CGβ genes in obtained cultures; some of PDMC-lines expressed fusogenic gene ERVW-1 and trophoblast related transcription factor GSM1. The colonies resulting from single cells culturing were either positive or negative for CK7. The expression of CK7 decreased during subculturing and disappeared at 2nd passage. Both CK7+- and CK7-- clones expressed mRNA of EOMES, CDX-2, CG. On the other hand those clones shared MSC specific properties to differentiate into adipogenic and osteogenic lineages. Single cell-derived clone (C1) expressed cytoskeleton protein Vim, a-SMA, pCK, CK18, CK19. The transcription factors CDX-2, Eomes were detected in cytoplasm at 3rd and in nucleus at the 6th passage of C1 cells. ERG was started to be expressed at the 4th passage. C1 like PDMCs acquired multinuclearity and expression of ERVW-1, but no GCM1 under specific condition that distinguished them from trophoblast cells. To investigate the origin of PDMCs the use was made of immunohystochemistry of full term placenta, first trimester placental tissue and cultured full term placental explants. CDX-2 was not detected in full-term placental tissue. Furthermore we have not observed a-SMA+pCK+ or Vim+pCK+ cell populations in the term and first trimester chorion villi. ERG+a-SMA+ double positive cells were found in full-term placental tissue in walls of small blood vessels. We conclude that PDMCs belong to mesodermal cells possibly perivascular origin with some trophoblast features.

F-1062

COMPARISON OF ANGIOGENIC AND NEUROGENIC LINEAGE PROPENSITY OF STEM CELLS DERIVED FROM DECIDUOUS PULP AND PERIODONTAL LIGAMENT TISSUE FROM THE SAME DONOR

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Recent advances related to the knowledge of dental tissue derived-stem cells have revealed their unique plasticity and greater regenerative capacity. While pursuing technical excellence in regenerative medicine, it is essential to have an in depth interrogation and a thorough understanding on the mechanisms underlying the cellular characteristics and differentiation potential in stem cells prior to its clinical application. It is particularly important to reduce the biological noise that occurs donor based-heterogeneity in order to comprehend the biology integrated at cellular level. Therefore, in the current study we have attempted to identify and compare the profile of lineage specific-gene expression between stem cells from two different dental tissues from the same donor; stem cells from the human extracted deciduous tooth (SHED) and periodontal ligament stem cells (PDLSCs) to elucidate their differentiation potential, lineage favouritism, regulatory networks between signalling pathways and lineage-specific transcription factors underlying the lineage propensity. In the present work, both SHED and PDLSCs were isolated and basic stem cell characterization was carried out prior to the laboratory-based scientific investigation. Our results showed that both dental tissues derived stem cells met the criteria of mesenchymal stem cells (MSCs) suggested by International Society of Cellular Therapy. Results also revealed that, genes were differentially expressed in stem cells derived from these two different dental tissue sources, of which 78% of 84 genes related to human cell lineage were up regulated in PDLSCs compared with SHED. Specifically, these up-regulated genes in PDLSCs were found to be associated with pathways governing cellular differentiation, angiogenesis and neurogenesis. Our findings highlighted differences in terms of cellular and molecular signatures of MSCs being influenced by the tissue type derived from the same physiological condition thus eliminating donor variation in defining their differentiation propensity. In conclusion, the regenerative potentials of adult stem cells residing in the periodontal ligament may open a new way forward for cell-based therapies for many related degenerative diseases and disorders.

F-1063

IN VIVO THERAPEUTIC POTENTIAL OF MESENCHYMAL STROMAL CELLS DEPENDS ON SOURCE AND ISOLATION PROCEDURE

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Over the last several years, mesenchymal stromal cells (MSCs) have been isolated from different tissues following a large variety of procedures. However, very limited information is available on the most appropriate methods and sources to obtain MSCs with therapeutic potential, in particular providing benefit upon injection into ischemic tissues. Here, we comparatively assess the ex vivo and in vivo properties of C57/BL6 derived MSCs, isolated from the adipose tissue by tissue digestion (AT-MSCs) or from the bone marrow by either frequent medium changes (BM-MSCs) or immunodepletion (iBM-MSC). Our results show that the three MSC types acquire similar phenotypic and functional properties after the first passages, exhibiting a similar differentiation capacity. Despite presenting this similar behavior in cell culture, the three MSC types showed a different therapeutic potential once injected in vivo in a mouse model of hind limb ischemia. All cell types attenuated ischemic lesions in comparison to control animals, although at a different extent. Clinical and histological analysis revealed that BM-MSCs purified on adhesive substrates exerted the best therapeutic activity, preserving tissue viability, improving muscle perfusion and promoting formation of new arterioles without directly differentiating into vascular cells. In keeping with these observations, these cells abundantly expressed cytokines involved in vessel maturation; major differences compared to the other MSC types were in the levels of genes involved in smooth muscle cell recruitment and matrix remodeling (TGF-β, PDGF-β and MMP9). The repeated injection of conditioned medium from MSCs in ischemic tissues in vivo improved functional outcome, consistent with the results observed upon cell transplantation. In conclusion, our findings indicate that BM-MSCs stand as the most effective MSC type to improve perfusion and functional recovery after hind limb ischemia. Overall, these findings indicate that the choice of MSC source and purification protocol is critical in determining the therapeutic potential of the cells and warrants the standardization of an optimal MSC isolation procedure in order to select the best conditions to move forward to more effective clinical experimentation.

F-1064

DISCRIMINATION OF UNDIFFERENTIATED CLONAL HUMAN BONE MARROW STROMAL CELL LINES USING RAMAN SPECTROSCOPY

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Human bone marrow-derived stromal cells (BMSCs) are a heterogeneous population, containing progenitor cells of varied skeletogenic potencies. The application of BMSCs in regenerative medicine has been limited through the inability to reliably characterise BMSC sub-types using standard methods such as surface cell markers, or gene expression profiling. In this study, we explore the potential for Raman spectroscopy (RS), a non-destructive and label-free method, to discriminate clonally-derived BMSC lines following hTERT-immortalisation to enable in-depth repeat analyses. Four human hTERT BMSC lines – Y101, Y102, Y201 and Y202 were selected. The Y101/201 BMSCs have tri-lineage potential of differing capacity, whereas Y102/202 BMSCs are effectively differentiation incompetent. To characterise each cell line, 100 averaged spectra were collected using a laser spot sampling size of ~1µm from the nucleus of 20 randomly chosen cells (5 spectra/cell line). Spatially resolved, single-cell Raman maps were also obtained for each cell line. Raman markers were determined from the set of two-peak intensity ratios (PIRs) derived from key peaks in the averaged cell spectra. The most discriminatory PIR markers were found to be against the 1088.6 cm^{-1} peak assigned to the DNA symmetric backbone stretching vibration. The 966 cm^{-1} nucleic acid peak was the most distinctive across all cell lines, and thus the 966/1088.6 PIR was shown to clearly distinguish the four hTERT BMSC lines. PIR markers obtained from proteins and lipids in the >999.6 cm^{-1} range taken relative to the 1088.6 cm^{-1} peak were clearly separated for the '01 and '02 cell-types (1.6±0.4 difference in PIR magnitude), thus showing RS to clearly distinguish the Y101/201 and Y102/202 BMSCs. Spatially resolved cell-line discrimination was also achieved using the PIR Raman maps. In summary, RS has been applied in non-destructive, molecular-scale labelling and is demonstrated as a sensitive and discriminatory tool for BMSC subtype identification.

F-1065

IN-DEPTH CHARACTERIZATION OF MESENCHYMAL STEM CELL-DERIVED MICROVESICLES USING QUANTITATIVE PROTEOMICS

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Mesenchymal stem cells (MSC) are considered a promising tool for many therapeutic applications, as they home in on injured tissue, modulate the immune system and support tissue repair. In vitro, MSC have been shown to differentiate into multiple cell types mainly of the mesodermal, and more rarely of the endodermal and ectodermal lineage under appropriate conditions. In vivo, however, the beneficial effects mediated by MSC are mainly attributed to paracrine factors they secrete. MSC are known to secrete large amounts of extracellular microvesicles (EMV). EMV are thought to play an important role in intercellular communication transferring proteins, nucleic acids and lipids to acceptor cells. The aim of this study was the proteomic characterization of MSC-derived EMV (MSC-EMV). In our experiments we used human bone marrow-derived MSC. MSC-EMV were isolated by filtration and ultra-centrifugation. We performed mass spectrometry-based proteomic analysis using reductive dimethylation labeling for quantitation. Vesicles were directly compared to their donor cell to gain insight into proteinaceous factors enriched in them.

F-1066

IDENTIFICATION AND CHARACTERISATION OF DISCRETE MESENCHYMAL PROGENITOR SUBSETS IN ANATOMICAL COMPARTMENTS OF THE BONE

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Lineage negative PDGFRa+/Sca1+ cells (PaS cells) were shown to represent a highly clonogenic population of bone marrow mesenchymal stem cells (MSCs) capable of bone, fat and cartilage differentiation. Whole bone enzymatic digestion currently used to isolated bone marrow MSCs also releases a large pool of MSCs found in the periosteum which are entirely different MSC subsets. In our study, we aim to demonstrate the intrinsic differences and hierarchical relationship between discrete subsets of PaS cells isolated from the different bone compartments. Flow cytometry performed on cell suspensions of periosteum compared to bone marrow revealed the presence of distinct subsets of PaS cells based on Sca1 expression and mitochondrial content (SSC). The bone marrow contains PaS^{low}/SSC^{low} subsets whilst the periosteum contains PaS^{low}/ SSC^{low}, PaS^{low}/ SSC^{high} and PaS^{high}/ SSC^{high} subsets representing 0.08%, 1.5%, 1.2%, and 8.6% of total bone marrow and periosteum cells respectively. Moreover, PaS cells from the periosteum exhibited higher frequency of colony forming units in comparison to PaS cells found in the bone marrow. Spontaneous cell differentiation further demonstrated the distinction between subsets as bone marrow PaS cells gave rise predominantly to bone and cartilage, while periosteum PaS^{low}/ SSC^{low} differentiated toward cartilage, PaS^{high}/ SSC^{high} to fat and PaS^{low}/ SSC^{high} to all three lineages. Metabolomic analysis indicated that all subsets utilize glycolysis as their main energy source, representative of their immature state, but presented differential metabolomic profiles. Furthermore, following hind-limb ischemic injury in a mouse model, only the periosteal PaS^{low}/ SSC^{high} cells increase 3.2 fold in the injured tibias 5 days post injury. Overall, these data indicate the presence of discrete PaS cells subsets within the bone with varying degrees of pluripotency and mesenchymal plasticity. Furthermore, we hypothesis that periosteal PaS^{low}/ SSC^{high} cells may in fact represent a more primitive mesenchymal progenitor capable of spontaneous tri-lineage differentiation and responding to injury. Transcriptomic analysis currently underway will provide more insight to the hierarchical and developmental relationship between subsets and their biological functions in homeostasis and injury.

F-1067

HUMAN DENTAL PULP STEM CELLS FROM OLDER DONORS FOR AUTOLOGOUS TRANSPLANTATION IN CHRONIC STROKE

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Currently, there is no treatment for chronic stroke outside of rehabilitation. Adult human Dental Pulp Stem Cells (DPSC) from impacted third molars have previously been shown to improve disability in a rat model of stroke, making them a potential adjunct

therapy to rehabilitation. The clinical ease of isolating DPSC also makes them ideal for autologous transplantation. However, as 69% of stroke victims are over the age of 65, it must first be investigated whether the properties important to DPSC therapeutic potential remain unchanged with donor age. DPSC were isolated from donors from 13 to 90 years of age and grouped according to donor age: <25 years, 40-50 years and >65 years. Donor age had no effect on DPSC proliferation, expression of stem cell markers (CD73, CD90, CD105, CD146 and p75) or differentiation into mineral-producing osteoblasts, lipid droplet-producing adipocytes or neuron-like cells expressing beta-III tubulin and GFAP. These findings support the clinical use of autologous transplantation of DPSC as a future treatment for stroke patients living with chronic disability.

F-1068

ANALYSIS OF PLURIPOTENCY IN MUSE CELLS DERIVED FROM HUMAN UMBILICAL CORD TISSUE

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Mesenchymal stem cells (MSCs) have been considered to contain a subpopulation of pluripotent cells. We previously identified multilineage-differentiating stress enduring (Muse) cell in adult human mesenchymal tissue such as bone marrow, adipose tissue, and dermis. Muse cells can be isolated as cells that are double positive for pluripotent marker SSEA-3 and for a mesenchymal marker CD105. These cells are characterized by stress tolerance, self-renewal, generation of cell cluster that is very similar to ES cell-derived embryoid bodies formed in suspension culture, expression of pluripotency markers, the ability to differentiate into endodermal-, mesodermal-, and ectodermal-lineage cells from a single cell, non-tumorigenic, and low telomerase activities. However, little is known about Muse cells derived from fetal appendages such as umbilical cord (UC), placenta and cord blood. Here, we explored the basic properties of UC tissue-derived Muse cells. About 2% of human UC-derived MSCs from healthy individuals after informed consent were SSEA-3 positive cells, which located in the Wharton's jelly. These cells expressed the pluripotency markers such as Nanog, Oct3/4, Sox2, and PAR4. When each Muse cell was cultured in a single-cell suspension culture, cell clusters that expressed the pluripotency markers generated by day 7. To observe their differentiation ability, single cell clusters were transferred onto gelatin-coated plates. After 14 days, the cells expanding from the cluster spontaneous differentiated into ectodermal- (neurofilament, microtubule associated protein-2), mesodermal- (smooth muscle actin, Nkx2.5), and endodermal-lineage cells (cytokeratin 7, α-fetoprotein). Using specific cytokine induction system, Muse cells differentiated into cells positive for ectodermal- (Nestin, Musashi, and NeuroD), mesodermal- (FABP-4 and osteocalcin), endodermal-marker (albumin). These results suggested that UC-derived Muse cells have pluripotency as well as adult tissue derived Muse cells.

F-1069

COMPARISON OF HUMAN AND CANINE ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS

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Veterinary regenerative medicine is an emerging field. Animal stem cells have their own characteristics different from human stem cells. In this regard we performed comparative characterization of canine and human adipose stem cells. Canine and human adipose stem cells showed differences in cell surface antigen expression profile and differentiation potential. Basal medium immensely affected growth and differentiation potential in canine adipose stem cells. We therefore optimized culture condition of canine adipose stem cells and parameters for quality control of production of animal cell therapy products. *This study was supported by a grant (N01090009) from Global R and D Project of the Korea Institute for Advancement of Technology and by 'Agricultural Biotechnology Development Program', Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.*

F-1070

KINDLIN-2 MODULATES ADHESION, DIFFERENTIATION AND IMMUNE-SUPPRESSION IN MSCS GENERATED FROM HUMAN IPS CELLS

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Invasive procedure for harvesting MSCs and their limited proliferation ability and early senescence lead researchers to pluripotent or somatic cell induction of MSCs. We have previously shown that human iPSCs can differentiate toward MSCs and in this study we explored the role of Kindlin-2 to modulate adhesion, differentiation and immune-suppression capabilities of iPS-derived MSCs. (i) When compared to BM-MSCs after transfection (Lipofectamin LTX) with kindlin-2 shRNA and overexpressing constructs along with control plasmids iPS-MSC had increased proliferation capability and less apoptotic bodies. Disruption of kindlin-2 expression with shRNA effectively dampened proliferation (investigated with cell count, BrdU assay), however kindlin-2 overexpression increased proliferation by ~40% vs. control vector. (ii) We observed that post kindlin-2 overexpression CD-90+ and CD105+ cells increases (~1.5 and 3 folds respectively) but decreases the osteogenic (~5 folds for Osteocalcin and ~10 folds for Alkline phosphatase), chondrogenic (~20 folds for Collagen type II and Agrecan) and adipogenic (~10 folds for PPARγ and ~12 folds for LPL). (iii) Adhesion assay was done by flow chamber slides representing significantly higher attachment potential of iPS-MSCs after kindlin-2 overexpression. (iv) Moreover kindlin-2 overexpressing iPS-MSCs significantly dampened proliferation of CD4+ and CD8+ T-Lymphocytes in mixed lymphocyte reaction assay compared to iPSC-MSCs transfected by kindlin-2 shRNA. We here demonstrate the effects of kindlin-2 on increasing proliferation, migration and dampening apoptosis in iPSC-MSCs. In addition we demonstrated that kindlin-2 overexpression results in an increased expression of

mesenchymal surface markers such as CD-90 and CD-105 however decreases expression of osteogenic, chondreogenic and adipogenic markers. Moreover kindlin-2 overexpression in iPS-MSCs leads to more adhesion and less immune reaction in MLR assay. In conclusion, kindlin-2 may be one of proteins which will lead to harvesting highly potent MSCs in vitro to address proliferation and age related issues in BM-MSCs.

F-1071

CURE OF LIFE-THREATENING ACUTE GRAFT-VERSUS-HOST DISEASE USING PLACENTA-DERIVED DECIDUAL STROMAL CELLS

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Mesenchymal stem cells (MSCs) may cure acute GVHD in some patients, but long-term survival has been unsatisfactory. The placenta protects the fetus from the mother's immune system, and placental tissues have been used for over 100 years in Africa to successfully treat burn injuries. Decidual stromal cells (DSCs) are easily accessible. The placenta is discarded after delivery. We isolate, culture, expand, and store DSCs from term placentas. DSCs differ to MSCs and do not differentiate as well to bone or cartilage. DSCs need cell-to-cell contact to be immunosuppressive and to induce FoxP3 T-cells. Blocking of IDO, prostaglandin E2, PD-L1, and interferon impairs the immunosuppressive capacity of DSCs in MLC. Human DSCs inhibit MLC in mice. MCSs and DSCs induce xenoreactivity. We treated 59 patients with 136 doses of DSCs following allogeneic hematopoietic stem cell transplantation (ASCT). With early treatment (within median seven days, n=17) of steroid refractory acute GVHD, response rate was 100%, no death by GVHD, and one-year survival was 76%_as opposed to 6% in controls (n=34) not treated with stromal cells (p<0.001). A partial response was seen in two of three patients with severe chronic GVHD. Eleven patients were treated for hemorrhagic cystitis, and now we proceed with a prospective double-blind randomized study. A 33-year-old man developed acute respiratory distress syndrome (ARDS) after septicemia and ASCT. He required 15 L/min oxygen by mask. After infusion of 1 × 10^6 DSCs, oxygen saturation increased instantly from 92% to 98%, requirement for oxygen was discontinued, chest radiography improved and elevations in G-CSF, IL-6, IL-8, MCP-1, and TNF-alfa decreased. The patient is now alive and well. We reversed paresis in the arms and legs (respectively) of two patients with polyneuropathies. In a toxicity study survey of hospital charts, laboratory values, and autopsies, we found no major side-effects. Mild side-effects were seen during three DSC infusions. With our most recent protocol, DSCs cured steroid-refractory acute GVHD in all patients treated. DSCs may also be explored for chronic GVHD, hemorrhagic cystitis, ARDS, and neuropathy.

F-1072

IDENTIFICATION AND TARGETED INHIBITION OF A LINEAGE OF STROMAL CELLS RESPONSIBLE FOR SCARING AND CANCER STROMA

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Dermal fibroblasts represent a heterogeneous population of cells with diverse features that remain largely undefined due to a lack of functional subclasses. Here we reveal the presence of multiple embryonic lineages of dermal fibroblasts within the dorsal back. Genetic lineage tracing and transplantation assays identify a highly fibrogenic lineage defined by embryonic expression of Engrailed-1 that plays a central role in dermal development, wound healing, radiation-induced fibrosis, and cancer stroma formation. Using flow cytometry and in silico approaches, we identify CD26/DPP4 as a surface marker that allows for the isolation of this fibrogenic, scar-forming lineage. Reciprocal transplantation of distinct fibroblast lineages between the dorsal back and oral cavity induced ectopic dermal architectures that mimic their place-of-origin, indicating that intra and inter-site diversity of dermal architectures are set embryonically and maintained postnatally by distinct lineages of fibroblasts. We further demonstrate that targeted inhibition of this lineage results in reduced melanoma growth and scar formation with no effect on the structural integrity of the healed skin. These findings hold promise for development of therapeutic approaches aimed at in vivo modulation of their fibrogenic behavior.

F-1073

FUNCTIONALLY DISTINCT SUBPOPULATIONS OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS EXPRESS DIFFERENT SUBTYPES OF ADRENERGIC RECEPTORS

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Catecholamines regulate proliferation, differentiation and secretion of bone marrow mesenchymal stromal cells (MSCs) acting through β1- and β2-adrenergic receptors. However, the expression of adrenergic receptors (AR) on adipose-derived MSCs (ADSCs) as well as their role on those cells remains poorly understood. Previously we showed that ADSC population exhibited functional heterogeneity. A variety of first messengers was capable of stimulating Ca2+ signaling in ADSCs, including specific agonists of α1, α2 and β ARs. Only a relatively small group of cells was nevertheless specifically responsive to the particular GPCR agonist (Biochim Biophys Acta, 2014, 1843(9), p1899). Here, we explored the molecular mechanisms responsible for functional heterogeneity of ADSC. ADSC were isolated from subcutaneous fat tissue of healthy donors and analyzed at the next day after isolation and upon culturing up to 2-3 passages. Subpopulations of ADSCs expressing particular ARs were evaluated by flow cytometry and purified using FACS. ADSC phenotype characterized by flow cytometry was CD90+/CD73+/CD105+/CD45-/CD31- and cells were capable of adipogenic and osteogenic differentiation. We found that 3-5% of ADSC contained α1B, α2B or β2 ARs, using real-time PCR and immunofluorescence. ADSC subpopulations expressing α-ARs disappear during cultivation.

ADSC functional analysis with Fluo8 Ca2+ indicator and specific antagonists showed that α1, α2 and β AR isoforms were functionally active in primary ADSC cultures. Proliferation, migration and secretory activity of isolated α1B, α2B and β2 ARs containing ADSC were determined. The motility and proliferation of α2B-expressing ADSC were 1,5-2 times lower as compared to other AR-positive subpopulations and non-adrenergic ADSC. The secretory activity of the α1B and α2B expressing ADSC was dramatically increased as measured by the T-lymphocyte immunnosupression assay. Taken together, our data indicate that human ADSC contain distinct subpopulations of adrenergic cells. Remarkably, α1- and α2-AR expressing ADSCs can serve an immunosuppressive function. We further suggest that ADSC are functinally heterogenous and activities of distinct subpopulations depend on their hormonal sensitivity. *This work was supported by RSF grants 14-15-00439 and 14-14-00687.*

HEMATOPOIETIC CELLS

F-1075

IMPROVED EX VIVO EXPANSION OF HUMAN UMBILICAL CORD BLOOD HEMATOPOIETIC PROGENITORS WITH CORD PERICYTES

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Ex vivo expansion of hematopoietic progenitos is curently under investigation as a mean to accelerate hematological recovery following stem cell transplantation. The objective of this study was to increase the expansion of hematopoietic progenitors by the combine use of optimized cytokine cocktails and feeder cells (Pericyte derived umbilical cord vein or Mesenchymal Stem Cells derived Osteoblasts) normally present within the umbilical cord blood (UCB) environment. Osteoblasts (OST) derived from UCB Mesenchymal Stem Cells (MSC) and human umbilical vein pericyte (PER) were tested as feeder cells for CD34+ UCB cells. The cultures were complemented with the cytokine cocktails OMPC (TPO, SCF, FLT-3) used for megakaryocyte expansion (day 0-7). Two co-culture conditions were tested; OST and PER without contact in trans-wells. The control conditions consisted of CD34+ cells grown in 24-well (CTL). Hematopoietic progenitor cells (HPCs) frequencies were measured using standard progenitor assays. Three independent experiments were done for all tests. First, the impact of co-culture with the PER or OST feeder cells on the proliferation of UCB CD34+ cells was assessed. Total cell expansion in PER and OST cultures was increased by 2.8-fold and 2.2-fold compared to the CTL at day 7, respectively. Co-culture with PER and OST increased the frequencies of CD34+ by 1.9 and 1.7-fold ($p>0.05$ and $p<0.01$), and CD34+CD41+ cells by 4.5 and 1.5-fold ($p>0.05$) respectively, compared to the CTL at day 7. Next, the impact of the feeder cells on HPC expansions were investigated by colony assays. Co-culture with PER increased the expansion of myeloid HPC by 1.9-fold CFU-E ($p>0.05$), 1,5-fold CFU-G/M/GM ($p<0.05$), 1.3-fold CFU-GEMM ($p>0.05$) compared to the CTL at day 7, respectively. These results demonstrate that co-culture of HPCs with PER in the presence of optimized cytokine cocktails provides an interesting strategy to increase the amount of HPCs and megakaryocyte progenitor cells available for transplantation.

F-1076

COBALT PROTOPORPHYRIN IX INDUCES MOBILIZATION OF CELLS FROM BONE MARROW

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Cobalt protoporphyrin IX (CoPP) is commonly used to activate Nrf2 transcription factor and induce expression of heme oxygenase-1 (HMOX1). Induction of HMOX1 can enhance activity of VEGF or SDF1 and decrease production of TNF or MCP1, thereby improving endothelial cell function and ameliorating inflammatory response. Our aim was to evaluate the effect of CoPP administration on mobilization of hematopoietic cells in mice, in respect to the classical treatment with GSCF. Wild type mice were injected intraperitoneally with CoPP (15 µmol/kg) or GCSF (250 µg/kg) for 5 consecutive days. All analyses were performed on day 6th. Both compounds increased to a similar extent the number of total leukocytes, monocytes and granulocytes in the peripheral blood. However, while GCSF mobilized mainly immature granulocytes (CD11b+ CD11c- Ly6C+ Ly6G-mid SSC-mid), CoPP preferentially increased the number of mature granulocytes in the blood (CD11b+ CD11c- Ly6C+ Ly6G-hi SSC-hi), which were not elevated in response to GCSF. Treatment with CoPP was also more effective in mobilization of hematopoietic stem and progenitor cells (HSPC), including LT-HSC (KLS CD48- CD150+), ST-HSC (KLS CD48+ CD150+), multipotent progenitors (KLS CD48+ CD150-), and granulocyte-monocyte progenitors (KLS- CD48+ CD150- CD34+). Furthermore, administration of CoPP led to increased production of GCSF (76.5-fold increase in the blood), MCP1, IP-10, and IL-6. Surprisingly, treatment of mice with tin protoporphyrin (SnPP), inhibitor of HMOX1, did not exert any effect. Moreover, CoPP was equally effective in HMOX1-/- and Nrf2-/- mice. Hence, its effects were HMOX1- and Nrf2-independent. Performing experiments in C3H, C57BL6 and C57BL6xFVB mouse strains we noticed that CoPP is a strong mobilizer even in C57BL6 mice, known to be relatively poor responders to GCSF. In summary, treatment of mice with CoPP leads to increased level of endogenous GCSF in the blood, and causes mobilization of hematopoietic cells, especially cells of myeloid lineage. CoPP-mobilized granulocytes have a more mature phenotype than that mobilized by exogenous GCSF.

F-1077

M-CSF INDUCED HSC COMMITTED MYELOID CELLS PROTECT AGAINST LETHAL INFECTIONS OF PSEUDOMONAS AERUGINOSA OR ASPERGILLUS FUMIGATUS POST-HS/PC TRANSPLANTATION

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Myeloablative doses of chemoradiation therapy used in preparation

for hematopoietic cell transplantation (HCT) lead to depletion of hematopoietic stem cells (HSCs), progenitor cells, and mature cells. The resulting immunodeficiency, specifically neutropenia leads to a profound susceptibility to bacterial, fungal and viral infections. Numerous clinical and animal studies were undertaken to characterize the complex immune responses and to develop treatment strategies involving drugs as well as cellular therapies and vaccination. Modifying grafts by inclusion of committed progenitor cells, such as CMP/GMP or CLP, can be used to protect against bacterial and fungal or viral infections, respectively. In our lab, we previously showed that M-CSF, a myeloid cytokine released during infection and inflammation, could directly induce the myeloid master regulator PU.1 and instruct myeloid cell fate change in HSC, independently of selective survival or proliferation. In the present study we have tested the functional consequence of this increased M-CSF induced myeloid commitment of HSC in the immune response to infection. To do this, post-irradiation and HS/PC transplantation, M-CSF treated or untreated mice were infected with lethal doses of the opportunistic pathogens such as bacteria *Pseudomonas aeruginosa* or fungus *Aspergillus fumigatus*. M-CSF treatment presented increased survival of mice infected with *Pseudomonas aeruginosa* or *Aspergillus fumigatus* and also showed a significant decrease in the pathogen load in various tissues. Further, we have seen that M-CSF treatment increased splenic mature myeloid cells and progenitors (GMPs) 9-14 days after HS/PC transplantation, though there was no difference in the circulating levels of myeloid cells. Furthermore, M-CSF treatment showed normal long-term donor contribution to all lineages. These results demonstrate that the M-CSF treatment enhances the myeloid commitment of HSC and protects from lethal infections of opportunistic pathogens. These results further encourage potential clinical application of M-CSF post-HS/PC transplantation.

F-1078

OUTSIDE-IN SIGNALING VIA PERIOSTIN-INTEGRIN-ALPHA V BETA 3 INTERACTION REGULATES STEMNESS AND AGEING OF MURINE HEMATOPOIETIC STEM CELLS

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Functional regulation of hematopoietic stem cells (HSCs) is mediated by the microenvironment (niche) wherein they reside. Integrins expressed on HSCs not only play important roles in the physical retention of HSCs within the niche, but can, via outside-in signaling, also play crucial roles in a variety of cellular functions. We demonstrate here that Periostin (Postn), following binding to Integrin-$\alpha v\beta 3$ (Itgav-b3) regulates HSC proliferation and stemness. When added to SCF and Tpo-mediated cultures in vitro, Postn inhibited culture induced proliferation of HSCs, and maintained the long-term engraftability of HSC. Blood of young adult Postn-/- mice displayed elevated levels of myelopoiesis with mild effects on lymphopoiesis and erythropoiesis. These effects were more pronounced in older (>20 weeks) mice, where we observed significantly decreased erythropoiesis and lymphopoiesis. Although, the number of CD150+CD48-Lin-c-kit+Sca-1+ (SLAM KLS) cells increased in BM of Postn-/- mice, long-term engraftability decreased. We could relate these effects to early exhaustion of HSCs in Postn-/- mice, wherein increased proliferation of HSPCs was seen. Hematopoietic tissue specific deletion of Itgav using Vav-iCre and Itgav-floxed mice confirmed these results. Vav-iCre;Itgavfl/fl mice showed more pronounced increase in myelopoiesis and decrease in lymphopoiesis, compared with Postn-/- mice, and long-term engraftment of Vav-iCre;Itgavfl/fl HSCs was also significantly decreased. Importantly, Postn could not inhibit culture-induced proliferation of Itgav-/- HSCs, which proliferated faster compared with Itgav+/+ cells. Finally, we demonstrated that Postn binding to Integrin-$\alpha v\beta 3$ inactivates FAK mediated outside-in integrin based activation of p27Kip1 dependent regulation. Overall, we identified Postn as an important regulator of hematopoiesis that affects HSC proliferation and senescence though outside-in Itgav signaling.

F-1079

ANALYSIS OF BONE MARROW FUNCTION IN ALS PATIENTS

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Introduction: Neurodegenerative disorders are accompanied by elevated Transforming Growth Factor beta (TGF-β) levels in CSF and serum. Hence, inhibition of the TGF-β pathway might exhibit a new therapeutic approach for amyotrophic lateral sclerosis (ALS), a progressive, fatal neurological disease without effective therapy. TGF-β also influences hematopoiesis, thus ALS patients may display reduced hematopoietic stem cell (HSC) resources and limited endogenous repair mechanisms within the central nervous system. Therefore we sought to analyze the bone marrow (BM) function and the effect of TGF-β on HSC of ALS patients in vitro. Methods: Circulating CD34+ cells in peripheral blood (PB) and BM of ALS patients and healthy donors were measured by flow cytometry. Colony-forming cells (CFC) were concurrently analyzed in methylcellulose-based assays. To test the effect of TGF-β on HSC, a co-culture model with mesenchymal stromal cells (MSC) as feeders was developed. Results: Individual circulating CD34+ cell numbers in PB of healthy donors ranged from 0.9 - 3.5 CD34+ cells/µl blood (median = 1.7). In comparison, ALS patients displayed a lower content (median = 1.1). CFC numbers in PB and BM of healthy donors ranged from 0.35 - 1.76 CFC x 102/ml blood (median = 0.7) and 9.5 - 18.5/102 CD34+ (median = 11.3), respectively. Again, ALS patients revealed lower numbers in PB (median = 0.5) and BM (median = 9.0). TGF-β significantly reduced the proliferation of HSC in co-culture after 7 weeks. Conclusions: CD34+ cell numbers in PB as well as CFC numbers in PB and BM were reduced, which might indicate an impaired BM function in ALS patients compared to healthy donors. Co-culture experiments of BM HSC and MSC confirmed the anti-proliferative effect of TGF-β. This experimental setup is therefore a suitable model to investigate the efficacy and safety of TGF-βRII blocking molecules that are currently under development as new therapeutic agents for ALS treatment.

F-1080

SCREENING FOR HSC REGENERATING GENES USING THE MPL-DEFICIENT MOUSE MODEL

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Mpl represents the cytokine receptor for thrombopoietin (Thpo) and Thpo/Mpl-signaling regulates megakaryopoiesis, hematopoietic stem cell (HSC) maintenance and post-transplant expansion. Importance of Thpo/Mpl-signaling in HSC is demonstrated by MPL deficiency in human patients, causing thrombocytopenia and lethal aplastic anemia, called congenital amegakaryocytic thrombocytopenia. To further elucidate the molecular mechanism we performed transcriptome analysis of the stem cell enriched Lin-, Sca-1+ and c-Kit+ (LSK) cell population in two different transplantation models. In one model, the HSC defects of the Mpl-/- mouse were corrected by lentiviral overexpression of Mpl. In the other model, expression of an intracellular-truncated, signaling-deficient Mpl receptor (termed dominant-negative (dn)Mpl) in wildtype mice induced thrombocytopenia and HSC defects. dnMpl mice had 4-fold reduced LSK cells and bone marrow cells did not engraft in secondary recipients. The expression profile of dnMpl LSK cells negatively correlated with known HSC stemness signatures and the Wnt-, Jak/Stat-, and PI3K/Akt-signaling pathways, while expression of genes involved in cell cycle progression were positively correlated. The lower percentage of quiescent HSC was confirmed by flow cytometry and the expression of typical mouse HSC markers (Tie2, EPCR (CD201) and Esam1) was significantly reduced (**$p<0.005$) in dnMpl mice compared to control mice. In vitro experiments revealed receptor competition for Thpo binding and intracellular inhibition of wtMpl-signaling as the underlying mechanisms. By comparing the transcriptome of Mpl corrected Mpl-/- LSK cells and Thpo/Mpl-signaling inhibited LSK cells we could identify Thpo/Mpl-induced genes and pathways that are potential therapeutic targets for the regeneration of HSC. Candidate genes, including transcription factors and aforementioned surface marker with unknown function, are currently investigated in the Mpl-/- transplantation model for their effects on hematopoiesis. Lentiviral overexpression of selected candidates improved engraftment potential of Mpl-/- cells, altered progenitor cell proliferation or corrected thrombocytopenia confirming the Thpo target genes as potential therapeutic targets for the regeneration of HSC.

F-1081

NON-PLATELET RNA-CONTAINING PARTICLES ARE THE BASIC SUBSTANCES FOR ASSEMBLING STEM CELLS

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Non-platelet RNA-containing particles (NPRCPs) are newly identified subcellular particles in human and mouse blood. They are about 1 to 5 µm and express Oct4, Sox-2, DDX4 and actin. NPRCPs can aggregate, fuse, and undergo cellularization to become nucleated stem cells in vitro and in vivo. However, the origin of NPRCPs is still unclear because it is difficult to separate NPRCPs from similar sized platelets and other blood subcellular particles. In the present studies, we have found that some large cellular clumps that can release small particles are existed in mouse blood. In these large cellular clumps, we have identified one type of cells that releases short-rod shaped Oct4-, Sox2- and DDX4-expressing NPRCPs. Released short-rod shaped NPRCPs can be single, or linked as dimmer, trimmer or polymer. In addition, a group of sperm-like NPRCPs that have a thin head and a long tail was identified. These NPRCPs can aggregate, coil to each other and fuse into small spheroids, multiple of that can further fuse into large spheroids. In these spheroids, exogenous-derived nuclei can quickly expend DNA contents. Finally, the spheroids become newly formed cellular clumps containing NPRCP-releasing cells. Our data provide the evidence that stem cells are regenerated. NPRCPs are the essential substances for stem cell formation.

F-1082

ENDOCANNABINOIDS AND THEIR RECEPTORS ARE LOCATED ON HUMAN BONE MARROW NICHE MESENCHYMAL STEM AND HEMATOPOIETIC PROGENITOR CELLS

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Endocannabinoids are endogenous morphine ligands and present widespread receptor-mediated effects at physiological and pathological levels on the nervous system as well as many other systems. These effects are partially realized through mechanisms affecting cell growth, differentiation, apoptosis and migration. The hematopoietic progenitor cells (HPCs) and mesenchymal stem cells (MSCs) are located in the bone marrow at distinct niches and interact with each other. Research questions of this study were whether human HPCs and MSCs synthesize major endocannabinoids (anandamide-AEA, acyl glycerol-AG), express their receptors (CB1, CB2), and if these play a role in HPC and MSC interaction within the human bone marrow niche. For this purpose, CD34 positive (CD34+) bone marrow HPCs were isolated from healthy donors mononuclear cells (MNCs) with magnetic cell isolation and cell separation (MACS) method. Isolated MNCs, CD34+ cells and characterized MSCs were analyzed for CB1 and CB2 receptor expression using qRT-PCR and flow cytometry. The AEA and total-AG (1-AG + 2-AG) levels in human bone marrow plasma and MSC culture supernatants were determined using liquid chromatography-electrospray ionization/multi-stage mass spectrometry (LC-ESI-MS/MS). Passage 1 MSC supernatant levels of AEA and total-AG were 115,468 ± 41,5 ng/mL , and 3124,438 ± 705,5 ng/mL, respectively. AEA level in passage 2 was significantly lower than that of passage 1. Plasma levels of AEA and total-AG were 1,432 ± 0,26 µg/mL, and 41,608 ± 21,6 µg/mL, respectively. Flow cytometry and qRT-PCR analyses revealed endocannabinoid

receptor expression in CD34+ cells. CB1 and CB2 receptor percentage averaged as 1.6 ± 1,24 and 1.3 ± 1,8 for MNCs; 27.3 ± 10,7 and 16.4 ± 7,5 for CD34+ cells; 0.2 ± 0,15 and 0.07 ± 0,25 for MSCs, respectively. CB1 and CB2 receptor gene expression was low but detectable in all the cells. These results present that, MSCs secrete endocannabinoid ligands, AEA and 2-AG and bone marrow HPCs expressed endocannabinoid receptors, CB1 and CB2. In conclusion HPCs and MSCs have a mutual receptor-ligand relationship in bone marrow microenvironment (niche) through the endocannabinoidal system.

F-1083

HUMAN IN VITRO HEMATOPOIETIC DIFFERENTIATION REVEALS THE INFLUENCE OF MICRORNAS ON HEMATOPOIETIC DEVELOPMENT AND SPECIFICATION

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While microRNAs (miRNA) constitute important regulators of hematopoietic differentiation, their exact role during hematopoietic development remains ill-defined. Thus, analyzing the miRNA profile of induced pluripotent stem cell (iPSC)-derived hematopoietic cells and comparing it to related in vivo populations would improve our understanding of hematopoietic development. To this end we performed hematopoietic differentiation of human iPSC (hiPSC) using an embryoid body (EB)-based myeloid differentiation protocol adding cytokines from day 5 onwards and yielding so-called "myeloid cell forming complexes" (MCFCs) within 7-10 days. We analyzed the expression profile of hematopoiesis-associated miRNAs-125b, -142-3p and -223 during in vitro differentiation of human iPSC towards monocytes and macrophages and in comparison to the corresponding in vivo populations. When compared to hiPSC, expression of miRNA-125b and miRNA-223 increased 10 to 1000 - fold during early hematopoietic specification, with maximum expression in hiPSC-derived macrophages similar to physiological differentiation in vivo. Also miRNA-142-3p accelerated 500-fold upon terminal differentiation towards CD14+, CD163+ macrophages. Since all investigated miRNAs showed a distinct expression pattern during hematopoietic differentiation they may also play a role in the early endothelial versus hematopoietic specification. Interestingly, miRNA-142-3p was down-regulated in the early phases of hematopoietic differentiation which correlated with the upregulation of the early mesodermal markers T and MIXL1, suggesting a role of this miRNA in early mesodermal priming. In order to investigate this finely tuned process, we generated 3rd generation, SIN-lentiviral vectors containing the respective miRNA target sequences. MiRNA-142T and -223T sponges were able to downregulate the corresponding miRNA in U937 cells compared to a scrambled target sequence containing vector. Using this technology, the influence of miR-142 and -223 on early hematopoietic specification will be analyzed in our hematopoietic differentiation protocol. We here demonstrate that human hematopoietic differentiation of pluripotent stem cells represents a valuable tool to study hematopoietic development and the role of miRNAs within this process.

F-1084

BCL11A IS ESSENTIAL FOR HEMATOPOIETIC STEM CELL MAINTENANCE AND FUNCTION

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The zinc-finger protein BCL11A is a critical transcriptional regulator in hemoglobin switching and fetal hemoglobin (HbF) silencing. In vivo modeling of sickle cell disease using a humanized mouse model has demonstrated that inactivation of Bcl11a can correct the disease phenotype through reactivation of HbF. Thus, BCL11A has become a highly attractive target for therapeutic induction of HbF in β-globin disorders. Bcl11a is expressed in multiple hematopoietic lineages. Targeting Bcl11a in erythroid cells have demonstrated a non-essential role for Bcl11a in normal erythropoiesis. However, knockout studies have shown that Bcl11a is essential in B cell and lymphoid progenitor development. The complete role of Bcl11a and its function in different hematopoietic lineages, particularly in hematopoietic stem cells (HSCs) has not been fully characterized. Establishing the lineage specific effects of Bcl11a inactivation in hematopoietic cells is critically important in evaluating potential toxicities that may arise as a result of therapeutic targeting of Bcl11a. Here, we have deleted Bcl11a in hematopoietic cells by crossing a conditional Bcl11a strain to the Mx1-Cre mouse strain, and inducing gene deletion by Poly I:C treatment. Following the acute deletion of Bcl11a, there is a rapid loss of B cells and lymphoid progenitors, whereas other lineages remain unchanged. Notably, phenotypic HSCs (LSK CD48- CD150+) are increased. Assessment of the HSC function in transplantation experiments revealed a dramatic reduction in donor chimerism with loss of all hematopoietic lineages. These results suggest that Bcl11a is critical for normal HSC maintenance and function, highlighting the need for understanding the molecular mechanisms by which Bcl11a regulates HSCs and other blood lineages. A better understanding of the regulatory role of Bcl11a will help to identify more specific targets for HbF reactivation and minimizing the impact on other blood lineages.

F-1085

RETROSPECTIVE INFERENCE OF DIFFERENTIATION STIMULI AND PROSPECTIVE IDENTIFICATION OF LINEAGE CHOICE FROM TIME-LAPSE MICROSCOPY DATA

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Time-lapse microscopy allows to continuously observe cells during decision making processes. However, while markers for specific cell states can be used as final readouts of a decision making process, it remains a challenging task to determine the mechanism and the exact timepoint of a cell state transition. Here, we present two computational methods for the analysis and interpretation of time-lapse microscopy data. Using cellular genealogies of murine hematopoietic stem cells and models for the execution of a

differentiation decision, we first show that a decision stimulus can be inferred from the correlated onsets of blood lineage markers. This allows for a retrospective analysis of single cell features at the timepoint of the decision stimulus. Second, we present a machine learning method to prospectively predict the lineage decisions of single cells based on morphodynamics and cell motility. In hematopoietic stem cells, we achieve an error rate below 20% up to 3 generations before the onset of blood lineage markers. Our approach thus allows the identification of lineage choice much earlier than current techniques and could be used to identify novel molecular factors that are involved in hematopoietic differentiation.

F-1086

FOXA3 IS A NOVEL REGULATOR OF STRESS HEMATOPOIESIS IN MURINE HEMATOPOIETIC STEM CELLS

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The use of hematopoietic stem cells (HSC) to treat disease is limited by their scarcity. One way to overcome this limitation is to enhance HSC engraftment efficiency. We recently performed a functional screen involving >1300 mice for novel regulators of stable HSC repopulation and identified Foxa3. Knockdown of Foxa3 in HSC perturbed their ability to repopulate recipient mice. Foxa genes have never before been implicated in hematopoiesis. Foxa3 expression is restricted to long-term HSC (LT-HSC) in the hematopoietic compartment. Although Foxa3-/- mice displayed normal blood counts and frequencies of hematopoietic stem and progenitor populations, Foxa3-/- LT-HSC generate 50% fewer CFU than Foxa3+/+ HSC ($p<0.0001$). Further, in competitive transplant studies, Foxa3-/- WBM displayed reduced repopulating potential relative to Foxa3+/+ WBM ($p=0.03$) and a complete loss of secondary repopulating potential ($p=0.0001$). Limiting dilution transplants revealed that Foxa3-/- WBM contains about 5-fold fewer HSC than control WBM ($p=0.033$). These data suggest that Foxa3-/- bone marrow contains fewer functional HSC than Foxa3+/+ marrow and that self-renewal is compromised in Foxa3-/- HSC. FOXA binding motifs were enriched in active enhancers in LT-HSC ($p=0.00027$). We used IM-PET (Integrated Method for Predicting Enhancer Targets) to identify the promoters targeted by FOXA motif+ LT-HSC enhancers. These targets were expressed higher in Foxa3+/+ HSC than Foxa3-/- HSC, indicating likely regulation by Foxa3 ($p<0.0001$). Pathway analysis of these genes revealed a strong enrichment for pathways regulating metabolic stress and survival (e.g. "Thrombopoietin Signaling", "Myc Mediated Apoptosis Signaling", and "Endoplasmic Reticulum Stress Pathways"). Top predicted regulators included Myc, TP53, and TGFβ. These analyses reveal that metabolic and stress pathways are compromised in Foxa3-/- HSC. Indeed, Foxa3-/- HSC fail to repopulate most dramatically when transplanted under conditions that place high proliferative stress on individual cells (e.g. in limiting dilution and serial transplantation). Thus, Foxa3 is essential for HSC function during hematologic stress and this work highlights the importance of these pathways in enabling the effective reconstitution of an ablated hematopoietic system by HSC.

F-1087

MEF2C PROTECTS BONE MARROW B LYMPHOID PROGENITORS BY ENHANCING DNA REPAIR AND V(D)J RECOMBINATION

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B lymphocytes are central mediators of humoral immunity. To generate functional B cell receptor (BCR), B cell progenitors in the bone marrow (BM) go through V(D)J recombination, which involves generation and repair of DNA double strand breaks (DSBs). We identified transcription factor MEF2C as a novel guardian of DNA repair and V(D)J recombination in B cell progenitors. Hematopoietic deletion of Mef2c in mice reduced the survival and cellularity of BM B cell progenitors downstream of CLP (common lymphoid progenitor), while peripheral B cells remained unaltered in homeostatic conditions. Intriguingly, this phenotype was reminiscent of the B lymphoid defects observed during aging. Loss of Mef2c severely compromised the recovery of BM and peripheral B cells after sub-lethal irradiation, while the recovery of T-lymphoid and myeloid cells was unaffected. These data imply that MEF2C protects B cell progenitor survival, especially upon proliferative stress. Microarray of Mef2c deficient B cell progenitors showed down-regulation of DNA repair genes, including sensors of DSB and effectors in homologous recombination (HR) and non-homologous end joining (NHEJ) repair pathways. Comet assay revealed excessive DNA damage specifically in B cell progenitors in Mef2c deficient BM, while CLPs, mature B cells, T cell progenitors or myeloid cells were unaffected. γH2AX staining showed increased DSBs in Mef2c deficient pre-B cells. These data show that MEF2C regulates DNA repair specifically in B cell progenitors. NHEJ repair is also required for V(D)J recombination in BM. Loss of Mef2c impaired the proper induction of Rag initiators and key NHEJ factors during B cell progenitor transition, and reduced the recombination efficiency of both heavy and light chains, uncovering a novel function for MEF2C in V(D)J recombination. ChIP-Seq in human B lymphoblasts showed that MEF2C directly binds to genes encoding critical factors of DSB repair and V(D)J machinery. MEF2C binding strongly correlated with the binding of co-activator, p300, and enhancer epigenetic marks, H3K4me1 and H3K27ac, suggesting that MEF2C functions by boosting gene activation through of these enhancers. These data define MEF2C as a lineage specific DNA repair regulator that protects B lymphoid progenitor homeostasis.

F-1088

EPIGENETIC FUNCTION OF BMI-1 IN THE REGULATION OF HEMATOPOIETIC STEM CELL SELF-RENEWAL

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Hematopoietic stem cell (HSC) is a multipotent cell type, which can self-renew and differentiate into all blood cell lineages. These unique features of HSCs are vital for replenishing the stem cell pool within an individual. However, the underlying molecular mechanisms of HSC function remain to be elucidated. Recent studies have

revealed the polycomb group protein, Bmi-1, has an essential role in regulating HSC function. Bmi-1 is a core component of the polycomb repressive complex 1, which silences gene expression by introducing histone H2A ubiquitination (H2AK119ub) mark at target gene loci. We have established a primary cell system to study the function of Bmi-1 in hematopoiesis. Overexpression of Bmi-1 led to the suppression of known Bmi-1 target genes p16Ink4a and p19Arf in primary isolated murine HSCs. The colony forming potential was significantly enhanced through the promotion of HSC proliferation. In contrast, Bmi-1 knockdown in HSCs was accompanied by de-repression of p16Ink4a and p19Arf genes and by a significant reduction of colony formation due to inhibited cell proliferation. Bmi-1 enhances HSC self-renewal is associated with its epigenetic function on H2A ubiquitination. Treatment of HSC with a BMI-1-RING1A/B E3 ubiquitin ligase inhibitor PRT4165, which efficiently and specifically depletes global H2AK119ub level, led to over 50% reduction of colony number. However, overexpression of Bmi-1 in HSCs can maintain the colony forming potential after the inhibitor treatment, indicating that H2A ubiquitination is crucial for HSC self-renewal. To investigate how Bmi-1 regulates gene transcriptional machinery in HSCs, we performed RNA-Seq analysis of HSCs with Bmi-1 overexpression. Bmi-1 overexpression mediated the downregulation of 722 genes and upregulation of 541 genes. Gene Ontology analysis indicated that Bmi-1 was involved in the regulation of proliferation, differentiation and various cell signaling pathways, including Wnt, cytokine-associated and Mapk pathways, which are crucial for the HSC biology. Further investigation of the Bmi-1 targeted regions and the genome-wide H2K119ub pattern would allow us to dissect the underlying epigenetic mechanism that controls the global transcription machinery of HSCs and provides novel insights on the role of Bmi-1 in HSC self-renewal.

F-1089

IDH MUTANTS ARE PROMISING THERAPEUTIC TARGETS FOR ACUTE MYELOID LEUKEMIA

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Mutations in isocitrate dehydrogenase (IDH) 1 and 2 are frequently observed in acute myeloid leukemia (AML), glioma, and many other cancers. Mutant IDHs acquire new function, enabling it to convert α-KG to oncometabolite 2-hydroxyglutarate (2-HG), which dysregulates a set of α-KG-dependent dioxygenases, such as TETs, histone demethylases, EGLNs, and other enzymes. Inhibitors directed against mutant IDH are not expected to have the side effects as those of anti-cancer agents. To determine whether mutant IDH enzymes are valid targets for cancer therapy, we created a mouse model of mutant IDH-dependent AML. Previously, the IDH mutation alone was shown to be insufficient for the induction of AML. We found that NPM+/- hematopoietic progenitor cells transduced with IDH2/R140Q and three highly co-occuring genes (NPMc, DNMT3A/R882H, and FLT3/ITD) cooperatively induced AML in a mouse model. All four mutations are necessary for the efficient induction of AML. By using a combination of AML model mice with cre-loxp, we conditionally deleted IDH2/R140Q from AML mice, which blocked 2-HG production and resulted in the loss of leukemia stem cells. Accordingly, the progression of AML was significantly delayed. Because IDH mutations and TET2 mutations are mutually exclusive in AML, the inhibition of TET-mediated conversion of 5mC to 5hmC is considered one of the main roles of mutant IDH. We found that IDH2/R140Q decreased the level of 5hmC and the expression of differentiation-inducing genes, including Ebf1, Spib and Pax5. Gene expression analysis revealed that IDH2/R140Q activated the hypoxia pathway and the expression of Meis1. These results indicate that the function of IDH2 mutation is critical for the development and maintenance of AML stem cells, and that mutant IDHs are promising targets for anticancer therapy. Based on these findings, we developed potent and specific inhibitors of mutant IDH1 and tested their effects in the mutant IDH1-dependent AML mouse model, created by introducing four mutant genes including mutant IDH1. The 2HG level was promptly and dramatically decreased in AML cells soon after treatment with the mutant IDH1 inhibitors, and the number of leukemia cells was reduced after a 4-week treatment. These results indicate that IDH1 mutant inhibitors are effective for the treatment for AML.

F-1090

A ROLE FOR ENDOGLIN IN HEMATOPOIETIC STEM CELL QUIESCENCE

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During vertebrate embryogenesis, the establishment of the hematopoietic system is highly complex, involving a number of key anatomical sites, cellular interactions as well as intrinsic and extrinsic regulators. We have recently shown that endoglin (Eng), a receptor for the TGF-β superfamily, is required for proper yolk sac hematopoiesis. Absence of Eng leads to embryonic lethality at E10.5, and analysis of E8.5-E9.5 embryos shows severely reduced erythropoiesis. Hematopoietic progenitor activity in wild-type embryos is restricted to Eng^{+} cells. Because of the early lethality, the role of endoglin in hematopoiesis beyond the YS stage is currently unknown. To test the hypothesis that endoglin may play an important function in hematopoietic stem cell (HSC) regulation *in vivo*, we have generated a conditional knockout mouse for this receptor by combining Eng-floxed with Mx1-cre alleles, allowing us to delete endoglin specifically in HSCs. Bone marrow cells from pIpC-treated conditional Eng knockout and control mice (CD45.2 background)) were transplanted into lethally irradiated CD45.1 recipient mice, and engraftment levels were investigated for short- and long-term engraftment (6 and 16 weeks, respectively). With the goal to assess the self-renewing capability of Eng-deleted HSCs, engrafted BM cells were then serially transplanted. Our results show a drastic reduction in the multi-lineage repopulation ability of $Eng^{fl/\Delta}$*; Mx1-Cre* BM cells when compared to the controls in the tertiary (≈ 3 times) and quaternary (≈ 10 times) grafts, suggesting that Eng is important to maintain the HSC pool. To determine whether the exhaustion of Eng-deleted HSCs was due to loss of quiescence, we analyzed the cell cycle status of LSK SLAM+ cells of primary transplanted mice. Our results showed a significant decrease in the frequency of quiescent HSC of $Eng^{fl/\Delta}$*; Mx1-Cre* transplanted mice (p=0.0026) when compared to control mice. These findings point to an important role for endoglin in the maintenance of HSC quiescence. We are currently dissecting the phenotypic and molecular changes resulting from endoglin deletion in the adult HSC pool, and these data will be discussed.

F-1091

LINKING THE ENDOCYTIC PROTEIN AP2A2 TO LIPID METABOLISM IN HAEMATOPOIESIS

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The long-term repopulating HSC (LT-HSC) function of self-renewal and lifelong blood replenishment remains a mechanistic mystery but asymmetric cell division (ACD) is integral to the process. We have previously demonstrated that the endocytic protein, Ap2a2 distinguishes an asymmetrical from a symmetrical HSC division and when overexpressed enhances mouse HSC activity. The tetracycline-inducible H2B-GFP mouse has revealed functional heterogeneity within the $CD150^+48^-$ $Lin^-Sca^+Kit^+$(LSK) HSC population, such that only the GFP^{high} as opposed to the GFP^{low} subpopulation retains true LT-HSC functions. Using this mouse model, we have shown that Ap2a2 is not only more highly expressed in the GFP^{high} LT-HSCs but that Ap2a2 overexpression increases the fraction of this specific subpopulation from 20% to 60% at plus 20 weeks post-transplantation. To further investigate Ap2a2 in haematopoiesis, we have constructed an Ap2a2 conditional knockout mouse line, which has a LacZ reporter expressed from the endogenous Ap2a2 promoter. Using this Ap2a2-LacZ reporter mouse with β-galactosidase flow cytometry methodology we have shown relative increased staining of Ap2a2 within the bone marrow $CD150^+48^-$LSK subpopulation. Moreover, the specific function of Ap2a2 in developmental (Vav-Cre) and adult (Mx-Cre) haematopoiesis is being investigated by respective tissue specific-Cre matings with the $Ap2a2^{fl/fl}$ mice. Mechanistically, a recent publication identified the importance of PPAR-δ/β-fatty acid oxidation regulation for both maintenance and ACD of HSCs. Interestingly, Ap2a2 is a target of the PPAR transcription factors. Our hypothesis states that the role of Ap2a2 in LT-HSCs involves lipid metabolic pathways. To this end, in our Ap2a2-LacZ reporter mouse we have shown specific and high LacZ expression in adipocytes of the bone marrow and other lipid containing organs; and increased expression of genes involved in fatty acid synthesis and metabolism in the H2B-GFP $CD150^+48^-$LSK GFP^{high} ($Ap2a2^{high}$) HSC subpopulation. In addition, studies with fibroblasts transfected with Ap2a2 and the H2B-GFP (Ap2a2)/GFP^{high} have both detected less lipid droplet formation compared to respective controls of vector-transduced and GFP^{low} HSCs, suggesting that Ap2a2 may be involved in priming the LT-HSCs for lipid metabolism.

F-1092

IMPROVED OUTPUT AND FUNCTIONALITY OF IPS DERIVED HEMATOPOIETIC PROGENITORS BY MULTI-LAYERED STRESS INHIBITION

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In the hematopoietic hierarchy the hematopoietic stem cells (HSCs) are most critically sensitive to stress, where many sources of increased stress, including irradiation, inflammation, and a lack of a supportive niche, can cause exhaustion of the HSC fraction either through apoptosis, or by lowered functionality by decreased self-renewal and proliferative capacity. Using a human iPS-to-blood differentiation protocol, together with a small molecule screen for inhibition of separate stress signaling pathways, we evaluate the nature of stress that could cause limited functionality in generated cells with an HSC-like phenotype. Inhibitors of ER-stress, mitochondrial mediated apoptosis, non-mitochondrial mediated apoptosis, and p38 mediated senescence all demonstrate a improvement in the frequency of the HSC-like cells (CD45/43+CD34+CD38-CD90+) by up to 400% ($p: <0.05, n=3$), and significantly increased the colony forming capacity by 200 % ($p: <0.05, n=3$). Our findings demonstrate that separate stress pathways are simultaneously active, interconnected, and that inhibition of stress, mediated through these separate stress pathways, can increase both the generation, and the functionality, of hematopoietic progenitors from human pluripotent stem cells. We are currently evaluating the potential of simultaneous multi-pathway stress inhibition for the purpose of generating repopulating HSC-like cells.

F-1093

HOXA3 INDUCES LIGAND CIS-INHIBITION OF THE NOTCH PATHWAY TO BLOCK BLOOD DEVELOPMENT

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Definitive hematopoiesis occurs in the AGM region during development. In this area, a specialized subpopulation of Endothelial Cells, the Hemogenic Endothelium (HE), gives rise to Hematopoietic Stem Cells (HSC) in a process tightly controlled by several signals sent from the subaortic mesenchyme including the Notch pathway. We demonstrated that HoxA3, an important transcription factor controlling organogenesis, restrains the cell at the hemogenic endothelium stage by direct repression of Runx1, a transcription factor necessary for blood formation. In addition to inhibiting hematopoietic transcription factors, preliminary data show that HoxA3 is able to modulate the Notch pathway. We demonstrated here that overexpressing HoxA3 in endothelial cells, results in ligands Jag1 and Dll1 induction. Despite the increase in ligands, Notch target genes are unchanged or show a trend in repression. These data suggest that in presence of HoxA3 endothelial cells are unable to receive the Notch signal as a consequence of ligand cis-inhibition resulting in arrest of blood formation. In order to test this hypothesis we evaluate the effect of Notch inhibition (DAPT treatment) or activation during blood development. Inhibition of Notch results in decrease of blood progenitors originating from hemogenic endothelium. When HoxA3 is upregulated, Notch inhibition cannot promote blood formation. Moreover Runx1 upregulation is not sufficient to rescue blood progenitors when Notch pathway is inhibited. We demonstrate that HoxA3-dependent ligand upregulation lead to cis-inhibition rendering hemogenic endothelium not responsive to Notch signals. Forced activation of the pathway

in ligand independent fashion, drives hemogenic endothelial cells to lose their endothelial phenotype but is not sufficient to fully promote blood formation. Taken together, these results demonstrate that: 1) HoxA3 inhibits endothelial cells from receiving the Notch signal by promoting ligands cis-inhibition; 2) Runx1 requires an active Notch pathway to generate blood progenitors in presence of HoxA3; 3) Notch pathway activation in hemogenic endothelium acts to downregulates endothelial phenotype, but it requires Runx1 expression to fully promote endothelial cells conversion to blood.

F-1094

DECONSTRUCTING NEURAL NICHE COMPONENTS TO GENERATE HEMATOPOIETIC PROGENITORS FROM PLURIPOTENT STEM CELLS

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Hematopoietic stem cell transplantation is a treatment option in several hematological malignancies and disorders. However, the shortage of human leukocyte antigen (HLA)-matched transplantable hematopoietic stem cells (HSCs) leads to insufficient treatment of patients. This project aspires to elucidate the molecular determinants of HSC development by modelling embryonic development in vitro, where differentiation of pluripotent stem cells (PSCs) to blood is performed using a novel feeder layer system. Towards this, we used human embryonic kidney cells (HEK293) as a co-culture model. HEK cells share many features with neuronal cell types, including expression of catecholaminergic key enzymes and adrenergic receptors, despite being developmentally mesodermal. We asked if HEK293 could provide a supportive niche to generate HSCs in vitro. Combining our efficient PSC-to-blood differentiation system with HEK293 co-culture, we have achieved an increase in the HSC-like phenotype (CD43+CD34+CD38-CD45RA-CD90+). We further aim to characterize the novel factors influencing HSC development under the regulation of neural niche. The generated HSCs will be analysed for their functional capacity to reconstitute hematopoietic system in mice. Taken together, this proposal will decipher the key factors influencing HSC development and will lead towards finding alternative ways to upscale HSC production, critical for treating haematological malignancies and disorders.

F-1095

DELTA-LIKE-4 IMMOBILIZED METHYLCELLULOSE HYDROGEL FOR PROGENITOR T CELL DIFFERENTIATION FROM MURINE HEMATOPOIETIC STEM CELLS

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The thymus integrates multiple niche effector molecules such as cytokines, cell surface ligands and extracellular matrix to potentiate T lymphocyte development in vivo. However, the lack of a defined in vitro thymic model has prevented the deconvolution of the pleiotropic roles of these niche molecules in T cell development. Developing better technologies to generate progenitor T (pro-T) cells could provide novel tools for quantifying T cell potential from blood progenitor cells and enable the robust production of these cells for clinical applications. Thus, we investigated the minimum essential components needed to replicate early T cell development in vitro. We first demonstrated the production of CD25+CD90+CD45+ pro-T cells in a defined IMDM media-based serum-free culture with adsorbed Delta-like-4 (DL4). The transition to serum-free media enabled us to define several key assay design criteria. Tuning the input sca1+ckit+ hematopoietic stem cells (HSCs) cell density yielded a 150-200 total fold expansion of pro-T cells after 7 days of culture. Using a luciferase-based surrogate NIH3T3 cell assay to quantify Notch activation, we found that addition of soluble DL4 actively inhibited the translocation of the intracellular domain of the Notch1 receptor (NICD) into the nucleus even in the presence of adsorbed DL4. We also increased the efficiency of T cell differentiation by reducing the amount of cytokines used (IL7, SCF and Flt3L) and by eliminating the need for media exchange during culture. Using these defined pro-T cell growth conditions, we transitioned DL4 presentation to a 3D methylcellulose hydrogel. We derivatized free hydroxyls on the hydrogel to thiols to immobilize DL4 via thiol-maleimide chemistry. DL4-immobilized hydrogels exhibited a dose-dependent increase in Notch activation in the NIH3T3 luciferase assay. We demonstrated the utility of DL4 hydrogels as colony-forming cell (CFC) assays for quantifying T cell potential from HSCs. Lastly, incorporation of thymic niche molecules fibronectin and VCAM-1 enabled a reduction in the quantity of DL4 used by augmenting cell motility and/or enhancing Notch activation. Thus, by systematically engineering the minimum essential thymic niche components we demonstrate for the first time generation of pro-T cells in a 3D functionalized hydrogel.

F-1096

MODELING IRF8 DEFICIENT DENDRITIC CELL DEVELOPMENT AND FUNCTION WITH ENGINEERED HUMAN INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem (iPS) cells provide excellent opportunities for knockout models to study gene function in human hematopoiesis. Interferon regulatory factor 8 (IRF8), also known as interferon consensus sequence-binding protein (ICSBP), is a transcription factor, which acts as tumor suppressor and lineage determining factor for myeloid cells, including dendritic cells (DC). Loss of IRF8 function in patients causes severe monocytic and DC immunodeficiency. Here we generated a human IRF8 knockout model based on IRF8 deficient iPS cells by employing RNA guided CRISPR/Cas genome editing. IRF8 knockout cells were induced to differentiate into hematopoietic stem/progenitor cells and further into DC to study the impact of IRF8 on human DC development and function. DC are professional antigen presenting cells with a pivotal role in immunity and maintenance of immune tolerance. DC comprise different subsets: classical/conventional DC (cDC), which capture, process and present antigens to T cells, and plasmacytoid DC (pDC), which produce large amounts of interferon alpha in response to pathogens.

We demonstrate that human iPS cells can be differentiated into BDCA1+ and BDCA3+ cDCs and BDCA2+ pDCs in a GM-CSF/ FLT3L/ IL-4/ SCF culture system. Differentiated cells express DC markers, such as MHC class II and CD11c, and DC specific markers, like Clec9A for BDCA3+ cDCs or CD123 for pDCs. They express DC genes, including PU.1, BATF3, ID2 or TCF4. iPS cell-derived DC are fully functional and effectively upregulate costimulatory molecules and chemokine receptors upon Toll-like receptor stimulation. Initial data show that development of BDCA3+ cDC and pDC from IRF8 knockout iPS cells is impaired whereas BDCA1+ cDC are unaffected. Taken together, we engineered a human IRF8 knockout model that allows studying molecular mechanisms of human DC development in vitro, including the pathophysiology of IRF8 deficient DC.

F-1097

THE DNA-DAMAGE RESPONSE GENE GADD45A INDUCES DIFFERENTIATION IN HEMATOPOIETIC STEM CELLS WITHOUT INHIBITING THE CELL CYCLE PROGRESSION OR SURVIVAL

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Hematopoietic stem cells (HSCs) maintain blood cell production life-long by their unique abilities of self-renewal and differentiation into all blood cell lineages. Growth arrest and DNA-damage-inducible 45 alpha (GADD45A) is induced by genotoxic stress in HSC. GADD45A has been implicated in cell cycle control, cell death and senescence, as well as in DNA damage repair. In general, GADD45A provides cellular stability by either arresting the cell cycle progression until DNA damage is repaired or, in cases of fatal damage, by inducing apoptosis. However, the function of GADD45A in hematopoiesis remains controversial. We revealed the changes in murine HSC fate control orchestrated by the expression of GADD45A at single cell resolution. In contrast to other cellular systems, GADD45A expression did not cause a cell cycle arrest or an alteration in the decision between cell survival and apoptosis in HSCs. Though GADD45A strongly induced and accelerated the differentiation program in HSCs. Continuous tracking of individual HSCs and their progeny via time-lapse microscopy elucidated that once GADD45A was expressed, HSCs differentiate into committed progenitors within 29 h. GADD45A-expressing HSCs failed to long-term reconstitute the blood of recipients by inducing multi-lineage differentiation in vivo. The GADD45A-induced differentiation program allowed the generation of megakaryocytic-erythroid, myeloid and lymphoid lineages. The differentiation induction by GADD45A was transmitted by activating p38 MAPK signaling. These data indicate that genotoxic stress-induced GADD45A expression in HSCs prevents their fatal transformation by directing them into differentiation and thereby clearing them from the system.

F-1098

AN SCF c-KIT MYC SLUG REGULATORY FEEDBACK CIRCUIT GOVERNS THE SELF RENEWAL OF HEMATOPOIETIC STEM CELLS

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As an extrinsic regulator, stem cell factor (SCF) and its transmembrane tyrosine kinase receptor c-Kit play critical roles in hematopoietic stem cell (HSC) functions. Either mutations in the SCF or c-Kit loci result in hematopoietic deficiency and anemia. HSCs with low levels of surface c-Kit expression exhibit enhanced self-renewal and long-term reconstitution potential, whereas high expression levels of HSCs show restricted self-renewal capacity with an intrinsic megakaryocytic lineage bias. Despite its profound effects on HSC functions, the molecular mechanisms for how SCF/c-Kit signaling pathway finely modulate HSC self-renewal and its downstream targets in HSCs have not been fully elucidated. Slug is a highly conserved zinc-finger transcriptional repressor. Our previous studies show that Slug deficiency enhances self-renewal of HSCs during hematopoietic regeneration. In the present study, we investigated how Slug regulates the self-renewal capacity of HSCs through the SCF/c-Kit signaling pathway. We demonstrated that Slug negatively regulates c-Kit expression both in vivo and in vitro. Consistent with this finding, we found that the inhibition of endogenous c-Kit impaired the engraftment enhancement of Slug-deficient HSCs after transplantation. To further study the fine modulatory feedback loop between Slug and c-Kit in vivo, we force-expressed Slug, c-Kit, or both Slug/ c-Kit in HSCs and performed serial BM transplantations. Our results indicated that either overexpression of Slug or c-Kit impairs the self-renewal of HSCs, indicating that the balance between Slug and c-Kit controls HSC long-term repopulating potential. By performing a luciferase reporter assay and chromatin immunoprecipitation (ChIP) assay, we confirmed that c-Kit is a direct target gene of Slug. Furthermore, we found that SCF treatment induces expression of endogenous Slug in wild-type HSCs and distinctly elevates c-Kit expression only in Slug-deficient HSCs. Finally, we showed that knockdown of endogenous c-Myc impairs SCF/ c-Kit-mediated induction of Slug, suggesting that c-Myc is a mediator that is required for Slug/ SCF/ c-Kit regulatory circuit. In summary, our findings uncover the previously unknown molecular mechanisms of the SCF /c-Kit-Myc-Slug regulatory circuit in HSCs.

F-1099

ROLE OF NOTCH SIGNALING IN THE GENERATION OF CD34+ PRE-HEMATOPOIETIC PROGENITORS CAPABLE OF T-LYMPHOPOIESIS IN VITRO

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The generation of hematopoietic cells capable of efficiently giving rise to lymphoid cells from human embryonic stem cells (hESCs) in vitro remains an important challenge in the field. In the embryo, hematopoietic stem cells (HSC) arise from hemogenic endothelium, a specialized subset of endothelial cells that undergo endothelial-to-hematopoietic transition (EHT). The temporal and spatial

expression of Notch ligands has implicated this signaling pathway in HSC development, primarily by promoting EHT. A requirement for Notch signaling has been demonstrated in intra-embryonic definitive hematopoiesis, while this signaling pathway has been shown to be dispensable for the formation of erythromyeloid progenitors (EMPs) and primitive extraembryonic hematopoiesis, which generate hematopoietic cells that are devoid of lymphoid potential. To examine the role of Notch signaling, we cultured induced pluripotent stem cells (iPSCs) on OP9 cells that ectopically express GFP or DL1, a Notch ligand. The resulting iPSC-derived CD34+ cells were harvested from each condition and placed on OP9-DL4 cells to induce T-lymphopoiesis. Harvested CD34+ cells that were differentiated in the presence of the Notch ligand, DL1, gave rise to committed CD34+CD7+CD5+, immature single positive CD7+CD4+CD8-, double positive CD4+CD8+ and mature CD3-bearing T-cells; while OP9-GFP differentiated CD34+ cells generated only CD45+CD235a+ and CD45-CD235a+ red blood cells when co-cultured on OP9-DL4 cells. These findings highlight the limited multi-lineage potential as well as the absence of lymphoid potential of CD34+ hematopoietic progenitors generated in the absence of Notch signals. The resulting T-lymphoid capacity of CD34+ cells that had received Notch signals suggests that engaging this pathway replicates the intra-embryonic microenvironment that normally supports the development of definitive hematopoiesis.

F-1100

DIFFERENTIATION OF HUMAN IPS CELL-DERIVED HEMATOPOIETIC PROGENITOR CELLS INTO MAST CELLS

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Mast cells are tissue-resident cells best known for their role in the pathogenesis of allergic diseases. Much of our knowledge regarding the mechanisms of mast cell activation comes from the studies of mouse bone marrow-derived mast cells. However, clear differences have been observed between human and mouse mast cells. The number of mast cells that can be obtained from human adult tissues is limited because mast cells reside in peripheral tissues including the skin but not in circulating blood, making it difficult to study the function of human mast cells. To address this limitation, we generated the mast cell-like cells from human iPS cells. CD34+CD43+ hematopoietic progenitor cells, CD43+ cells, and CD34+ cells were induced from human iPS cells by embryoid body formation method, and they were subsequently isolated and cultured in methylcellulose medium containing stem cell factor, IL-6, and IL-3. iPS cell-derived CD43+ cells could not form colonies. On the other hand, iPS cell-derived CD34+CD43+ and CD34+ cells formed mast cell-like colonies at 5 to 6 weeks of culture. These results suggest that mast cell progenitor cells include in not only CD34+CD43+ cells but also CD34+ cells. We analyzed the expression levels of mast cell marker genes in iPS cells, iPS-derived CD34+CD43+ cells, and iPS-derived mast cells by quantitative RT-PCR. The expression levels of mast cell marker genes were significantly up-regulated in iPS-derived mast cells as compared with iPS-derived CD34+CD43+ cells. In addition, we found that iPS-derived mast cells showed positive staining for tryptase and negative staining for chymase. Previous studies demonstrated that fibroblasts promote the maturation of mouse mast cells. Therefore, iPS cell-dived mast cells were co-culturing with mouse dermal fibroblasts (MDF) to generate tryptase/chymase-double positive mast cells. The expression level of chymase in iPS-derived mast cells co-cultured with MDF was significantly increased as compared with that in control cells. These data indicate that fibroblasts could promote the maturation of iPS-derived mast cells. In addition, iPS-derived mast cells displayed b-hexosaminidase release for IgE-mediated antigen stimulation. Thus, iPS-derived mast cells can be used as a model of human mast cells to further investigate their functions.

F-1101

EFFECTS OF EXPOSURE TO ELECTROMAGNETIC FIELDS EMITTED BY NUCLEAR MAGNETIC RESONANCE DEVICES ON HEMATOPOIETIC STEM CELLS

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We previously showed that extremely low-frequency electromagnetic fields (EMFs) promote proliferation and differentiation of rodent neural stem cells. In order to identify possible risks for the health of hospital workers, we explored the effects of exposures to EMFs emitted by nuclear magnetic resonance (NMR) devices on hematopoietic stem cells. First, we measured exposure to EMFs of staff working in NMR units to subsequently stimulate our cell models through an experimental device reproducing the same sequence of stimuli experienced by workers. Normal CD34+ hematopoietic cells, isolated by fluorescence-activated cell sorting from buffy coats of healthy blood donors, were exposed for 72 hours to EMFs (EMF+ samples). Control samples were maintained in the same conditions (IMDM + 10% fetal bovine serum), in the absence of EMFs (EMF- samples). Cells were then recovered and expanded for 7 days in the Stem Spam medium containing stem cell factor, thrombopoietin, Flt3-ligand and GM-CSF. Soon after the exposure (day 3) and after the culture (day 10), the clonogenic potential (colony number/103 cells) in EMF+ and EMF- samples was evaluated. On the whole, 47.2 ± 2.8% CD34+ viable cells were recovered after the first 72 hours, with no difference between EMF+ and EMF- samples (p=0.717). The EMF exposure produced an early selective favorable effect on the expansion of erytroid and monocyte progenitors. In fact, at day 3, the BFU-E content was 71 ± 5 in EMF+ cells and 39 ± 4 in controls (p=0.001). Similarly, the CFU-M content was 30 ± 5 and 16 ± 2 in EMF+ and EMF- samples, respectively (p=0.004). No effects were detected on immature progenitors such as GFU-GEMM (p=0.416) and CFU-GM (p=0.295). At day 10, EMF+ and EMF- samples produced similar amounts of various colonies. These data suggest that EMFs promote a transient expansion and differentiation of unilineage-committed hematopoietic progenitors. *This work was supported by grant from the Italian Ministry of Health (RF-2009-1543811).*

CARDIAC CELLS

F-1102

FUNCTIONAL REPROGRAMMING OF HUMAN EMBRYONIC STEM CELL-DERIVED VENTRICULAR CARDIOMYOCYTES INTO BIO-ARTIFICIAL PACEMAKER

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Normal heart rhythms originate in the sino-atrial node, a specialized cardiac tissue consisting of only a few thousands nodal pacemaker (Pm) cardiomyocytes (CMs). Malfunction of PmCMs due to diseases or aging leads to rhythm generation disorders, necessitating the implantation of electronic Pm, with such shortcomings as limited battery life, permanent implantation of leads, lead dislodging (particularly to pediatric patients due to somatic growth), the lack of autonomic responses, etc. As such, the engineering of bio-artificial Pm (BPm) as an alternative or supplement to electronic devices has been pursued. Human pluripotent stem cell (hPSC) can self-renew and differentiate into all lineages, serving as an unlimited CM source. However, the yield of PmCMs is always typically poor (<3%), independent of hPSC lines and even with directed cardiac differentiation protocols; recently, we reported a highly efficient hPSC specification protocol, enabling mass generation of ventricular (V) CMs with ~100% yield and purity. Building upon our series of previous studies of the crucial PmCM protein hyperpolarization-activated cyclic nucleotide-gated channel 1 (HCN1), here we functionally reprogrammed hPSC-VCMs into -PmCMs via adeno-associated virus (rAAV9, isoform chosen for cardiac tropism)-mediated overexpression of the engineered HCN1 channel (HCN1ΔΔΔ) whose S3-S4 linker residues 246-248 have been strategically deleted by design to promote cardiac pacemaking. rAAV9-HCN1ΔΔΔ-reprogrammed hPSC-PmCMs converted from -VCMs showed automaticity and action potential parameters typical of native nodal PmCMs. When tested in a long-term preclinical large animal porcine model of complete heart block (via atrio-ventricular node radiofrequency ablation), implantation of rAAV9-HCN1ΔΔΔ-based BPm via focal injection in the right ventricle significantly reduced the dependence on device-supported pacing from 89% observed in sham to 12% of our tested subjects. Electro-anatomical mapping further revealed the generation of spontaneous heart rhythms from the BPm, which remained stable for at least 4 weeks. Collectively, these results have taken our previous groundwork on BPm to the next translational level. Toxicity data collection for IND and the launch of a possible first-in-man trial of BPm are forthcoming.

F-1103

EARLY REPROGRAMMING-DERIVED GENE COCKTAIL INCREASES CARDIOMYOCYTE PROLIFERATION FOR HEART REGENERATION

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Although cardiomyocytes (CMs) possess a certain degree of proliferative ability, their efficiency is too low for regeneration. Examining changes occurring during cell reprogramming may yield useful information for re-enabling CM proliferation. As a result, we identified a unique combination of genes which can increase CM proliferation for regeneration after myocardial infarction (MI) in mice. Post-natal CMs and non-CMs were isolated non-genetically based upon TMRM staining. We then employed a modified reprogramming protocol optimized for CMs, and the resulting induced pluripotent stem cells (iPSCs) were characterized and found to be fully pluripotent. During the early stages, we observed differences between CMs and non-CMs in terms of their reprogramming efficiency and the timings of key events. For example, morphological changes caused by mesenchymal-epithelial transition were detected in non-CMs on day 2, whereas this process began on day 4 in CMs. Nevertheless, both cell types produced iPSC-like colonies after 6 days of reprogramming. In order to identify the key events preceding colony formation, total RNA was purified for microarray analysis on day 0, 2, 4, and 6 of the reprogramming. Genes associated with tight junctions and early pluripotency were observed for non-CMs on day 4, but again delayed to day 6 for CMs. Most importantly, we detected a significant increase in expression of mitosis associated genes in CMs on day 2. Thus, we hypothesized that a carefully selected cocktail of candidate genes could be delivered to CMs in order to restore their proliferation. Several candidates were selected from the day 2 microarray results and a combination of three genes, delivered as a cocktail, was able to increase post-natal murine CM proliferation in vitro by a factor of 8, according to higher H3P+ population %. When delivered to the normal heart in vivo, this gene cocktail can increase the heart-to-weight ratio and H3P+ population. Furthermore, when the gene cocktail was delivered to adult mice after MI, heart function was significantly improved and less fibrosis was observed. In conclusion, by examining gene expression changes during CM reprogramming, we have identified delivering a unique combination of genes which can restore CM proliferation for heart regeneration.

F-1104

METABOLIC MATURATION OF HIPS DERIVED CARDIOMYOCYTES IS REQUIRED FOR IN VITRO ISCHEMIA REPERFUSION MODELING

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Reperfusion after heart ischemia causes massive cardiomyocyte death as a result of ROS generation by a congested mitochondrial respiratory chain. Modeling in vitro the acute phases of an ischemic

event is made difficult by the challenges of pO2 control in culture, especially if fast dynamics are required and in line cell imaging is desired. Moreover, hiPS-derived cardiomyocytes, representing the only human cardiac source easily available, display a fetal hypoxia-resistant glycolytic metabolism far from an adult-like phenotype and are unsuitable for ischemia modeling. The aim of this work is to integrate metabolically induced human cardiomyocytes with a microfluidic platform for pO2 control in order to provide a suitable human cardiac in vitro model for ischemia/reperfusion events. We optimized a simple protocol based on metabolite-restriction in the culture media to induce intracellular glycogen stores depletion first, and then energetic substrate switch towards fatty acids utilization. The metabolic maturation induced a marked mitochondrial intracellular relocalization and structural reorganization. The proteins associated with fatty acid metabolism were up-regulated while the glycolytic pathway was shut down to minimal levels. Metabolic assays showed differential substrate utilization and significant improvement in respiratory reserve and maximum capacity. From a technological point of view, we present a novel microfluidic platform developed to provide very fast and controlled regulation of cell culture gaseous environment, designed for high resolution live imaging. The device allows equilibration of cell cultures with any desired pO2 within 60 seconds, without the slightestest perturbation of the culture medium. The system was first validated with neonatal rat cardiac cultures, in which 3 hours of hypoxia produce a 70% death rate. The metabolic induction transitioned the hiPS-CMs from a fetal phenotype insensitive to the hypoxic stresses provided in the device, to a hypoxia-sensitive adult-like oxidative metabolism. This resulted in similar death rates of the murine primary cardiac cultures after 3 hours of hypoxia, thus providing a good human cellular substrate for ischemia/reperfusion studies in vitro.

F-1105

PROBING FUNCTIONAL MATURATION OF SINGLE HUMAN PLURIPOTENT STEM CELLS-DERIVED CARDIOMYOCYTE

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The most important outputs of heart tissue are force generation and contraction characteristics, which are directly related to the oscillation-like increases and decreases in calcium concentration in the cytosol, termed Ca2+ transients. The aim of this work was to realize an in vitro platform that acquires the physiological parameters of human pluripotent stem cells derived cardiomyocytes (hPSC-CMs) and simultaneously induces a functional maturation of the cells. To this, we propose a method to measure the global force of contraction combined with the simultaneous detection of calcium dynamics on hPSC-CMs with an elongated shape induced by a specific chemistry of the substrate. Thanks to the use of an innovative substrate fabricated with an elastomeric photo-patternable material that can be easily functionalized through UV-light exposure, we fabricated micropillar elastomeric substrates with confined rectangular cell-adhesive areas. Elongated hPSC-CMs anchor between two micropillars inducing pillar deflections. Confocal analysis allowed the simultaneous detection of calcium dynamics and micropillar deflection due to cell contraction. The recorded deflection was employed to quantify the exerted force through the use of a proper finite elements model (FEM). This platform has been applied to evaluate calcium dynamics and contraction force on hESCs-CMs after 1 week and after 5 weeks of culture. Our results show that the duration of the transient of calcium decreases while contraction force increases on cells cultured for 5 weeks compared to cells cultured for 1 week, reinforcing the hypothesis that functional and structural maturation of hESCs-CMs is strictly related not only to the endogenous and exogenous factors but to the mechanical stimuli of the environment and to cell shape.

F-1106

DISSECTION OF HETEROGENEITY DURING CARDIAC MATURATION PROCESS BY SINGLE CELL RNA SEQUENCING

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Human induced pluripotent stem cells-derived cardiomyocytes (iPSC-CMs) are expected to be clinically applicable, especially in the field of disease modeling and drug screening. However, one of the major problems to be overcome is their immaturity and heterogeneity, because these problems hinder the acquisition of stable experimental results for the clinical use. To overcome these problems, we performed the single cell RNA sequencing during cardiac differentiation in vitro and explored deeply the insights underlining cardiac differentiation. We successfully defined the differentiation state of cells at single cell level by principle component analysis (PCA) and largely recapitulated the cardiac development process with dynamic changes of transcriptome during the differentiation. In addition, this analysis clarified the heterogeneity of differentiated-CMs. For scoring maturation level of these heterogeneous CMs, we uniquely defined maturation index as a barometer of CMs maturation. By ordering cells according to maturation index, we identified important gene networks for cardiac maturation process and also discovered a useful surface marker, by which we could enrich well-matured CMs from heterogeneous population of differentiated CMs. Taken together, these results suggested that high resolution analyses by single cell RNA sequencing was useful to discover important gene regulations for understanding heterogeneity during cardiac maturation process in vitro.

F-1107

NON-CELL AUTONOMOUS CUES FOR ENHANCED FUNCTIONALITY OF HUMAN PLURIPOTENT STEM CELL-DERIVED VENTRICULAR CARDIOMYOCYTES VIA MATURATION OF SARCOLEMMAL AND MITOCHONDRIAL KATP CHANNELS

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Human embryonic stem cells (hESCs) are a potential unlimited ex vivo source of ventricular (V) cardiomyocytes (CMs). However, hESC-VCMs display many immature traits. In adult VCMs, sarcolemmal (sarc) and mitochondrial (mito) ATP-sensitive potassium (KATP) channels play crucial roles in excitability and

cardioprotection. However, their biological roles and use in hESC-VCM have not been exploited. In this study, we investigated the role of KATP channels and their maturation in hESC-VCMs. SarcIK, ATP in single hESC-VCMs was dormant under baseline conditions, but became markedly activated by cyanide (CN) or the known opener P1075 with a current density that was ~8-fold smaller than adult; CN initially accelerated spontaneous action potential (AP) firing by shortening AP duration before subsequently rendering hESC-VCMs silent. CN-silenced cells remained excitable and could elicit a single AP upon depolarization. These effects were reversible upon washout or the addition of GLI or HMR1098. Neither sarcIK, ATP nor AP was affected by mitoIK, ATP opener, diazoxide, or blocker, 5-HD. Interestingly, sarcIK, ATP displayed a ~3-fold increase after treatment with hypoxia (5% O2) but not electrical stimulation or thyroid hormone (T3). MitoIK, ATP was absent in hESC-VCMs. Consistently, neither diazoxide nor 5-HD affected hypoxia (1%O2)-induced apoptosis of hESC-VCMs. Interestingly, T3, rather than hypoxia or electrical field stimulation, up-regulated mitoIK, ATP, conferring diazoxide protective effect on T3-treated hESC-VCMs. We conclude that hypoxia and T3 enhance the functionality of hESC-VCMs by selectively acting on and promoting the maturation of sarc and mitoIK, ATP.

F-1108

A NEW LONG NONCODING RNA EVX1-AS IS TRANSIENTLY EXPRESSED DURING EARLY HUMAN CARDIAC DEVELOPMENT

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Heart development is a highly orchestrated process involving the gene regulatory networks and transcriptional mechanisms. Long noncoding RNAs (lncRNAs) are emerging as new players responsible for fine-tuning of gene expression in mammalian development. Although Braveheart and Fendrr were recently characterized in murine cardiac development, little is known about lncRNAs in human developmental process. Here, we report that EVX1-AS, EVX1 antisense RNA, was found to be transiently expressed during early human cardiac development. Human iPS cells were differentiated into cardiomyocytes using the RPMI/B27 monolayer directed differentiation protocol to recapitulate human heart development. Serial samples collected during differentiation protocol were analyzed by strand-specific RNA sequencing. The expression profile of lncRNAs was verified using real-time RT-PCR analyses. 3' and 5' rapid amplification of cDNA ends (RACE) were employed to obtain the full sequence. Protein coding gene expression pattern was similar to the previous reports in human cardiac differentiation using human embryonic stem or induced pluripotent stem cells. Totally, 6,453 lincRNAs and 4,577 antisense RNAs were identified during cardiac differentiation process. We found that a transcript derived from the antisense locus of the EVX1, a human homeobox gene homologous to even-skipped, were highly and transiently up-regulated in early stages for cardiac progenitor specification. RACE analyses identified a novel transcript of approximately 3.4 kb, whose expression is generally absent in pluripotent stem cells and differentiated cardiomyocytes. Real-time RT-PCR analyses revealed that EVX1AS shows a dynamic expression pattern resembling the genes involved in mesoderm formation and patterning. Our data suggested a novel long noncoding RNA EVX1-AS is a possible target for investigations designed to elucidate the mechanisms governing normal human cardiac development.

F-1109

COMBINATORIAL MODULATION OF CRUCIAL CA HANDLING PROTEINS IN HUMAN PLURIPOTENT STEM CELL-DERIVED VENTRICULAR CARDIOMYOCYTES

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Ventricular (V) cardiomyocytes (CMs) are generally considered non-regenerative while self-renewable human pluripotent stem cells such as embryonic stem cells (hESCs) can differentiate into VCMs as an unlimited ex vivo cell source for disease modeling, drug/cardiotoxicity screening and cell-based therapies. However, hESC-VCMs display immature Ca2+-handing properties with smaller Ca2+ transient amplitudes and slower kinetics compared to adult. These functional immaturities can be attributed to their differential expression of crucial Ca2+-handling proteins. For instance, sodium-calcium exchanger (NCX) is robustly expressed in hESC-CMs but much less so in the adult counterparts; by contrast, sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) is less expressed in hESC-CMs. By lentivirus-mediated SERCA overexpression and NCX shRNA suppression, we demonstrated that SERCA was the major Ca2+ remover during diastole. Furthermore, SERCA overexpression partially rescued the negative force-frequency response seen in immature hESC-VCMs. Interestingly, combinatorial overexpression of SERCA, calsequestrin and phospholamban reduced the amplitude of electrical stimulation-elicited Ca2+ transient, but restored a positive inotropic response to β-adrenergic stimulation in hESC-VCMs. Taken collectively, these findings provide a better mechanistic understanding of the immaturity of hESC-VCMs, which may facilitate their clinical and other applications.

F-1110

SALL1-MESP1 CORPORATELY FUNCTIONS FOR CARDIOMYOCYTE INDUCTION AND ITS DIFFERENTIATION IN MURINE EMBRYOS/HUMAN IPS MODELS

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Cardiac cells are mainly derived from mesoderm cells regulated by Mesp1. Mesp1-lineaged cells differentiate into several cell types such as cardiac cells, hematopoietic cells, skeletal muscle and so on. So our aim is to understand how cardiac cell fate is specified from mesoderm cells, and which defined factors act as key players into this lineage. One of genes that we screened out from non-Mesp1-lineaged cells was *Sall1*, which acted as a novel key factor committing cardiovascular call fate from mesodermal cells. *Sall1* expression strongly starts in the primitive mesoderm region at E6.5, and it was restricted in the *Isl1* positive cardiac progenitor region by E9.5. Lineage trace analysis showed that *Sall1*-derived cells substantially contributed into the entirely heart and differentiated into all type of cardiac cells. *Sall1*-expressing cells sorted from in vitro differentiating ES cells clearly differentiated into cardiomyocytes and conduction cells, but not other organ cells fate (ex; skeletal muscle, vascular, neuron, pancreas gene program). Interestingly, a part of *Sall1*+ cells differentiated into *Mesp1*- cardiomyocytes in vitro, suggesting that

Sall1 marks non-*Mesp1* derived new cardiac cell lineage. In addition, *Sall+Mesp1-* cells labeled by DiO was detected differentiated cTnT+ cardiomyocytes in vivo heart. Amazingly, *Sall1/Mesp1* DKO mice as loss-of-function study caused no cardiac field, and Dox inducible *Sall1-Mesp1-overexpressed* hiPS cells as gain-of-function study efficiently differentiate cardiomyocytes. These results indicate that *Sall1-Mesp1* coordinately regulates cardiac cell lineages.

F-1111

SINOATRIAL NODE PACEMAKER CELLS GENERATED FROM HUMAN PLURIPOTENT STEM CELLS CAN FUNCTION AS BIOLOGICAL PACEMAKER

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The human heart rate is established by the sinoatrial node (SAN) that functions as primary pacemaker throughout life. Impaired SAN function due to congenital disease or aging is routinely treated by implantation of an electronic pacemaker that has disadvantages including limited adaption to growth in pediatric patients and lack of autonomic responsiveness. Biological pacemakers derived from human pluripotent stem cells (hPSCs) represent a promising alternative. Although hPSC differentiation cultures directed to a cardiac fate contain some pacemaker cells in addition to other cardiomyocytes subtypes, strategies for directed differentiation of SAN myocytes do not exist. Using developmental biology as a guide, we developed a differentiation strategy that promotes the generation of hPSC-derived populations that contain up to 35% SAN-like pacemaker cells (SANLP). These SANLP express typical SAN markers including TBX3, TBX18, SHOX2 and HCN4 at significant higher levels than found in control hPSCs-derived ventricular cardiomyocytes. SANLP do not express the ventricular markers MLC2V or IRX4, the atrial marker NPPA or the atrioventricular node marker TBX2. Furthermore, SANLPs display typical functional pacemaker properties including appropriate ion current profile and chronotropic responses to autonomic signals. In order to evaluate the potential of SANLPs for biological pacemaker applications we tested their ability to pace cardiac-tissue both in-vitro and in-vivo. To this end we show that aggregates of SANLP can act as pacemaker for monolayers of hPSC-derived ventricular cardiomyocytes (VLCM). When SANLP were engrafted to the apex of the rat heart, subsequent optical mapping studies in the isolated hearts, confirmed the ability of the SANLP to pace the ventricle following induction of transient atrioventricular block. In contrast, transplanted control VLCM displayed no pacemaker capacity. Taken together, these findings show that it is possible to specifically generate hPSC-derived SAN-like pacemakers that can act as biological pacemakers.

F-1112

EICOSAPENTAENOIC ACID ENHANCES DIFFERENTIATION OF EMBRYONIC STEM CELL INTO CARDIOMYOCYTE

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Embryonic stem cells (ESCs) offer a reliable means to produce acceptable number of functional cardiomyocytes to exploit in cell therapy, however low efficiency of differentiation hampers its therapeutic use. Although a vast number of chemical compounds have been tested on efficiency of cardiac differentiation, the effect of fish oil components like eicosapentaenoic acid (EPA) remained unstudied. EPA has been reported to have several cardioprotective effects, but there is no study about its contribution in cardiac differentiation. In the present study, mouse ESCs were induced to differentiate using hanging drops to form embryoid bodies and treatment with ascorbic acid, and in order to examine the effect of EPA, they were treated with different concentration of EPA simultaneously. Gene and protein expression and functional properties of cardiomyocytes derived from ESCs were evaluated. EPA at low concentration increased percentage of beating and beating area. It could also upregulate mRNA expression of Nkx2.5, Mef2c, α-MHC, cTnT and CX43 significantly. Flowcytometric analysis showed that the percentage of α-MHC positive cells in EPA-treated group was higher than control group. However, these findings have not been observed at higher concentrations of EPA. In conclusion, we have demonstrated that treatment of mESCs undergoing cardiac differentiation with low concentration of EPA enhanced cardiac differentiation and exhibited synergistic effects with ascorbic acid.

F-1113

NANOFIBROUS PATCHE LOADING Tβ4-MSCS WITH EPICARDIAL GRAFT ACCELERATE INFARCT MYOCARDIAL REPAIR BY ACTIVATING ENDOGENOUS REGENERATION MECHANISMS

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Recent studies indicate that the epicardium plays a crucial role in tissue regeneration after myocardial infarction (MI). The epicardial cells may proliferate and undergo epithelial-to-mesenchymal transition to form epicardium-derived cells (EPDCs) which have potentials to differentiate towards various types of cardiac cells after MI. Thymosin beta 4 (Tβ4) was deemed to play an important role in activating epicardial cells. However, it is a key issue how to explore an approach activating effectively the epicardial cells. In this study, mesenchymal stem cells (MSCs) derived from mice were transfected with Tβ4. Release of Tβ4 from the cells was determined with reverse phase high-performance chromatography. To prepare cardiac patches, the transfected cells were seeded on PLACL/collagen nanofibers. Survival and proliferation of the cells on the nanofibers were examined with MTT assay and hypoxia treatment. In the MI models

of female transgenic mice, the patches were implanted on the epicardium of the infarcted region. An indelible GFP label was used to trace fate of the activated EPDCs. Differentiation of the EPDCs and engrafted MSCs towards cardiomyocytes and vascular cells was traced. At four weeks after implantation of the patches, cardiac functions were improved significantly, scar area in the infarcted region reduced obviously. EPDCs increased in subepicardium and expressed Wt1, and some GFP+ cells expressed CD31, α-SMA or cTnT. Moreover, c-kit+ cells were observed in subepicardium and myocardium. Fluorescence in situ hybridization showed that some engrafted MSCs migrated into the subepicardium and myocardium, and a few of them expressed CD31, α-SMA or cTnT. These results suggest that Tβ4 released from the transfected MSCs in PLACL/collagen nanofibrous patches may effectively attenuated left ventricular remodeling and improved cardiac function by activating the epicardial cells and recruiting endogenous stem cells. Our finding provided a novel strategy for myocardial regeneration by enhancing the endogenous regenerative mechanisms.

F-1114

ANGIOTENSIN II REGULATES THE CALCIUM TRANSIENTS OF EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES BY ACTIVATING CANONICAL TRANSIENT RECEPTOR POTENTIAL ISOFORM 3 CHANNELS

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Mechanisms underlying the spontaneous beating activity of early differentiating cardiomyocytes are largely unknown. Calcium transients (CaTs) are important determinants of excitation-contraction coupling of cardiomyocytes. Objectives of this study: 1) to investigate if mouse embryonic stem cell-derived cardiomyocytes (mESC-CMs) express Ang II receptors and canonical transient receptor potential isoform 3 (TRPC3); 2) to investigate if Ang II regulates CaTs of mESC-CMs via TRPC3 channels. Our study revealed that mESC-CMs expressed Ang II type 1 receptors (AT1R) on the cell membrane. Exogenous Ang II increased CaTs; pre-incubation with AT1R-specific blocker losartan but not Ang II type 2 receptors (AT2R)-specific blocker PD123319 attenuated the effect of Ang II on CaTs. In addition, TRPC3-specific blocker Pyr3 attenuated Ang II-induced changes in CaTs, suggesting that Ang II regulates CaTs by opening TRPC3. In conclusion, mESC-CMs expresses AT1R and TRPC3 and exogenous Ang II increases the CaTs through AT1R and TRPC3.

F-1116

LONG-TERM EXPANSION AND CHARACTERIZATION OF HUMAN PLURIPOTENT STEM CELLS-DERIVED CARDIAC PROGENITOR CELLS

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Cardiac progenitor cells (CPCs) are considered as one of the most promising cell sources to cure heart diseases which can replace the dead myocardium. Human pluripotent stem cells (hPSCs) as unlimited proliferative stem cells are used to isolate CPCs during differentiation of hPSCs into cardiomyocytes. Isolated CPCs can be used as building blocks for other applications including cardiac tissue engineering and pharmaceutical studies if we will be able to maintain and expand them in a scale-up manner. In this study it was tried to expand CPCs by manipulation of cell signaling pathways using small molecules in a top-down study design. Our CPCs were cultured in a suspension culture condition which is more suitable for large-scale expansion. To characterize expanded CPCs, their proliferation, differentiation and tumorigenicity were analyzed. In each experiment the number of selected chemicals was decreased and the most effective combination of chemicals was determined based on maintenance of CPCs specific markers and genes expression profile. Expanded CPCs in the maintenance medium were clonogenic with differentiation potential into all three cardiac lineages (cardiomyocytes, endothelial and smooth muscle cells). No teratoma was formed after transplantation of expanded CPCs in nude mice. In conclusion we were able to maintain and expand CPCs in suspension culture condition using effective signaling chemicals in defined medium.

F-1117

SIMPLIFIED CULTURE SYSTEM FOR CARDIOMYOCYTE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS IN BASIC AND TRANSLATIONAL RESEARCH

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A simplified and reliable media system for generating cardiomyocytes from donor- or disease-specific human pluripotent stem cells (PSC) would provide a valuable source of cells for basic and translational research. Current protocols have led to heterogeneous results with varying purity and long lead times for generation of cardiomyocytes. As a result, we developed, tested and manufactured a GMP-grade culture media system that is scalable and can be used to generate large numbers of continuously maintained or cryopreserved cardiomyocytes. On Day 0, continuously maintained PSC were enzymatically dissociated, plated on 1:100 Geltrex®-coated surface at a seeding density between 0.5 to 2×10^4 cells/cm^2 and expanded for three days under serum-free, feeder-free condition. On Day 3, after reaching target confluence of ~30 to 60%, an induction media was added and cultured for two days followed by addition of a second induction media and cultured for two days. After the induction steps, the media was replaced with maintenance media and re-fed every other day for six or more days. PSC-derived cardiomyocytes were analyzed by morphology, immunocytochemistry, flow cytometry, electrophysiology, gene expression and RNA-Seq.
Individual beating cells were observed as soon as Day 10 and contracting syncytia by Day 14. There was an over 100-fold increase in cell number from time of plating on Day 0 to contracting syncytia on Day 14. Quantitative flow cytometry detected populations of 90% and higher troponin T type 2 (TNNT2)-immunoreactive cells. Key to high percentage of TNNT2-immunoreactive cells is optimal seeding density, which was PSC line-dependent. PCR and RNA-Seq studies confirmed expression of mesoderm, cardiac mesoderm and mature cardiomyocyte genes as well as presence

of atrial, ventricular and sinus-nodal genes. Immunocytochemistry studies verified expression of early and late cardiac markers. Calcium fluor and microelectrode array studies supported the presence of electrically active cardiomyocytes sensitive to known cardioactive agents. A simple and robust differentiation media system can serve as a standardized culture system for generating large numbers of consistent, spontaneously active cardiomyocytes for basic and translational research studies.

F-1118

DISCOVERY OF COMPOUNDS ENHANCING PROLIFERATION OF HUMAN CARDIAC PROGENITOR CELLS AND EPICARDIUM-DERIVED CELLS

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Multipotent progenitor cell populations have been reported to exist in the heart, some of which can play a role in normal turnover and/or repair after injury. However, these progenitor cells are quite rare in number and despite their presence in the heart, true functional repair after a myocardial infarction does not spontaneously occur. Identification of compounds aimed at enhancing repair through expanding the progenitor populations is a promising approach to enable effective regeneration of cardiac tissue. We performed phenotypic screens for proliferation of epicardium-derived cells (EPDCs) isolated from adult human heart and Nkx2.5+ cardiac progenitor cells (CPCs) derived from human iPSCs. The screens were run as medium throughput assays with the same optimized 7.5-10K compound set, with hits tested on human cardiac fibroblasts to remove non-specific cardiac cell proliferative agents. Developing and running these screens in parallel has allowed us to identify interesting compounds such as inhibitors of glycogen synthase kinase 3 that specifically proliferate CPCs and/or EPDCs without affecting proliferation of fibroblasts. Many of the compounds also maintained or even increased the multi-lineage differentiation capacity of the CPCs. The identification of compounds that proliferate CPCs and EPDCs, but not cardiac fiborblasts, has allowed us to discover novel compounds and targets that have the potential to result in true cardiac regeneration.

F-1119

INTRAMYOCARDIALLY INJECTED HIGHLY PURIFIED AND FUNCTIONAL INDUCED PLURIPOTENT STEM CELL-DERIVED HUMAN CARDIOMYOCYTES SURVIVE IN MOUSE INJURED HEARTS AND EXHIBIT MYOCARDIAL REGENERATION POTENTIAL

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Myocardial infarction (MI) remains a leading cause of morbidity and mortality worldwide. Conventional management of MI does not replace lost cardiomyocytes (CMs) with new CMs. Induced pluripotent stem cells (iPSCs) reprogrammed from somatic cells can differentiate into any type of cells. Thus, patient-specific iPSC-derived CMs (iPSC-CMs) have significant potential in providing immune-matched CMs for future clinical use in cardiac repair. A previous study showed that majority of injected iPSC-CMs in pig hearts were lost, possibly resulted from insufficient immunosuppression by tacrolimus. Thus, the aim of this study was to investigate survival, therapeutic effect and safety of injected human iPSC-CMs using an immunodeficient (SCID) mouse model following ischemic heart injury. Human iPSC-CMs were generated by culturing luciferase-labeled iPSCs in an induction medium. The CM differentiation efficiency was found to be 94%. Differentiated CMs were characterized to exhibit spontaneous contraction and express CM markers using immunofluorescence staining. For in vivo study, mice (n=4 mice/group) were subjected to a permanent occlusion of the left anterior descending coronary artery. iPSC-CMs in phosphate-buffered saline (PBS) or PBS alone (as a contorl group) were then injected into the border zone of infarcted area. Survival and migration of injected cells in live mice and freshly harvested tissues were tracked using luciferase-based bioluminescence imaging. Infarct size and terotoma formation were measured using Trichrome staining, and hematoxylin and eosin staining, respectively. Strong bioluminescent signals were observed in the heart area both one and three weeks after cell injection. Bioluminescent images of live mice and tissue clearly showed that intramyocardially injected iPSC-CMs did not migrate to other organs up to 3 weeks after injection. Injected iPSC-CMs significantly decreased infarct size relative to the control group. No terotoma were observed in mice.
Conclusions: We demonstrated for the first time that intramyocaradially injected human iPSC-CMs survived in injured hearts and decreased infarct size. They did not form teratoma nor migrate to other organs, suggesting feasibility, safety and myocardial regenerative capacity of human iPSC-CMs.

F-1120

CANINE MITRAL VALVE INTERSTITIAL CELL GROWTH AND PHENOTYPE IS IMPROVED BY CANINE WHARTON'S JELLY MESENCHYMAL STEM CELL CONDITIONED MEDIA OR EXOSOMES

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Mitral valve prolapse is the most common adult valvular heart disease in industrialized countries. Histologic changes include disarray of collagen and elastin fibers. In dogs with myxomatous mitral valvular disease (MMVD), the histological changes and functional consequences are virtually identical to those in humans. Canine valve interstitial cells (VICs) from diseased heart valves have a greater prevalence of cells with a myofibroblastic phenotype and growth retardation in vitro. The changes seen in VICs in diseased valves suggest a progressive fibrotic process. TGFβ stimulation of VICs in vitro results in fibroblastic (vimentin high+, αSMA low+) to myofibrolastic (vimentin low+, αSMA high+) transition. Given that mesenchymal stem cells (MSC) can exert anti-fibrotic effects, we investigated the effects of conditioned media (CM) derived from canine Wharton's Jelly MSCs (WJ-MSC) on the growth potential of canine VICs and its ability to counter the effects of TGFβ. VICs

were isolated and cultured from normal and diseased valves. IHC and qPCR were used to evaluate the expression of αSMA, vimentin, elastin, and collagen in VICs. CM was collected from WJ-MSC cultures (24 hr), and CM exosomes (EXs) were isolated by either ultrafiltration or ultracentrifugation. Cell growth and replicative capacity were evaluated using MTT assay and colony forming units (CFU). Internalization of WJ-MSC EXs by VICs was imaged with membrane or RNA staining. qPCR showed that diseased valve VICs had increased myofibroblastic phenotype and markedly decreased cell growth by MTT and CFU. Culturing of VICs of either phenotype with WJ-MSC CM or EXs alone resulted in increased number of viable cells, and depletion of EX had the opposite effect. EX membrane and RNA labeling confirmed EX uptake into VIC cytoplasm and nucleus, although RNA containing EXs were internalized by <50% of VICs implying selective endocytosis of RNA containing EXs or they are less numerous. Preliminary data also showed decreased responsiveness to TGFβ1 stimulation when VICs were cultured in CM with decreased expression in αSMA, elastin, and collagen. WJ-MSC CM or its EXs improve cell growth for VICs isolated from both normal and diseased canine mitral valves and may exert protective effects on VICs to slow the progression of canine MMVD.

F-1121

CONTRIBUTION OF IP3R2 IN HUMAN ESC MAINTENANCE AND CARDIAC LINEAGE DIFFERENTIATION

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Ca^{2+} is a versatile signal-conductor in cells, and participates in various cell processes, from life to death. Under stimulations, Ca^{2+} concentrations change fast in global or local manner, and pass signals to downstream molecules. Inositol-1,4,5-trisphosphate receptors (IP_3Rs), working as Ca^{2+} channels, mainly locate on endoplasm reticulum membrane. IP_3R2 is the dominant type in mammalian hearts. IP_3R2 participates in rodent fertilization, early development and heart function. IP_3R1 and IP_3R2 double knockout mice suffer embryo lethal at E13.5, with developmental defects of ventricular myocardium and atrioventricular canal in heart. Single knockout of IP_3R2 loses the response ability to endothelin-1. These data indicate that IP_3R2 may play a role in embryonic development and heart function. However, the precise role of IP_3Rs in development, especially in human early development, is largely unknown. To determine the role of IP_3R2 in early cardiac development, we used human embryonic stem cells (hESCs) in vitro cardiac differentiation model in the present study, combined with IP_3R2 knockout ($IP_3R2^{-/-}$) hESCs using TALEN technology, we confirmed that lack of IP_3R2 did not alter the pluripotent ability by analyzing alkaline phosphatase activity, pluripotent marker expressions, and cell proliferation. Then, we tested the role of IP_3R2 in cardiac linage commitment. Little influence was detected in the yield of cardiovascular progenitor cells and the expression level of early cardiac marker genes in $IP_3R2^{-/-}$ cells. In addition, the expression of cardiac specific genes and generated cardiomyocyte numbers were comparable between the wild type and $IP_3R2^{-/-}$ cells. These results demonstrate a minimal contribution of IP_3R2 in hESC maintenance and cardiac linage differentiation. The findings provide new knowledge to the impact of subtypes of IP_3Rs in hESCs and cardiac lineage commitment. The roles of IP_3R2 need to be investigated to get full knowledge of this receptor in other specific lineage commitment during early differentiation of hESCs.

MUSCLE CELLS

F-1123

THE CELL POLARITY PROTEIN SCRIB CONTROLS MUSCLE STEM CELL FATE

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Satellite cells are resident muscle stem cells, supplying myonuclei for homeostasis, hypertrophy and regeneration in adult skeletal muscle. Satellite cells are activated in response to stimulation such as muscle injury. The majority of activated satellite cells rapidly proliferate and then undergo myogenic differentiation, whereas only a minority population returns to a quiescent state to self-renew for future requirements. Scrib is a cell polarity protein that plays an important role in maintaining cell adhesion and preventing cell growth in epithelial tissues, thus known as a potent tumour suppressor. Here, we investigated the role of Scrib in the satellite cell fate decisions during myogenic progression in adult mice. We found that Scrib is undetectable in quiescent cells, but becomes weakly expressed in activated/proliferative cells. Scrib is asymmetrically distributed in dividing daughter cells, with robust accumulation in cells committed to myogenic differentiation. Overexpression of Scrib disrupted both proliferation and self-renewal of satellite cells, whereas constitutive expression of Scrib prevented only self-renewal but not proliferation. Surprisingly, either knockdown of Scrib by siRNA or satellite cell-specific conditional knockout of Scrib (Scrib-scKO) in mice also inhibited population expansion, likely through deregulation of growth factor signalling in satellite cells. Indeed, Scrib-scKO mice exhibited a significant defect in muscle regeneration. Although Scrib is a well-known tumour suppressor in epithelial cells, our observations indicate that in satellite cells muscle, Scrib has functions distinct from those in epithelial cells. Taken together, our results suggest that Scrib is a regulator of myogenic progression, controlling population expansion and self-renewal with expression levels of Scrib potently directing satellite cell-fate decisions.

F-1124

EFFICIENT MUSCLE REGENERATION POTENTIAL OF HUMAN MUSCLE PRECURSOR CELLS EXPANDED IN HYPOXIA

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Skeletal muscle engineering would be highly beneficial to treat congenital and acquired diseases in children. We focused our efforts on (1) the procedure of human muscle precursors cell (hMPC) extraction from fresh and frozen muscle biopsies, (2) culture condition for cell expansion, in particular oxygen tension, and (3) the use of a platelet gel and hyaluronic acid support to improve in vivo muscle regeneration. Twenty human muscle biopsies (age from

20 to 65, male and female) from Tibialis and Peroneous muscles were used after informed consent. Biopsies were finely minced and alternatively frozen in liquid nitrogen for later use or straight digested with type I collagenase and trypsin-EDTA. Cells were then cultured at 20% and 5% oxygen. The effect of 1 μM bpV(phen) and 1 μM Q-VD-OPh addition was also assessed. Doubling time analysis, myogenic index evaluation and immunoflorescence staining for Myf5, MyoD, Pax7 and Ki67 were carried out. In vivo injection of hMPC in damaged Tibialis Anterior muscle of Rag 2-/- γc-/- mice were performed using platelet gel and hyluronic acid as support. We firstly demonstrated that hMPC from frozen biopsies did not display any difference compared to cells obtained from fresh samples, in terms of proliferation, myogenic marker expression and in vitro myotube formation. Both at 20% and 5% of oxygen tension, unselected cells homogenously expressed CD56 after two or more passages (more than 90% CD56+ cells), without FACS selection. We observed that in hypoxia and after addition of chemicals the doubling time was shorter in respect to normoxia, whereas the expression of myogenic markers and the myogenic index did not change according to oxygen condition. In vivo in muscle treated with platelet gel, hyaluronic acid and hMPC cultured in hypoxia there was significant muscle regeneration. We identify a simple and efficient culture protocol that allows obtaining a good population of hMPC also after treatment with bpV(phen) and Q-VD-Oph in low oxygen tension. When injected in vivo in a damaged muscle treated with a tissue engineering approach hMPC possess good mobility, proliferation rate and regeneration ability. Taking together, these findings may have important implications for clinical application of skeletal muscle tissue engineering.

F-1125

MIR195 AND MIR497 INDUCE POSTNATAL QUIESCENCE OF SKELETAL MUSCLE STEM CELLS

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Skeletal muscle stem cells (MuSCs), the major source for skeletal muscle regeneration in vertebrates, are in a state of cell cycle arrest in adult skeletal muscles. Prior evidence suggests that embryonic muscle progenitors proliferate and differentiate to form myofibres and also self-renew, implying that MuSCs, derived from these cells, acquire quiescence later during development. Depletion of Dicer in adult MuSCs promoted their exit from quiescence, suggesting microRNAs are involved in the maintenance of quiescence. Here we identified miR-195 and miR-497 that induce cell cycle arrest by targeting cell cycle genes, Cdc25 and Ccnd. Reduced expression of MyoD in juvenile MuSCs, as a result of overexpressed miR-195/497 or attenuated Cdc25/Ccnd, revealed an intimate link between quiescence and suppression of myogenesis in MuSCs. Transplantation of cultured MuSCs treated with miR-195/497 contributed more efficiently to regenerating muscles of dystrophin-deficient mice, indicating the potential utility of miR-195/497 for stem cell therapies.

F-1126

THE EFFECTS OF TNF-ALPHA ON THE MYOGENIC DIFFERENTIATION OF HUMAN RS MYOGENIC PROGENITOR CELLS

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The external urethral sphincter, also known as the urethral rhabdosphincter (RS), is a key structure involved in urinary continence. Although it has been considered that human RS cell apoptosis and/or RS impairment are induced with aging, the mechanism remains unclear. Meanwhile, it is widely considered that aging is accompanied by a chronic, low grade inflammatory condition. We previously reported that TNF-α led growth inhibition and apotosis induction in human RS myogenic progenitor cells. In the present study, we explored the effects of TNF-α on the myogenic differentiation of these cells. Human urethral sphincter samples were obtained from patients who underwent radical cystectomy for bladder cancer. Human myogenic cells were immmortalized on the protocol; mutated cyclin-dependent kinase4, cyclinD1 and teromerase to expand their life span. Firstly, we examined differentiation potential of human RS myogenic progenitor cells by real-time RT-PCR, Western blot and immunostaining. Subsequently, we examined the effects of TNF-α on their differentiation. Immortalized RS progenitor cells highly expressed striated muscle markers such as desmin, and could differentiate into myotubes under myogenic differentiation medium. Even in molecular analysis, these cells highly expressed differentiation marker such as myosin heavy chain (MHC). TNF-α significantly attenuated the MHCs expression of these cells in a dose-dependent fashion. The present study indicated that TNF-α has a potential to suppress not only cell proliferation but also myogenic differentiation in RS myogenic progenitor cells. Although further verification studies are warranted, TNF-α may be involved in age related decreases in the number of human RS cells and be a causative factor for urinary incontinence in the elderly population.

F-1127

EXPRESSION OF TNF-ALPHA-STIMULATED GENE 6 PRODUCT IN MESENCHYMAL STEM CELLS REQUIRED FOR THEIR SUCCESSFUL TRANSPLANTATION INTO THE MOUSE SKELETAL MUSCLE

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Our previous report demonstrated that when mesenchymal stem cells (MSCs) from mouse ES cells were transplanted into mouse skeletal muscle MSCs showed high potential for differentiation in the crush injured tibialis anterior muscle in vivo, although MSCs neither

differentiated nor settled in the intact muscle. Microenvironments including extracellular matrix (ECM) between the injured and intact muscle were quite different. In the injured muscle, hyaluronan (HA), heavy chains of inter-α-inhibitor (IαI), CD44 and TNF-α-stimulated gene 6 product (TSG-6) increased 24-48h after injury, while basement membrane components of differentiated muscle such as perlecan, laminin, and type IV collagen increased gradually from 4 days after the crush. We then investigated micro environments crucial for the cell transplantation, using the lysate of C2C12 myotubules for mimicking injured circumstances in vivo. MSCs settled in the intact muscle when they were transplanted together with the C2C12 lysate or TSG6. We demonstrated MSCs suddenly produced and released TSG6 when they were cultured with C2C12 lysate in vitro by real-time PCR, immunohistochemistry and western blotting. MSCs pretreated with the lysate also settled in the intact muscle. Further, MSCs who's TSG6 was knocked-down by shRNA, even if transplanted or pretreated with the lysate, could not settle in the muscle. On the other hand, neither HA nor IαI could settle MSCs without TSG6 in the intact muscle. Immunofluorescent staining showed that HA and IαI always co-localized or distributed closely, suggesting formation of covalent complexes, i.e., the SHAP-HA complex in the presence of TSG6. Thus, TSG6, HA and IαI were crucial factors for the settlement and probably subsequent differentiation of MSCs.

F-1128

PDGFR-ALPHA+MUSCLE-DERIVED MESENCHYMAL STEM CELLS CONTRIBUTE TO THE FORMATION OF HETEROTOPIC OSSIFICATION

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Heterotopic ossification (HO) is a pathological condition that muscles calcify. Local muscle damage and fracture/osteotomy are often associated with the development of HO. It is unclear, however, the source of cells in the muscles that develop into bone. In muscles, there are two types of cells which are potent in differentiation: satellite cells and mesenchymal stem cells. Both of them have been demonstrated are capable of osteogenic differentiation. This study was designed to differentiate the source of cells that participate in the development of HO. HO surgical model: Surgery that included osteotomy of great trochanter and muscle injury (O+M) was performed on the right hip of mice to simulate the tissue condition for HO formation. Muscle injury was done by clamping the gluteus maximus and gluteus medius for 5 min. For controls, either osteotomy of great trochanter (O) or muscle injury (M) was performed on mice. Mice in groups O+M, O and M, were euthanized at days 1, 3, 5 and 10. Gluteus maximus and gluteus medius were collected for muscle digestion. The isolated cells were cultured in an osteogenic medium for 3 weeks and osteogenesis was examined with gene expression of type I collagen, osteocalcin and Runx2. Notably, the expression of type I collagen, Runx2 and osteocalcin by the cells of M+O group harvested on days 3, 5 and 10 was significantly increased, compared with the corresponding ones in M and O groups. Flow cytometry was performed on cells isolated from M, O, and M+O (day 10) for the expression of CD73, CD90, CD105, CD56 and PDGFRα (CD140). In general, M-MSCs isolated from M, O and M+O groups (day 10) had similar expressions profiles of common MSC surface markers, except the expression of CD90 was lower in the O and M+O groups than in the M group. CD56, representing myogenic progenitors, was highly expressed in M-MSCs isolated from M group (day 10), as compared with O and M+O groups (day 10) ($p<0.05$). CD140, also known as platelet-derived growth factor receptor α (PDGFRα), is a specific marker of muscle derived MSCs. Among the three experimental groups, CD140 was expressed the highest in the M+O group (day10), followed by the O group and M group ($p<0.05$). The data indicate that muscle-derived MSCs, rather than muscle satellite cells, participate in HO formation in the muscles.

KIDNEY CELLS

F-1129

FUNCTIONAL RECONSTRUCTION OF THE NEPHRON PROGENITOR NICHE

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FGF, BMP and WNT balance embryonic nephron progenitor cell renewal and differentiation. By modulating these pathways we have created an in vitro niche in which nephron progenitor cells from embryonic kidneys or derived from human embryonic stem cells can be propagated. Nephron progenitor cell cultures expanded several thousand-fold in this environment can be induced to form tubules expressing nephron markers. Single cell culture reveals phenotypic variability within the early CITED1-expressing nephron progenitor cell compartment indicating that it is a mixture of cells with varying progenitor potential. Furthermore, we find that the developmental age of nephron progenitor cells does not correlate with propagation capacity, indicating that cessation of nephrogenesis is related to factors other than an intrinsic clock. This in vitro nephron progenitor niche will have important applications for expansion of cells for engraftment and will facilitate investigation of mechanisms that determine the balance between renewal and differentiation in these cells.

F-1130

KIDNEY ORGANOIDS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS CONTAIN PATTERNING NEPHRONS WITHIN AND APPROPRIATE STROMAL ENVIRONMENT

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We have previously reported the successful differentiation of human pluripotent stem cells, including hESC and iPSC, to a kidney endpoint. This involved the stepwise induction of appropriate patterning events known to result in kidney development, including differentiation into posterior primitive streak, intermediate mesoderm and ultimately both the mesenchymal (metanephric mesenchyme) and epithelial (ureteric bud) progenitor populations required to form the collecting duct and nephron progenitors. When these 2D cultures were aggregated and grown in 3D, we observed the spontaneous initiation of nephron formation, as evidenced by the formation of JAG1$^+$CDH6$^+$ renal vesicles. While this was strong evidence of a

kidney endpoint, the renal vesicle represents only the very initial stage of nephron formation with substantial additional patterning and segmentation required for a functional filtration unit to form. Here we report modifications to the differentiation protocol with respect to culture duration and growth factor conditions. As a result, we can now generate mini-kidneys within which nephrons pattern and segment into four distinct segments, representing a contiguous collecting duct, distal tubule, proximal tubule and glomerulus. These segments were marked by GATA3$^+$ ECAD$^+$, GATA3$^-$ECAD$^+$, LTL$^+$ and WT1$^+$NPHS1$^+$, respectively. Branching collecting ducts were observed along the bottom layer of each organoid with many nephrons present in the upper layers which are connected to these branches. We also show the presence of an appropriate MEIS1+ stromal population surrounding the nephrons as well as evidence for endothelial cells, based upon CD31 and KDR immunofluorescence. Despite the presence of endothelium, we observed no evidence of vascularization in glomeruli. This data suggests that these organoids, which are 3-5 mm in size, continue to represent a developing structure rather than a mature organ. However, the complexity observed suggests that such an organoid may prove useful for applications such as nephrotoxicity screening, disease modelling, tissue bioengineering or the generation of specific renal cell types for cellular therapy.

PANCREATIC, LIVER, LUNG OR INTESTINAL/GUT CELLS

F-1131

ENDODERM PROGENITORS DEVELOP INTO ENTERIC NEURONS DURING MOUSE EMBRYONIC DEVELOPMENT AND PERSIST IN THE ADULT ENTERIC NERVOUS SYSTEM

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The enteric nervous system (ENS) is thought to develop from the ectodermal neural crest cells in mammals, while the ENS originates from both the endoderm and the ectoderm in the invertebrate sea anemone. We thus asked whether any neurons of the mammalian ENS might be derived from the endoderm. We previously have shown that pancreatic multipotent precursors are capable of generating small numbers of neurons in vitro, indicating a cross-germ layer potential. Moreover, pancreatic β-cells and neurons share master regulatory transcription factors during development. We thus analyzed the development of ENS in mice using a lineage tracing method by crossing pancreatic duodenal homeobox1 (Pdx1)-Cre transgenic mice with Rosa-YFP reporter mice. Our results revealed a subpopulation of enteric neurons that were derived from endoderm pancreatic lineage. The Pdx1 lineage derived neurons appeared at E11.5 and migrated to colonize the gut following neural crest derived neurons. In adult, the Pdx1 progenitor derived neurons comprised 29.5 ± 5% of total neurons in the duodenum, 6.5 ± 1% in the ileum and 5 ± 2% in the colon. Using a second Pdx1-Cre transgenic line and a Sox17-2A-iCre knock-in line, we confirmed the endodermal origin of this subset of enteric neurons. Similar to neural crest stem cells, Pdx1 derived progenitors reside in the muscle layer of the adult gut and were able to form clonal spheres upon culturing in vitro. Next, we cultured single cells from the gut smooth muscle layers from Pdx1-Cre x Rosa-YFP mice in neurosphere assays. Clonal YFP positive spheres comprised 11% and 17% of total spheres from ileum and colon, respectively. Pdx1 lineage derived spheres differentiated primarily into neurons, but did not form any GFAP positive progeny. They also gave rise to pancreatic cell types, expressing endocrine markers Pdx1 and C-peptide, and exocrine marker amylase; whereas YFP negative spheres (from the neural crest lineage) generated neurons and GFAP positive progeny, but not pancreatic progeny. In summary, our data revealed that a subset of ENS neurons originate from the endodermal Pdx1/Sox17 lineages, challenging the single origin theory of mammalian ENS. We also identified a novel population of the endoderm-origin neuronal progenitors that persisted in adult and gave rise to neurons along with pancreatic cells in vitro.

F-1132

GLIAL CELL-DERIVED NEUROTROPHIC FACTOR PROTECTS HUMAN ISLETS FROM APOPTOSIS INDUCED BY SERUM STARVATION AND ENDOPLASMIC RETICULUM STRESS

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One of the most promising new treatments for patients with type 1- diabetes is islets transplantation. However, widespread application of procedure is hindered by different obstacles such as shortage of donor pancreas, low rate of islets proliferation and also high rate of apoptosis through islets isolation and pre-transplant culture. Therefore, we have investigated the effect of Glial Cell-derived Neurotrophic Factor (GDNF) on human islets proliferation and whether GDNF can eliminate serum starvation-induced apoptosis and ER stress-induced cell death in vitro. Human islets were cultured in CMRL medium containing 5.5 mM glucose supplemented with 0.5% AB serum, in order to mimic starvation condition, with or without GDNF (200 ng/ml) for 72 hours. In vitro islets function and survival were assessed by analyzing glucose stimulated insulin secretion (GSIS) and cell death detection ELISA respectively. Proliferative effect of GDNF was studied by culturing human islets in complete CMRL containing 10 uM of EDU for 6 days. Proliferation rate of islets were assessed using EDU-incorporated click-iT assay. In parallel and with the aim of studying protective effect of GDNF against ER stress-induced apoptosis, human islets were exposed to Thapsigargin (Tg) (1uM) with or without GDNF for 48 hours followed by cell death assessment and proteins analysis of potential responsible apoptosis signaling pathway. We have found that, GDNF was able to improve GSIS (n=7, $p<0.01$ vs control) and reduce apoptosis (n=7, $p<0.0001$ vs control) in human islets under starvation condition. However, there was no significant difference

in islets proliferation between GDNF-treated and control groups. In addition, Tg significantly elevated human islets apoptosis (n=6, $p<0.0001$ vs control) whereas GDNF were able to rescue islets and decrease apoptosis (n=6, $p<0.01$ vs tg-treated islets). Western blot/Bioplex analysis revealed that GDNF survival effect could be through reduction of Chop and increase in p-GSK3β (n=3, $p<.05$ vs Tg-treated islets). GDNF has a protective effect on starved and also ER stressed human islets in vitro. GDNF anti-apoptotic effect in ER stressed-islets is through Chop and GSK3β signaling pathway.

F-1133

TRANSWELL® FOR ENHANCING HEPATOCYTE DIFFERENTIATION FROM HUMAN EMBRYONIC STEM CELLS

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Primary human hepatocytes are considered as the gold standard model for xenobiotic metabolism and cytotoxicity studies. However, their limited availability and inter-individual variability seriously constrain the use in in vitro systems in screening [1]. Therefore, hES-derived hepatocyte is an attractive alternative cells source to primary hepatocyte as the cells have unlimited renewability and can potentially generate consistent hepatocytes. In recent years, several 3D culture differentiation protocols have been reported to enhance hepatic function of the ES-derived cell [2-6]. Among them, Matsui demonstrated that placing hepatocytes on a gas-permeable membrane resulted in polarity restoration and formation of bile canaliculus in the cultured hepatocytes [7]. Other studies utilized Corning Transwell® to co-culture with other cells to promote/hamper stem cells or progenitors differentiate into certain lineages [8-9]. Transwell® is composed of a porous membrane which does not only create an environment for cell-cell communication, but also enable high oxygenation in cell culture. Here we describe a method using Corning Transwell® as a platform to differentiate hepatocyte progenitors (derived from human embryonic stem cells line, TW6) into hepatocytes of improved drug metabolism functions. Hepatocyte progenitors were generated by exposing TW6 cells, plated on collagen-coated plates, to activin A for 3 days then FGF4 and HGF for 5 days. The progenitors were harvested and seeded on semipermeable membrane inserts coated with Matrigel and cultured in DMEM based differentiation medium for 10 days and in Corning Hepatocyte Maintenance Medium for another 7 days. CYP1A2 and CYP3A4 were determined using Promega's P450-Glo™ Assays as described by the manufacturer. Culturing hepatocyte progenitors on Transwell® inserts significantly enhanced basal and induced CYP1A2 and CYP3A4 enzymatic activity 2-3 fold relative to cells cultured on tissue-culture dishes coated with Matrigel. The expression of both CYPs was comparable to CYP activities observed using to the cell line HepaRG™. We conclude that Transwell® culture may be used to promote the differentiation of stem cell derived hepatocytes without co-culture.

F-1134

MITCHONDRIAL TRANSFER THROUGH TUNNELING NANOTUBE-LIKE STRUCTURES FROM HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM/STROMAL CELLS TO HUMAN PRIMARY HEPATOCYTES IN CO-CULTURE

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Hepatocyte transplantation using encapsulated cells in alginate beads is a promising alternative to whole liver transplantation for patients with acute liver failure diseases. Nevertheless, poor viability and function of primary human hepatocytes (HCs) after extraction from their native niche limit the effective duration of this therapy. We and others have shown that Mesenchymal Stem/Stromal Cells (MSCs) enhance HC viability and metabolism, thus highlighting a likely effect on HC mitochondrial function. As MSCs have been reported to transfer mitochondria to other cell types through actin-based nanostructures called Tunnelling Nanotubes (TNT), we hypothesise that MSCs affect HC function due to TNT-based mitochondria transfer. Aim: To confirm that MSCs can enhance HC viability and functionality and assess if TNT-based transfer of mitochondria occurs between MSCs and HCs. Cryopreserved HCs were thawed and stained with CFSE (2μM, 10min, 37°C), and MSCs were stained with MitoTracker Red™ (0.1μM, 15min, 37°C). HCs were cultured on collagen-coated plates with or without MSCs (ratio: 10/1). Cell function was quantified for 23 days by measuring albumin synthesis and total protein; viability/attachment was assessed using an SRB assay. Mitochondrial transfer from MSC to HCs was detected over 24h by co-localisation of CFSE and MitoTracker Red signals using live fluorescent microscopy, and FACS. The presence of TNT was studied by fluorescent microscopy after cell fixation. MSCs increased HCs initial attachment 7-fold ($p<0.05$) and albumin production 3-fold at day 3 and 40-fold at day 23: respectively 188 ± 39 vs 58 ± 11 and 756 ± 24 vs 17 ± 2 ng/10^6cells/24h, (mean ± SEM, n=3, $p<0.002$). Mitochondrial transfer from MSCs to HCs was progressively detected after 2h using live microscopy and confirmed by FACS (n=3), where a significant increase in the percentage of double stained HCs was detected in the co-cultures after 4 and 6h (27.6 ± 10.3 and 27.7 ± 10.8% respectively, $p<0.05$ vs monocultures). TNT structures containing MitoTracker-labelled mitochondria were observed between MSCs in mono-cultures, as well as MSCs/HCs in co-cultures. Conclusion: MSCs can transfer mitochondria to HCs through TNT, which may explain the beneficial effects on HCs viability and function in direct co-culture.

F-1135

GENERATION OF THE HUMANIZED LIVER MOUSE FROM HEPARP3 CELLS

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Humanized-liver mice, in which the liver has been repopulated

with human hepatocytes, have been used to study aspects of human liver physiology such as drug metabolism, toxicology and hepatitis infection. The procurement of human hepatocytes is a major problem in producing humanized-liver mice because of the finite nature of the patient-derived resource. We previously reported that the human hepatic cell line HepaRG cells were a possible cell source for generating humanized-liver mice. HepaRG cells differentiate into CYP3A4-expressing mature hepatocytes in the liver of TK-NOG mouse. However, replacement index of HepaRG cell is significantly low. Here we describe the transplantation studies of new cell line, Hepa-RP3. Hepa-RP3 is one of the human hepatic cell line derived from the Hepa-SC cells carrying a stem cell-like phenotype, following genomic engineering of the parental HepaRG cells. Transplanted Hepa-RP3 cells retained proliferation activity in vivo, and Hepa-RP3 derivatives replaced about 20% of recipient mouse liver (cf. HepaRG cells: ~0.2%). On the other hand, Hepa-RP3 derivatives demonstrated duct-like morphology and sustained CK-19 expression. Immunohistochemical analysis revealed that Hepa-RP3 derivatives had not expressed mature hepatocyte markers, such as CYP3A4 and MRP2. These results indicated that Hepa-RP3 derivatives remain the hepatic stem cell characteristics in the recipient mouse liver. We then expect that in vivo induction of Hepa-RP3 cells would generate highly replaced humanized-liver mice, which are a useful model for in vivo studies of liver physiology.

F-1136

EFFECTS OF TNF-ALPHA AND NICOTINE ON DIFFERENTIATION OF HUMAN ENDODERMAL PROGENITOR CELLS INTO INTESTINAL EPITHELIAL CELLS

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It was suggested that TNF-alpha may prevent mucosal healing in patients with inflammatory bowel diseases. On the other hand, it was shown that nicotine exerts protective effects on progression of ulcerative colitis. The present study examined whether stimulation with TNF-alpha or nicotine affects differentiation of human endodermal progenitor cells into intestinal epithelial cells. Human endodermal progenitor cells were induced in differentiation of human iPS cells by stimulation with BMP-4, activin A, bFGF, and LY294002 (a PI3K inhibitor). Wnt3a (0.25 nM), TNF-alpha (3-100 U/ml), and/or nicotine (3-30 microM) were added to the endodermal progenitor cells on gelatin-coated plates and were cultured for 4-7 days. The differentiation potential from human endodermal progenitor cells into intestinal epithelial cells was evaluated by CDX2 expression using immunofluorescence staining and western blot analysis. The differentiation of human iPS cells into endodermal progenitor cells was confirmed by examining the expression of specific markers such as SOX17 and FOXA2. In addition, stimulation with Wnt3a significantly induced CDX2 expression in the endodermal progenitor cells cultured with the differentiation protocol. On the other hand, treatment with TNF-alpha (100 U/ml) significantly inhibited Wnt3a-induced CDX2 expression. Simultaneous treatment with nicotine (3 or 10 microM) significantly reversed the inhibitory effect of TNF-alpha. In addition, pretreatment with alpha-bungarotoxin (an alpha7 nicotinic acetylcholine receptor antagonist) blocked the reverse effect of nicotine. [Conclusion] While the stimulation with TNF-alpha inhibits the differentiation of human endodermal progenitor cells into intestinal epithelial cells, the stimulation with nicotine may protect the inhibitory effect of TNF-alpha.

F-1137

A NOVEL SOURCE OF MESENCHYMAL STEM CELLS FROM THE HUMAN NEONATAL PANCREAS

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Understanding β-cell neogenesis for diabetes research currently relies upon the use of rodent models or lengthy pluripotent stem cell protocols. Human pancreatic mesenchymal stem cells (pMSCs) are resident stem cells with limited capacity for differentiation and provide an alternative source for β-cell neogenesis research. In this study, pMSC lines were derived from three patients with congenital hyperinsulinism in infancy (CHI). CHI is a disorder of uncontrolled insulin release and pancreatic hyperproliferation in neonates which requires removal of part of the infant's pancreas. Both CHI and adult control pMSCs were grown out from pancreas digests as monolayer cultures according to published methods. Here, three unique cell lines were derived from patients of different ages with different forms of the disease. Two of the patients were positive for mutations in ABCC8, which encodes the SUR1 subunit of ATP-sensitive potassium channels. In the third patient, the genetic cause of the disease is unknown. Single tandem repeat profiling and genotype analysis were undertaken for each cell line to authenticate originality and confirm the patient source. Using RT-PCR and western blot, all three cell lines were found to express Islet1, MafB, Pax6 and Sox9, markers associated with pancreatic development. CD29, CD44, CD45, CD90 and CD105 expression profiling by flow cytometry was similar to adult islet-derived pMSCs and published criteria. Morphology of all three lines was consistent and, as with adult islet-derived pMSCs, CHI-derived cell lines were readily able to form islet-like clusters in low adherence cell culture plates. CHI-derived cell lines also proliferated faster than adult pMSCs (doubling time of 90 hours vs 195 hours) and continued to proliferate beyond P20 in contrast to their adult counterparts. Under standard cell culture conditions with the extracellular matrix proteins fibronectin and vitronectin, CHI-derived pMSCs maintained expression of the insulin gene for >3months, whereas this was lost in adult islet-derived pMSCS. Our data show that derivation of pMSCs from CHI patients is feasible, reproducible, and that this source of tissue has several advantages over adult islet-derived pMSC lines, including insulin gene expression and longer-term stability and viability.

F-1138

HYPOXIA-INDUCED STEM-LIKE PROPERTIES MEDIATED BY DNA DEMETHYLATION IN LUNG CANCER

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Tumors exhibiting extensive hypoxia have been shown to be more aggressive than corresponding tumors that are better oxygenized, which suggests that hypoxic condition induces stem-like properties. The purpose of the present study was to investigate whether hypoxic stress induces acquisition of stem-like properties, and which is involved with DNA demethylation in lung cancer. Normal epithelial cell line (BEAS-2B) and human lung cancer cell lines (A549, H292, H226 and H460) were incubated in either normoxic or hypoxic (below 1% O2) condition. The cell lines were treated with a DNA methyltransferase inhibitor (5-azacytidine, AZA) to determine whether the expression of stem cell markers (CD44, CD133, CXCR4, ABCG2, CD117, ALDH1A1, EpCAM, CD90, Oct4, Nanog, SOX2, SSEA4 and CD166) was reactivated. Methylation-specific PCR and bisulfite sequencing were used to analyze the methylation status, and real-time RT-PCR and western blotting were performed to analyze the expression of the stem cell markers. Cell migration and Matrigel invasion assay were performed for functional analysis. Among the 13 stem cell markers, CXCR4, Oct4 and Nanog were increased at least one lung cancer cell line in hypoxic condition compared with in normoxic condition. These three stem cell markers were reactivated by treatment with AZA. Methylation-specific PCR showed decreased promoter methylation of these three stem cell markers in hypoxic condition compared with in normoxic condition, which was further validated by bisulfite sequencing. Migration and invasion were increase in hypoxic condition compared with in normoxic condition. These results suggest that under the hypoxic condition, reactivation of stem-like properties was related with promoter demethylation of stem cell markers. Further studies are needed to assess its value as a prognostic factor and potential therapeutic applications. *This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. 2014R1A2A1A11052422).*

F-1139

SUBSTANCE P ALLEVIATES HEPATIC FIBROSIS VIA INDUCTION OF STEM CELL MOBILIZATION AND ANTI INFLAMMATORY RESPONSE

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The repetitive hepatic damage by viral hepatitis, toxins and alcohol triggers chronic inflammation and the excessive accumulation of extracellular matrixes, which causes scar tissue formation to make difficult for the liver to function. Although mortality from liver cirrhosis has been consistently declining, liver diseases have still been hailed as the global burden. In recent, stem cell therapy has been proposed as a promising treatment for liver diseases and thus, numerous clinical trials with stem cell transplantation or stem cell-mobilizing factors have been attempted. Previous reports demonstrated that substance-p (SP) promotes tissue repair by mobilizing bone marrow stem cells and stimulating anti-inflammatory response. Our approach is based on the hypothesis that SP can exert the therapeutic effect to liver fibrosis by recruiting endogenous stem cells and modulating immune response. In order to explore the effect of SP, liver fibrosis was induced in mice by injection of thioacetamide (TAA) and recombinant leptin, three times a week for 10 weeks. After induction of liver disease, SP was intravenously administrated three times a week for 6 more weeks with injection of TAA+leptin. In results, SP decreased the formation of hepatic micronodule on the liver external surface. Histological analysis revealed SP is able to reduce the area of collagen deposition, extent of portal-portal bridge and number of α-SMA (+) hepatic stellate cells, accompanied by reduction of TGF-β1 expression and increase in the activity of MMP-2 and 9 in the liver tissue. Also, SP treatment suppressed inflammatory responses entirely by decreasing the number of infiltrated immune cells within liver tissue and blocking enlargement of spleen. Additionally, stem cell recruitment was promoted by SP treatment. Thus, the alleviation of hepatic fibrosis by SP might be attributable to anti-inflammatory effect and stem cell mobilization in liver cirrhosis model. Our study suggests a promising effect of SP as a reparative agent to prevent progression of hepatic fibrosis.

F-1140

MONITORING OF LUNG TISSUE CELLS DIFFERENTIATED FROM HUMAN PLURIPOTENT STEM CELLS BY A SECRETED PROTEIN

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Human pluripotent stem cells-derived terminally differentiated cells mimics in vivo functional abilities, and facilitates basic clinical applications such as disease modelling and regenerative medicine. Recent progress of differentiation research have developed several efficient derivation methods of lineage specific progenitors, and succeeded in generating hind gut, mid gut, and anterior foregut endoderm. Especially, lung and airway progenitors, those derived from anterior foregut endoderm, have been differentiated quite efficiently by screening numerous cytokines or mimicking in vivo development.

Comparing to differentiation of lung progenitors, however, preparation of functionally differentiated lung tissue cells from pluripotent stem cells is still challenging, and some reports have shown that the efficiency of terminally differentiated cells from pluripotent stem cells was only several percent. For improving this problem, here, we present a secreted protein that can monitor the differentiation of lung cells from pluripotent stem cells. Secreted proteins have been used as a disease-specific markers, as they can be analyzed with small amount of blood samples without serious invasion or genetic modification. Some secreted proteins are functionally stable in cultured medium, and can be quantitated by ELISA to monitor the presence of functional cells. This secreted protein marker could be a new probe to develop efficient differentiation methods, and useful for a both safe and easy quality control of monitoring of lung cell differentiation from pluripotent

stem cells in future clinical application. We will discuss the usefulness of our marker protein to monitor lung tissue cell differentiation from human pluripotent stem cells.

F-1141

LIVER STEM CELL MEDIATED GENE THERAPY FOR LYSOSOMAL STORAGE DISEASE

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Lysosomal storage diseases (LSDs) are inherited metabolic disorders caused by a deficiency in one of the approximately 50 lysosomal enzymes. The estimated cumulative prevalence of LSDs is 1 in 5000 live births and patients are characterized by the intralysosomal accumulation of undegraded substrates, which ultimately leads to tissue damage, subsequent organ dysfunction and neurological complications. Although progress has been made in the treatment of some LSDs by the use of enzyme replacement therapy (ERT) and hematopoietic stem cell (HSC) transplantation, there is still a large unmet medical need for better and new treatments. In this study we explore the opportunities to use a liver stem cell-mediated gene therapy as an alternative approach for the treatment of LSDs. With the recent advances in the establishment of long-term culture of adult Lrg5+ liver stem cells, it is now possible to make targeted genetic modifications and select genetically stable clones that overexpress and secrete the missing lysosomal enzyme. As proof-of-principle we make use of α-L-iduronidase (IDUA) deficient mouse, which serves as a model for the LSD named Hurler's syndrome (mucopolysaccharidosis type I). We established a culture of liver stem cells from IDUA-/- mice and by lentiviral transduction we obtained cells that expressed and secreted supranormal levels of IDUA. We are currently transplanting the modified liver stem cells into IDUA-deficient mice and monitoring the efficacy of the treatment. Simultaneously, we are optimizing protocols to improve the transplantation efficiency and for this we make use of GFP-labeled liver stem cells that can be easily traced back and quantified after transplantation. In addition, we aim to target IDUA via CRISPR/Cas9 technology to a locus, such as the albumin locus, by which the enzyme becomes highly expressed and the use of lentiviruses and their side-effects is avoided. Given our proof-of-principle study is successful, liver stem cell-mediated gene therapy could be applicable to other forms of LSDs and other diseases that respond to recombinant proteins, such as hemophilia.

F-1142

A NOVEL MODEL OF LIVER INJURY TO INVESTIGATE THE LIVER REPOPULATING CAPACITY OF A DEFINED POPULATION OF HPCS ORIGINATE FROM THE BILIARY ORIGIN

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Hepatic Progenitor Cells (HPCs) are thought to differentiate towards both hepatocyte and biliary (cholangiocyte) lineages during liver repair. The ability of HPCs to repopulate the liver parenchyma is currently under debate in several studies using conventional liver injury models. Here, we describe a novel model of hepatocyte regeneration where over 98% of hepatocytes are irreversibly damaged through hepatocyte specific loss of Mdm2 and the remaining hepatocytes express high levels of p21. This large scale hepatocyte senescence results in activation of HPCs and complete, functional reconstitution of the liver from endogenous HPC population, whereas inhibition of HPC activation results in mortality. Furthermore, we identify a highly defined population of HPCs which originate from the biliary lineage, can be isolated based on surface marker expression (EpCAM+/CD24+/CD133+/CD45-/CD31-/Ter119-) by FACS. This population has high colony forming capacity and can be expanded to a large scale in vitro, and remain phenotypically stable. We show that these ex vivo HPCs can be transplanted into a non-competitive repopulation assay in the injured adult liver where they significantly contribute to restoration of the liver parenchyma, with improved liver function characterised by the significant increase in serum albumin levels and 50% reduction in scarring. In addition, transplanted cells incorporate into bile ducts highlighting their potency in vivo. Here we demonstrate for the first time that large-scale expansion of endogenous HPCs is sufficient to reconstitute the liver in vivo and can be expanded in vitro, offering a viable alternative to hepatocyte replacement therapy or liver transplantation following chronic disease.

F-1143

A GENETIC CIRCUIT FOR PLURIPOTENT VS. DIFFERENTIATED CELL FATE SELECTION IN THE DROSOPHILA AIRWAYS

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The developmental potential of cells, ranging from stem cells to terminally differentiated cell types, is tightly controlled at multiple levels. Understanding the genetic and molecular mechanisms balancing pluripotency and differentiation in vivo is a crucial challenge. The embryonic Drosophila airway tree can be roughly subdivided into 2 types of cell populations with distinct developmental potentials: 1) a proximally located, pluripotent adult precursor cell population (P-fate) and 2) the distally located, more differentiated cells (D-fate). We show that a zinc finger transcription factor (TF), Grain (grn) promotes the P-fate while a POU-homeobox TF Ventral veinless (vvl, also called drifter or u-turned) promotes the D-fate. Hedgehog (hh) and receptor tyrosine kinase (RTK) signaling cooperate with vvl to drive the D-fate at the expense of the P-fate, while negative regulators of either of the signaling pathways ensure

P-fate specification. The requirement of grn for P-fate specification is partially alleviated by vvl loss suggesting that the default fate of airway cells could be the P fate. Removal of all major determinants for both the P-and the D-fates leads to reduction of the airway cell fate identity monitored by the expression of a master regulator, trachealess (a bHLH-PAS protein). We present a regulatory model, where the locally expressed morphogens: TGF-beta (Dpp), Wnt (Wingless) and Hh differentially regulate the expression of 3 TFs and RTK signaling activation to transform an equipotent developmental field into a 2D centro-peripheral morphogenetic fate pattern. This relatively simple patterning rule gradually gives rise to the distal-proximal organization of differentiated and pluripotent cells in the mature airway.

F-1144

MULTIPOTENT ENDODERMAL PROGENITORS (EPS) AS AN IN VITRO MODEL FOR DISSECTING ENDODERMAL CELL FATE DECISIONS

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Human embryonic stem cells (hESCs) are the gold standard in pluripotent stem cell research. hESCs are derived from blastocysts, this raises ethical and immune rejection concerns. The use of human induced pluripotent stem cells (iPSC), which are similar to hESCs by-passes these concerns. We have generated episomal-derived and integration-free iPSC (E-iPSC) from human fetal foreskin fibroblast cells (HFF1). This viral-free method has the advantage over viral-based protocols because of the lack of integrations which otherwise leads to chromosomal re-arrangements of the host genome.
The derivation of integration free iPSC from somatic cells and differentiating them into a donor cell type of interest are promising approaches for (i) future application in tissue replacement therapies, (ii) generation of disease models in vitro, (iii) toxicology and drug screening. Based on these viral-free iPSC (undifferentiated stage) a stepwise protocol was used to generate first definitive endoderm (DE), transient cells (TC), then pre-endodermal progenitors (pre-EP) and finally the endodermal progenitors (EP). The EPs express lineage specific markers such as Albumin (liver), PDX1 (pancreas), CDX2 (intestine) and SOX2 (intestine and lung). This expression pattern indicates that these EPs are multipotent endodermal stem cells. Microarray analyses uncovered that definitive endoderm and primitive streak related genes were expressed most in DE and pre-EP samples. Transcription factors related to early liver (TBX3), pancreas (ISL1, RFX6), lung (FOXP2) as well as ID2 and MEIS2 were over expressed in EPs. A heat map of progenitor related genes identified the EP as a panendoderm progenitor. Further studies are planned involving the use of the E-iPSC derived EPs to generate hepatocyte and pancreatic cells. These studies will enable uncovering the cell fate decisions, uncovering the genes regulative network and associated pathways that specify a multipotential EPs to differentiate to liver, pancreas or intestine. Additionally, these E-iPSC and derived EPs provide unique resources for disease modeling, developmental studies, drug screening and toxicology studies.

F-1145

THE STUDY FOR SIMPLIFIED METHODS OF INDUCING PANCREATIC ENDOCRINE CELLS FROM HIPSC IN COMPLETELY DEFINED MEDIUM CONDITION

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Investigation how to induce and differentiate pancreatic tissues is crucial to realize regenerative therapy for diabetes. Many studies of induction methods have been extensively performed, but the method still remains to be improved for optimization. To maximize the ratio of induced pancreatic endocrine cells, for example, many cytokines and additives have to be used, but the high cost by using them is a serious problem for the remedy. Here we show the method with small number of factors by which endocrine cells can be highly induced in completely defined medium condition. At first we fixed a basic protocol consisting of four treatment steps. Our previous report showed that appropriate cell density at the start point is important for endoderm cell differentiation. And in endocrine cell differentiation, optimized cell density is necessary for the high efficient induction in our culture condition. By our protocol, we could induce about 90% of Pdx1 positive cells and 10% of insulin positive cells from hiPSC. Next we refined the experimental condition in the 4th step, indicating that addition with a cytokine in this step changed the ratio of c-peptide/glucagon positive cells. We further assessed the effect of extracellular matrix on beta cell induction. Matrigels showed most efficient induction and fibronectin is also comparable, indicating the possibility of Xeno-free induction system. As a result, we established high-efficient beta cell induction method by using a small number of chemicals/cytokines in completely defined medium. Our study makes it more practical for regenerative medicine especially in the point of the cost.

F-1146

MESENCHYMAL STEM CELLS AND LOCAL IRRADIATION FOR MODULATION OF IMPLANT SITE IN INTRA-BONE MARROW PANCREATIC ISLET TRANSPLANTATION

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Bone marrow (BM) has been recently proposed as an alternative site for islet transplantation (tx). The BM, for its structure and anatomical position, offers the possibility to modulate microenvironment by local interventions. . The aim of our study was to investigate whether local irradiation and/or mesenchymal stem cell (MSC) co-tx in mouse are able to improve islet engraftment and prevent rejection in BM. A model of BM local irradiation was set up: we established irradiation conditions (932 rad), verified selective cell depletion and chose the time point for tx (3 days after treatment). MSCs from bone marrow (BM-MSCs) or pancreatic tissue (pMSCs) were isolated and characterized for biological and immunomodulatory properties to choose the best candidate for in vivo co-tx. Gain of normoglycaemia and time to rejection were evaluated in a fully MHC mismatched model of intra BM islet tx (400 C57BL/6 IEQ in BALB/c). Islets

with or without 300,000 syngeneic MSCs, from pancreas or bone marrow, were alternatively infused into irradiated or control femur. Islet tx into locally irradiated BM had better outcome compared to not irradiated recipients in terms of capacity to gain normoglycaemia (100% vs 55% in irradiated vs not irradiated mice, P=0,069). Glycaemia in the first two weeks after tx was significantly lower in the group of irradiated mice (P=0,047) and local irradiation also delayed time of rejection (the median time to graft rejection were 12 ± 0.6 days for locally irradiated mice and 5 ± 3.3 days for not irradiated mice). Pancreatic MSCs showed morphology, phenotype and plasticity comparable to BM-MSC and strong immunomodulatory properties in vitro, resulting the best candidate for co-tx. In vivo preliminary results showed that single infusion co-tx of pMSCs or BM-MSC and islets in irradiated recipients did not significantly improve the probability to gain normoglycaemia and didn't delay the time of rejection in transplanted mice.
Conclusion. BM irradiation before islet tx favours islet engraftment. Co-transplantation of pMSC or BM MSC in irradiated BM does not significantly impact on islet survival and function.

F-1147

EX VIVO PANCREATIC REGENERATION OF BRANCHING MORPHOGENESIS BY THREE-DIMENSIONAL DUCTAL CELL CULTURE SYSTEM

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The pancreatic duct cells (PDCs) are a potential cell source for new islet formation. In previous reports with monolayer culture, however, dense cell-to-dish contact may inhibit the potential growth and differentiation of duct cells in vitro. We hypothesized that duct cells in three-dimensional (3D) culture can retain their inherent characteristics such as branching morphogenesis during pancreatic organogenesis. Primary PDCs isolated from adult DBA/2 mice were cultured in suspension to form spheroids, which were then embedded in the collagen gel and cultured three-dimensionally. After seven days of 3D culture in collagen gel, PDC spheroids formed 3D structures of ductal cysts with branching morphogenesis when cultured in the medium containing 10% fetal bovine serum, whereas no branch formation was observed in serum-free medium. Notably, epidermal growth factor (EGF) enhanced the branching morphogenesis, which was evaluated by total branch length and number of branch points. Moreover, EGF significantly increased the branching proliferation, when compared to other growth factors such as KGF, VEGF, FGF10 and HGF. Immunostaining confirmed that the branching morphogenesis expressed the ductal cell markers, cytokeratin (CK), E-cadherin and Sox9, suggesting multipotent progenitor cells. We have developed a particularly promising approach of a 3D culture system using a collagen gel. This allows ex vivo branching morphogenesis to produce newly developed ducts, including progenitors that could give rise to new islets. This approach may provide important insights into the pancreatic ductal differentiation and a potential attractive therapeutic strategy for diabetes.

F-1148

REPROGRAMMED GASTROINTESTINAL STEM CELLS CAN SERVE AS A RESERVOIR FOR CONTINUOUS PRODUCTION OF INSULIN+ BETA CELLS

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The adult gastrointestinal (GI) tract contains stem cells that generate a constant supply of new cells in the normal turnover of the GI epithelium. Among the GI epithelial cells are hormone-secreting enteroendocrine cells. We have devised a strategy to reprogram GI stem cells in vivo and in vitro with defined genetic factors to produce functional insulin+ cells at the expense of enteroendocrine cells. Using inducible expression of three beta-cell reprogramming factors (Ngn3, Pdx1, and MafA) in mouse genetic models, we discovered that both gastric and intestinal stem cells can be reprogrammed to produce insulin+ cells. The induced GI insulin+ cells express key beta cell genes and show robust glucose-responsiveness. Surprisingly, molecular and functional analyses indicate that gastric insulin+ cells more closely resemble endogenous pancreatic beta cells compared with intestinal insulin+ cells. Gene profiling studies suggest that this difference likely results from differences in the endogenous endocrine programs of the gastric versus the intestinal system. The induced GI beta cells can rapidly regenerate from the stems cells and are thus capable of maintaining normoglycemia for many months in diabetic animals even after repeated ablation. To explore therapeutic potential of the induced GI beta cells, we created bioengineered stomach tissues using polyglycolic acid (PGA) scaffolds seeded with isolated gastric units or cultured gastric stem cells engineered to express the reprogramming factors. Upon transplantation into diabetic animals, the engineered stomach spheres produced insulin+ cells and suppressed hyperglycemia. These studies indicate that engineered GI stem cell could provide a renewable source of functional beta cells for diabetes studies and treatment.

ENDOTHELIAL CELLS/ HEMANGIOBLASTS

F-1150

INTRACORONARY ADMINISTRATION OF GCSF-MOBILIZED HUMAN CD34+ CELLS PRESERVES LEFT VENTRCULAR FUNCTION AND PREVENTS INFARCT EXPANSION IN A SWINE MODEL OF ACUTE MYOCARDIAL INFARCTION

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A phase I and a randomized phase II (PreSERVE AMI) clinical trials for intracoronary (i.c.) infusion of bone marrow (BM) CD34+ cells revealed the safety and potential efficacy such as dose-dependent improvement of left ventricular ejection fraction (LVEF) in patients with acute myocardial infarction (AMI). Despite such clinical development, CD34+ cell therapy has never been investigated in large animal models of AMI. AMI was induced by 60 min-balloon inflation and reperfusion in the left anterior descending artery of swine. One week after AMI, swine were randomly allocated to receive i.c. infusion of 2x10^5/kg GCSF-mobilized human CD34+ cells (cell group, n=8) or saline (control group, n=7). Immunosuppression was maintained by intramuscular injection of cyclosporine daily from week 1 to 5. Magnetic resonance imaging was performed to assess cardiac function and infarct size at week 1 and 5. Chemical staining for isolectin B4 and HLA-A,B,C was performed to detect capillaries and transplanted human-derived cells, respectively in the cardiac tissue at week 5. LVEF significantly increased in cell group (42.9 ± 5.2 at week 1 to 48.5 ± 4.1% at week 5, p<0.05) but not control group (47.6 ± 2.4 to 44.8 ± 3.5%, p=NS). LV end-systolic volume (LVESV) decreased in cell group (38.3 ± 4.9 to 31.4 ± 5.3 ml, p<0.01) but not control group (35.4 ± 5.4 to 37.7 ± 5.4 ml, p=NS). Regional wall thickening (RWT) in the border zone increased in cell group (30.8 ± 14.9 to 45.5 ± 10.4%, p<0.01) but not control group (37.2 ± 16.4 to 26.4 ± 9.3%, P=NS). Infarct size by delayed enhancement imaging increased in control group (16.8 ± 7.5 to 22.35 ± 6.4% of LV mass, p<0.05) but not cell group (20.5 ± 10.2 to 20.87 ± 8.0%, p=NS). Changes in LVEF, LVESV and RWT between week 1 and 5 were better preserved in cell group than control group (p<0.05, 0.05 and 0.01, respectively). Histological capillary density in border zone at week 5 was greater in cell group than control group (1698.0 ± 474.2 vs 998.6 ± 269.6/mm2, p<0.01). Double immunostaining for HLA-A,B,C and isolectin B4 revealed that human CD34+ cell-derived endothelial cells were 15.0 ± 2.3% of all endothelial cells in the border zone. Favorable outcomes would support the further clinical development of the cell-based therapy in patients with AMI.

F-1151

VASCULOGENIC CONDITIONED PERIPHERAL BLOOD MONONUCLEAR CELLS RESCUE THE RADIATION-INDUCED SALIVARY GLAND HYPOFUNCTION

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Currently, there is no effective treatment available to patients with irreversible loss of salivary gland (SG) function caused after radiotherapy for head and neck cancer. Recently, we have shown the therapeutic potential of bone marrow derived cells, including mesenchymal stem cells (MSCs) or endothelial progenitor cells (EPCs), which contribute to prevent SG hypofunction depending on vasculogenesis, paracrine effect, and cell transdifferentiation. However, bone aspirates involve donor-site morbidity and an expansion process of these cells is required to obtain a sufficient number of capable MSCs and EPCs for transplantation. The reality is that these stem cells lose their plasticity severely during culture expansion and cell passaging. To solve these problems for future clinical setting of stem cell therapy, we investigate whether exogenous peripheral blood-derived mononuclear cells (PBMNCs), purified by recently developed quality and quantity (QQ) culture system of EPCs, can rescue the SG hypofunction. QQ culture-treated PB-MNCs (QQ-PBMNCs) that contained EPC population abundantly can be obtained after 5-days of culture, and display the enhanced angiovasculogenesis and anti-inflammatory effect. 5x104 QQ-PBMNCs were transplanted to submandibular glands in a mouse just after local-irradiation (IR). After 4, 8 and 12 weeks of IR, salivary outputs were measured and then the gland tissues were harvested for histological analyses to clarify the effects of cell transplantation. Non-transplanted mice after irradiation were employed as an experimental control. Salivary outputs in mice that transplanted QQ-PBMNCs were increased approximately 2- to 4-fold sequentially for 4-12 weeks after IR when compared with that of non-transplanted mice. On the histological analyses, QQ-PBMNCs-treated SGs showed the increased level of blood vessel formation prominently and the decreased area of damaged acinar cells. Our data suggests that exogenous QQ-PBMNCs reduce the radiogenic damages of SGs. In particular, this cell therapy may bring the therapeutic effects by a small number of stem/progenitor cells that cultured over a short time. We are currently investigating further mechanisms of preventive or regenerative effects displayed by this therapy.

F-1152

MIR-130 INCREASES THE VASCULOGENIC PROPERTIES OF HUMAN ENDOTHELIAL COLONY FORMING CELLS UNDER HYPOXIC CONDITIONS

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Endothelial Progenitor Cells promote vascular repair in ischaemic tissues and therefore hold therapeutic potential. A subtype of endothelial progenitors known as Endothelial Colony Forming Cells (ECFCs) can be isolated from human peripheral and umbilical cord blood. ECFCs delivered into ischaemic sites integrate with newly formed blood vessels in vivo. If this novel cell therapy is to be translated to clinics, ECFCs will be required to traffic to non-perfused tissues and therefore, it is critical to understand how they respond to hypoxic stimuli. MicroRNAs are important regulators of gene expression and a number of microRNAs have been shown to influence the angiogenic properties of endothelial cells. Expression of these 'angiomiRs' in ECFCs upon exposure to hypoxia (1% oxygen) were assessed using qRT-PCR. Mir-130a and Mir-130b were significantly upregulated as early as 4 hours after exposure to hypoxia. To study the role of Mir-130, its expression was modulated and ECFC proliferation was examined by Ki67 immunostaining, BrdU incorporation, and cell counts. Overexpression of miR-130 using microRNA mimics led to an increase in cell proliferation under hypoxic conditions, while inhibition of miR-130 family with LNA inhibitors significantly diminished ECFCs proliferative capacity under hypoxic conditions (22% decrease, p<0.05) when compared to LNA controls. In agreement with in vitro findings, cells overexpressing miR-130 led to significantly higher number of perfused blood

vessels in the in vivo subcutaneous matrigel angiogenesis assay. Under hypoxic conditions, CD34 expression in ECFCs is significantly reduced as demonstrated by flow cytometry. Interestingly, miR-130 overexpression partially prevented this hypoxia-induced CD34 down-regulation. Inhibition of miR-130 family reduced HIF-1α protein expression in ECFCs, which suggests that mir-130 acts upstream HIF-1α as a positive modulator to enhance angiogenic ECFC properties in hypoxia. Our results indicate that miR-130 plays a role in ECFC response to hypoxia. Furthermore, mir-130 modulation is suggested as a novel stratagem to improve ECFC reparative capacity when faced to a hypoxic environment.

F-1153

HUMAN PODOPLANIN$^+$/VEGFR-3$^+$/LYVE-1$^+$ MONOCYTES ARE LYMPHATIC ENDOTHELIAL PRECURSORS

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Lymphatic vessels are involved in the development of various inflammatory disorders, and lymphatic vessel regeneration has been increasingly investigated to develop therapies for lymphatic diseases. Here we report that Podoplanin$^+$/VEGFR-3$^+$/LYVE-1$^+$ is a valid marker for human lymphatic endothelial precursors and the triple-positive cells can be used in lymphatic regeneration. During 5-day culture on an ultra-low attachment surface dish, human peripheral blood mononuclear cells (PBMCs) underwent exponential growth, aggregating into a sphere-like structure and expressing several lymphatic endothelial cell (LEC) markers and lymphangiogenic transcription factors. When dissociated from the aggregate and cultured on a gelatin-coated dish, the cells were attached to the surface. The attached cells were triple positive for LEC markers e.g. Podoplanin, LYVE-1, VEGFR-3. Furthermore, seeded in Matrigel with LECs, the 5-day aggregate-derived cells were incorporated into lymphatic endothelial network. The 5-day aggregates were largely positive for CD14$^+$, a monocyte marker. The CD14$^+$ population was sorted into Podoplanin-positive and negative group for further characterization. Notably, CD14$^+$/Podoplanin$^+$ cells showed increased expression of lymphangiogenic molecules (e.g. VEGFR-3, LYVE-1) both at the genetic and protein levels. Also, CD14$^+$/Podoplanin$^+$ cells secreted higher levels of lymphangiogenic cytokines (VEGF, HGF, PDGF-BB). ELISA results showed that CD14$^+$/Podoplanin$^+$ cells produced more lymphangiogenic cytokines than CD14$^+$/Podoplanin$^-$ cells. Local injection of monocyte aggregates significantly increased lymphatic neovascularization and facilitated healing of the skin wound model of nude mice, with CD14$^+$/Podoplanin$^+$ group showing the most dramatic result. Our data suggests that Podoplanin-positive monocytes can be transdifferentiated into lymphatic endothelial precursor cells, and cells with triple positivity for Podoplanin, VEGFR-3, and LYVE-1 can be a promising cell source for therapy against human lymphatic vessel diseases.

F-1154

SEQUENTIAL SWITCHING OF MATRICES DIRECTS HUMAN PLURIPOTENT STEM CELLS INTO ENDOTHELIAL LINEAGE

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Obtaining highly-purified differentiated cells by directed differentiation from human pluripotent stem cells (hPSCs) is an essential step for clinical application of PSCs. We previously established a 2-dimensional serum-free culture for mesodermal differentiation which can generate blood cells and vascular endothelial cells. Modifying that culture, we noticed that re-plating of PSC-derived mesodermal progenitor cells on day 3 (hereafter referred to as PSC-MPCs) onto another Matrigel-coated plate significantly improved the purity of KDR+/CD34+/VE-cadherin+ PSC-derived endothelial progenitor cells (PSC-EPCs) Since Matrigel contains various extracellular matrix (ECM) components which are not defined, we next tested various defined matrices as secondary matrix and evaluated the purity of PSC-EPCs. We found that non-coated and Laminin 411 (LM411)-coated dishes reproducibly produced highly purified PSC-EPCs among multiple cell lines. Although the purity of PSC-EPCs between LM411 and non-coating conditions, the yield of those cells on LM411 was slightly higher than that on non-coating. Then, in vitro tube formation assay showed that PSC-derived endothelial cells (PSC-ECs) between the two conditions, only LM411 produced PSC-ECs capable of angiogenesis. And PSC-ECs on LM411 took up Ac-LDL. Transcriptional profiles of those PSC-EPCs were closer to primary endothelial cell-derived cell lines such as HUVECs and HAECs than parental PSCs. In aggregation, sequential matrix switching to LM411 produced highly purified PSC-EPCs with simple and robust procedure. However, for clinical application, we need to improve the yield of functional endothelial cells.

EPIDERMAL CELLS

F-1156

ENHANCING WNT SIGNALING PREVENTS GENOTOXIC INJURY-INDUCED HAIR LOSS BY AUGMENTING SPONTANEOUS ANAGEN REPAIR

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There is currently a lack of method to prevent chemotherapy and radiotherapy-induced alopecia. Whether and how anagen hair follicles attempt to repair themselves in response to these genotoxic insults have not been well characterized. We found that there is a dose-dependent effect of ionizing radiation on the severity of dystrophic change of hair follicles. According the severity of dystrophy, anagen hair follicles were able to initiate two spatially and temporally distinct early and late repair activities to restore their structure. We found that bulge stem cells were not activated for the repair. Instead, two precursor cell populations, K5+ and Lgr5+ cells respectively, compensatorily proliferate to restore the anagen hair follicle structure. Molecularly, we found this apoptosis-driven

dystrophy was p53-dependent and accompanied by inhibited wnt signaling. Restoration of wnt signaling preceded the compensatory cell proliferation for the repair attempts. We demonstrated that boosting wnt signaling was able to prevent hair loss by enhancing compensatory cell proliferation at an earlier stage to reduce dystrophy. Similar prevention of hair loss was also achieved in chemotherapy-induced alopecia. Thus, radiation and chemotherapy-induced alopecia can be prevented by modulating wnt signaling to enhance spontaneous anagen repair by activating non-conventional precursor cells.

F-1157

P63 IS ESSENTIAL FOR MOUSE EYE DEVELOPMENT

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The transcription factor p63, a member of p53 family, plays a significant role in skin development as indicated by p63-deficient mice that fail to develop a stratified epidermis. Point mutations in one allele of the human p63 gene are linked to a subset of Ectodermal Dysplasia (ED) syndromes which are hallmarked by various abnormalities in the skin and other ectodermal derivatives (e.g. teeth, limb, and nails). However, although p63 is a key regulator of limbal stem cells which regenerate the cornea, the role of p63 in the corneal and eye development has not been defined. Here, in order to test whether p63 is required for corneal and eye development and pathology, we investigated p63-null mice and two recently generated mouse strains containing knocked in alleles of mutated p63 gene, which recapitulate the most frequent p63-related ED syndromes, namely, Ankyloblepharon-Ectodermal defects-Cleft lip/palate (AEC) and Ectrodactyly, Ectodermal dysplasia and Cleft lip/palate (EEC). p63-null mice failed to express the corneal differentiation marker cytokeratin 12 (K12), corneal progenitor markers, K5 and K14, and remained positive for ectodermal markers K8/18, indicating that p63-null epithelia could not develop beyond ectodermal stage. Additional defects included failure in eyelids development, abnormal lens and corneal stromal size. Interestingly, p63-related ED mice displayed several embryonic defects in corneal-epithelial commitment as indicated by reduced expression levels of essential cytokeratins (i.e. K12 and K5) and Pax6, and in epithelial stem cell marker K15. Moreover, p63 mutations were associated with abnormal corneal epithelial proliferation and epithelial thickening. Altogether, these data indicate that p63 is essential for proper mouse eye development, and suggest that loss of vision in p63-related ED patients is due to autonomous role of p63 in the cornea rather than a secondary effect attributed to defects in other tissues (e.g. meibomian gland dysfunction). Future experiments will elucidate the molecular mechanism that underlies the phenotypes observed in p63-mutated mice. Such knowledge may unravel new pathways in corneal pathophysiology, and pave the way for future therapy of p63-related ED and other corneal diseases.

F-1158

LONG-TERM MAINTENANCE AND PROLIFERATION OF MURINE SKIN EPITHELIAL STEM CELLS BY WNT-3A IN VITRO

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Wnt signaling is critical for regulation of a number of basic cell functions and deeply involved in stem cell maintenance. To elucidate the role of Wnt-3a in stem cell maintenance in skin, we prepared CD49f+CD34+ cells, a skin epithelial stem cell (EpSC)-rich population, from adult mouse skin and examined the effects of Wnt-3a on them using sequential cultures *in vitro* as well as hair follicle reconstitution in vivo. CD34+CD49f+ cells were collected using fluorescence-activated cell sorting and cultured for 10 days in the presence of Wnt-3a. At the end of that culture period, CD34+CD49f+ cells were sorted and subjected to a second 10-day culture with Wnt-3a. Using the same procedure, sequential cultures were repeated a total of 15 times. Cells showed proliferation of approximately 1000-fold by day 10 and CD34 expression was retained in about 10% of them. CD49f+CD34+ cells sorted on day 10 retained canonical Wnt responsiveness, proliferated markedly in the presence of Wnt-3a, maintained undifferentiated epithelial cell marker expression, and promoted hair follicle development in vivo. Furthermore, CD49f+CD34+ cells obtained from each subsequent culture retained the same EpSC characteristics. CD34+ and CD34- cells were found to produce Wnt-3a and Wnt/β-catenin inhibitors, respectively. Also, CD34+ cells were seen residing as small cellular clusters surrounded by a large amount of CD34- cells. Exogenous Wnt-3a delayed the conversion of CD34+ to CD34- cells and suppressed the production of Wnt/ β-catenin inhibitors by CD34- cells. Our results suggest that Wnt-3a plays an important role in maintenance of epithelial stem cells in skin.

F-1159

STRESS RESPONSES IN MOUSE EPIDERMAL STEM CELLS ARE REGULATED BY NSUN2-MEDIATED RNA METHYLATION

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Post-transcriptional regulation allows the fast activation of stress pathways in response to adverse conditions. Whether modulation of post-transcriptional regulation is important to control stem cell function during the stress responses is unknown. Here we identify essential regulatory roles for the cytosine-5 RNA methyltransferase Nsun2 in skin stem cells after UV-induced stress, wounding and during tumourigenesis. Skin wounding and UVB exposure cause strong up-regulation of Nsun2 expression in the hyperproliferating epithelium around the affected areas. In addition, skin regeneration of Nsun2-knockout mice is impaired after both UVB irradiation and wounding. To address the question whether Nsun2 also protected tumour cells under stress, we treated Nsun2-positive and -negative skin tumours with the genotoxic therapeutic agent 5-fluorouracil (5FU), which is commonly used to treat cancer. In contrast to Nsun2-expressing tumours, Nsun2-negative skin cancers could not be maintained in the presence of 5FU. This failure to regenerate a tumour after 5FU-treatment was not due to a proliferation defect in the absence of Nsun2 as untreated Nsun2-/- tumours exhibit a

larger number of undifferentiated and dividing cells compared to Nsun2-expressing tumours. Instead we show that Nsun2-/- tumour initiating cells fail to activate survival pathways after 5FU treatment, loose invasive tumour markers and show pre-mature expression of differentiation markers. In conclusion, Nsun2-mediated RNA methylation pathways play essential roles in the cellular stress response during regenerative processes in both normal skin and skin cancer. Our data further suggest a beneficial outcome of a combinatorial treatment of cytosine-5 RNA methylation inhibitors with genotoxic agents to treat cancer.

F-1160

TRANSCRIPTION FACTOR FOXI3 IS REQUIRED FOR EMBRYONIC HAIR DOWNGROWTH AND MAINTENANCE OF HAIR FOLLICLE STEM CELLS

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Hair follicles are capable to self-renew due to the population of hair follicle stem cells (HFSC). We have studied the role of transcription factor Foxi3 in HFSCs . Previously, a heterozygous mutation in the Foxi3 gene was shown to cause hairless phenotype in dogs. We have analyzed the expression pattern of Foxi3 during hair follicle development and cycling. Foxi3 was expressed exclusively in the epithelial compartment from placode stage onwards. In postnatal hair follicles Foxi3 was found in the hair germ, which contains primed HFSCs that are the first to proliferate at onset of new hair cycle. Foxi3 null mice had no apparent defects in hair placode formation. However, hair downgrowth was delayed right after the initiation stage, resulting in fewer hair follicles. Foxi3 cKO mice were characterized by nonsynchronous progression of the hair cycle, impaired initiation of hair growth phase (anagen) and formation of cysts with destructed hair shaft material. In hair plucking experiments, hairs grew back more slowly in Foxi3 cKO mice than in controls, and the major portion of the hair follicles degenerated leaving only the sebaceous unit behind. A large portion of Foxi3 cKO hair follicles had a degenerated HFSC compartment (bulge and hair germ) with diminished or absent expression of HFSC markers. To search for the Foxi3 targets we performed microarray assay for differentially expressed genes in Foxi3-/- epithelium and validated the results with qRT-PCR and immunostaining. Several HFSC marker genes were downregulated in Foxi3-/- epithelium. Analysis of the same markers in postnatal hair follicles showed graduate loose of SC compartment in Foxi3 cKO after progression through several normal hair cycles. Our study identifies Foxi3 as a novel regulator of hair follicle growth during morphogenesis. In postnatal hair follicle Foxi3 activates self-renewal pathways and is required for the maintenance of HFSCs.

EPITHELIAL CELLS (NOT SKIN)

F-1162

RIBOSOME BIOGENESIS DEFECTS CAUSE P53 DEPENDENT AND INDEPENDENT EXHAUSTION OF INTESTINAL STEM/PROGENITOR CELLS

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Ribosome biogenesis is a complex process requiring hundreds of trans-acting factors. Its impairment is associated with developmental defects and increased risk of cancer. The in vivo cellular responses to defective ribosome biogenesis and the underlying molecular mechanisms are incompletely understood. Here, we used a conditional inactivation strategy to study the role Notchless (NLE), an essential actor of ribosome biogenesis, in the adult mouse intestinal lineage. Nle deficiency led to ribosome biogenesis defects in crypts cells and resulted in the rapid elimination of intestinal stem cells and progenitors through distinct types of cellular responses, including apoptosis, cell cycle arrest and biased differentiation towards the goblet cell lineage. p53 activation was responsible for most of the cellular responses observed. However, p53-independent mechanisms occurred in mutant crypts and contribute to the elimination of mutant stem cells Our data indicate that NLE is a crucial factor for intestinal homeostasis and provide new insights into how perturbations of ribosome biogenesis impact on cell fate decisions within the intestinal epithelium.

F-1163

THE LEVEL OF WNT6 DIFFERENTIALLY REGULATES HUMAN LIMBAL STEM/PROGENITOR CELLS

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Our purpose was to investigate the role of Wnt6 in the regulation of human limbal stem/progenitor cells. Stable Wnt6-overexpressing 3T3 cell lines (Wnt6-3T3) were generated using a lentiviral construct pRRL-sin-cPPT-CAG-Wnt6-IRES-GRP. The construct lacking the Wnt 6 served as a control. Wnt6-3T3 were sorted into three groups, low-, medium-, and high-Wnt6 according to their GFP expressing levels. Freshly isolated limbal epithelial cells (LECs) were cultured on the Wnt6-3T3 feeder cells. The cell proliferation rate, colony forming efficiency (CFE), percentage of p63-bright cells, and percentage of small cells (diameter12m) were analyzed. The GFP expression level correlated with the Wnt6 mRNA level in the Wnt6-3T3 cells by qRT-PCR. The proliferation rate of LECs cultured on Wnt6- 3T3 was reduced by 35% and 24% ($p<0.05$) in the low- and medium-Wnt6 groups, respectively compared to the control. However, the proliferation rate was increased by 29% in the high -Wnt6 culture. The CFEs on Wnt6-3T3 cultures were reduced by 21% and 14% in low- and medium-Wnt6 groups and increased by 26% ($p<0.05$) in the high-Wnt6 group compared to controls. LECs cultured on

Wnt6-3T3 tended to contain lower percentage (15% lower) of p63-bright cells in the low-Wnt6 cultures, similar percentage in the medium-Wnt6 culture, and a higher percentage (1.3-fold higher) in the high-Wnt6 culture. The amount of small cells was similar in the low-Wnt6 culture and higher in the medium- and high-wnt6 cultures (1.1- and 0.9-fold higher, respectively) compared to the control. Different levels of Wnt6 appear to have different effects on human LECs in vitro. High level of Wnt6 supports a faster proliferation rate and better maintains the stem/progenitor phenotype of LECs while low level decreases the proliferation and tends to lose the stem/progenitor phenotype of LECs.

F-1164

GMP ISOLATION AND PRECLINICAL EVALUATION OF HUMAN AMNION EPITHELIAL STEM CELL FOR CLINICAL THERAPY

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Placenta is a non-controversial and readily available source of stem cells that can be used in regenerative medicine. We previously reported that amnion epithelial cells (hAEC) from term human placenta express surface markers and genes characteristic of pluripotent stem cells. hAECs are not tumorigenic, have immunomodulatory properties and once transplanted differentiate to hepatocyte-like cells resulting in correction of acute liver failure and mouse models of metabolic liver disease. These successful results have motivated isolation and banking of hAEC at our Institute for clinical therapy.

During the last year we have standardized reagents and procedures in accordance with current Good Manufacturing Practice (cGMP). Release criteria have been designed as quality control, and biosafety distribution of infused cells has been evaluated in two different preclinical models. A complete profile of mRNA levels of stem cell markers (Oct-4, Nanog, Sox-2, Activin-A) was performed by qRT-PCR. Flow cytometry analysis for surface and nuclear markers was performed using several antibodies, including: SSEA-3, SSEA-4, TRA1-60, TRA1-81, CD24. CD29, CD31, CD34, CD44, CD45, CD49f, CD73, CD105, CD90, CD117, CD326, CDw338, HLA-ABC, Nanog, Sox2, Oct4. All preparations were negative (above 85%). Stem cell markers, SSEA-3, TRA1-60 and 1-81positive cells comprised 12-15% of the population. As part of preclinical safety studies, we investigated the distribution of hAECs, previously labeled using near IR fluorescent lipophilic dye (DiR), and infused into the portal vein in mice. There was almost complete localization of infused cells into the liver at 3 and 20 hours after injection, without obvious side effects. Similar findings were obtained in a large animal model, the pig. Preclinical studies suggest that amnion-derived epithelial stem cells are a useful alternative to hepatocytes for the treatment of liver-based inborn errors of metabolism. Isolations according to cGMP procedures in combination with completed biosafety studies will allow the translation of amnion-derived stem cells for clinical applications.

F-1165

OPTIMIZATION OF AN IN VITRO 3D COLONY FORMATION ASSAY FOR THE STUDY OF HUMAN AIRWAY AND DISTAL LUNG STEM CELLS

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Stem cell niche is the microenvironment in which stem cells exist. The niche interacts with stem cells to regulate their fate; during homeostasis, after injury and disease. Our knowledge regarding human airway and distal lung stem cells and their niche are lacking because of absence of an in vitro assay to study them. To establish and optimize a protocol to process human surgically resected lung and bronchus samples into single cells then sort and culture them to examine their stem cells' clonal growth, self-renewal, differentiation and niche interactions. Freshly resected samples were processed into single cell suspensions then cultured in Matrigel with or without fibroblast co-culture. Several enzyme digestion methods and culture conditions were examined to improve cell yield, viability and colony formation efficiency and differentiation. We found that a combination of elastase and dispase enzymes gave the largest number of epithelial cells with better viability from lung samples. Co-culture with fibroblasts and high seeding densities were required for the clonal growth of distal lung stem cells. Analysis of the differentiation markers in the resulting colonies revealed that most colonies were expressing surfactant protein C (SFTPC) in addition to other secretory cell markers. Bronchial airway epithelium was efficiently obtained from bronchial samples using dispase enzyme digestion. Basal cells were sorted based on their expression of NGFR and/or CD44 and were able to form colonies in MatrigelTM with low seeding density and without fibroblasts co-culture. Analysis of the differentiation markers in the resulting colonies revealed that most colonies were expressing Cytokeratin 5 (K5) in addition to other secretory cell markers. Treating both bronchial and lung cells with cigarette smoke or 100-300 uM of H_2O_2 as a source for aldehydes and reactive oxygen species (ROS) resulted in reduction of their colony formation efficiency. An agonist of aldehyde dehydrogenase-2 (ALDH2), Alda-1, could rescue this reduction; revealing an important role for endogenous ALDH2 in protecting lung stem cells against aldehydes and oxidant injuries. We have established an assay to study human airway and distal lung stem cells and their potential niche interactions in vitro.

F-1166

PARASYMPATHETIC GANGLIOGENESIS REQUIRES SPROUTY-DEPENDENT WNT SIGNALS FROM EPITHELIAL PROGENITORS

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Parasympathetic innervation is critical for submandibular gland (SMG) development and regeneration. Parasympathetic ganglia (PSG) are derived from Schwann cell precursors that migrate along nerves, differentiate into neurons, and coalesce within their target tissue to form ganglia. However, signals that initiate gangliogenesis after the precursors differentiate into neurons are unknown. We found deleting negative regulators of FGF signaling, Sprouty1 and

Sprouty2 (Spry1/2DKO), resulted in a striking loss of gangliogenesis, innervation and Keratin 5-positive (K5+) epithelial progenitors in the SMG. Here we identify Wnts produced by K5+ progenitors in the SMG as key mediators of gangliogenesis. Wnt signaling increases survival and proliferation of PSG neurons and inhibiting Wnt signaling disrupts gangliogenesis and organ innervation. Activating Wnt signaling and reducing FGF gene dosage rescues gangliogenesis and innervation in both the Spry1/2DKO SMG and pancreas. Thus K5+ progenitors produce Wnt signals to establish the PSG-epithelial communication required for organ innervation and progenitor cell maintenance.

F-1167

TRANSCRIPTOME ANALYSIS OF AUDITORY HAIR CELLS WITH RNA SEQUENCE

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About 360 million people are suffered from hearing loss in the world. There are two types of hearing loss. One of them, sensorineural hearing loss is permanent because auditory hair cells in the inner ear cannot generally regenerate in mammals. The auditory tissues can be hardly obtained from a living human because the inner ear is encapsulated with hard bone, and the puncture to that capsule for biopsy gives serious damage to the inner ear function. Even if biopsy could be done, the amount of the inner ear tissue would be limited. These problems prevent the progress of human hearing loss research. Therefore, it is useful to generate hair cells in vitro for analysis of hearing loss. Recently several reports demonstrated differentiation into hair cells from pluripotent stem cells. However, there is no protocol with reliable results using human iPS cells. Instead of stepwise differentiation into hair cells, we are challenging direct conversion into auditory hair cells. It is necessary to figure out the genetic expression patterns of hair cells for achievement of direct conversion. A cochlea has two types of auditory hair cells, inner hair cells and outer hair cells, and both types are difficult to distinguish morphologically in developing condition. Furthermore, there are not currently useful surface markers to divide premature hair cells. Therefore, we used single cell RNA sequence to distinguish the expression patterns of inner hair cells and outer hair cells. Single cells were isolated from cochlea in neonatal Atoh1 (developing hair cell marker) GFP mice with enzymatic treatment and a cell sorter. Eleven of 15 GFP positive cells showed Myo6, hair cell markers, and they also expressed other hair cell markers. Previous reports indicated that Fgf8 was up-regulated in only developing inner hair cells, and only one cell of 11 definitive hair cells expressed Fgf8.

F-1168

DERIVING INNER EAR SENSORY CELLS IN VITRO FROM MOUSE EMBRYONIC STEM CELLS AND HUMAN ADIPOSE-DERIVED STEM CELLS

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Inner ear sensory cells, comprising specialized receptor hair cells and sensory neurons, are vital for our ability to hear and balance. Hair cells are particularly challenging to study as they are few in number, very fragile and difficult to maintain in vitro. The aim of the project is to develop a stem cell-derived in vitro hair cell epithelium, containing both hair cells and supporting cells, that can be used as a model for study and drug screening. We are employing two strategies: the first investigates the potential of human adipose-derived stem cells (hADSCs) to differentiate into otic cells using previously published stepwise growth factor differentiation protocols. The second replicates a mouse embryonic stem cell (mESC) protocol developed by Koehler et al. with the view of further extending the model to be usable in experiments. hADSCs were chosen because of their stem cell characteristics, their capability to differentiate into neural-like cells, and clinical potential. The mESC protocol by Koehler et al. was chosen for the remarkable yield and similarities of the derived cells to the native hair cells found in the inner ear. We monitored the changes induced by the different treatments using brightfield microscopy and timelapse imaging. Differentiation was assayed by PCR and immunocytochemistry for neural and otic markers including neuron marker ßIII-tubulin, hair cell (Myo7a) and supporting cell markers (Sox2). Functional assays, including electrophysiology and calcium imaging were carried out on hADSC-derived cells. PCR analyses indicate that the untreated hADSCs already express a number of these markers, including ion channels KCND3 and SCN9a, and otic developmental genes Six1 and Gata3. One differentiation paradigm induced hADSCs cells to express Pax8, a key otic specification marker, and purinergic receptors P2X2 and P2Y2. However, other characteristic otic markers such as Pax2 and Ngn1 were not expressed. Using mESCs, we were able to replicate the many aspects of the data presented by Koehler et al. and were able to describe some cells expressing hair cell markers. We noted some differences between samples treated with FGF2 and BMP inhibitor on day 4 versus day 4.5. Further optimization is underway to improve the yield and quality of cells produced.

F-1169

RABBIT LIMBAL EPITHELIAL SIDE POPULATION CELLS HAVE A SOMATIC STEM AND/OR PROGENITOR CELL PHENOTYPE WITH ENDOTHELIAL CELL MARKERS

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Limbal epithelial stem cells are essential for maintaining homeostasis of the corneal epithelia. Our previous studies have demonstrated that rabbit limbal epithelial side population (rLE-SP) cells exhibit stem cell-like phenotypes, such as ATP-binding cassette sub-family G (Abcg2), polycomb complex protein BMI-1 (Bmi1), and Nestin. However, the unestablished rabbit genome sequences and the lack of available antibodies for rabbit antigens have complicated further characterization of rLE-SP cells. Here, we show the gene expression profiles of both rLE-SP and non SP cells through RNA sequencing in which reads were mapped to both rabbit-derived sequences in the public database and the contigs that were created by de novo assembly. Firstly, genes that were upregulated more than 10-fold in rLE-SP and non SP cells were identified using RNA sequencing, and were categorized by Gene Ontology (GO) analysis. GO analysis

revealed that the term of "ectoderm development" were identified in not rLE-SP cells but rLE-non SP cells. In rLE-SP cells, the term of "cell adhesion" including endothelial cell adhesion molecules, such as vascular cell adhesion molecule 1 (Vcam1), junctional adhesion molecule 2 (Jam2), and endothelial cell-selective adhesion molecule (Esam), was found and validated using real-time polymerase chain reaction (PCR). As a result, the expressions of these three genes that are known as somatic stem and/or progenitor cell markers were highly expressed in rLE-SP cells. Moreover, other stem and/or progenitor cell markers, ATP-binding cassette sub-family B1 (Abcb1), CD34, and Kit, were also detected in rLE-SP cells. These findings support the hypothesis that rLE-SP cells have stem and/or progenitor cell phenotype with endothelial cell markers rather than epithelial cell ones. Secondly, singe-cell PCR was performed to clarify which markers are adequate to isolate rLE-SP cells. Abcg2, which have been defined as a SP cell marker, was expressed in approximately 30% of rLE-SP cells, and the expressions of CD34, Esam, and Jam2 were enriched in the Abcg2 positive cells. In addition, almost all Vcam1 positive cells coexpressed Abcg2. These findings suggested that these endothelial cell markers contribute to identify the rLE-SP cells, which may imply a novel character of the stem cells in corneal epithelium.

EYE OR RETINAL CELLS

F-1170

DIFFERENTIATION OF HUMAN DENTAL PERIODONTAL LIGAMENT STEM CELLS TO CORNEAL STROMAL KERATOCYTES

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Corneal opacification is a leading cause of reversible blindness and the pathological feature is the reduced density and activity of corneal stromal keratocytes (CSK). Since normal CSK are quiescent and inefficient to proliferate *ex vivo*, a stem cell-based strategy would be ideal, to replace the dysfunctional CSK. This study investigated the CSK fate competence of postnatal periodontal ligament-derived stem cells (PDLSC) since both share the same developmental origin from post-migratory cranial neural crest (NC). Primary human PDLSC were harvested from the extracted third molars. The cells at passage 3 to 6 were plated as spheroid culture (added with N2 and basic fibroblast growth factor, bFGF) to enrich NC progenitors, which showed up-regulated Snail, Slug and nestin. The cells were then cultured in CSK induction medium containing amnion stromal extract, ascorbate-2-phosphate, ROCK inhibitor Y27632 and bFGF for 5 to 14 days. Majority of cells exhibited dendritic morphology and expressed stromal crystallins (ALDH1A1, ALDH3A1), lumican, B3GNT7 and CHST6. Flow cytometry revealed that >80% cells expressed keratocan, a unique marker of CSK. When further treated with transforming growth factor β3, the differentiated cells synthesized collagen I, similar to normal CSK. Through marker screening, these cells did not differentiate towards myogenesis, neurogenesis, vasculogenesis and fibroblast development. Our results established the potential of adult human PDLSC as an autologous cell source of CSK in corneal cell therapy and stromal reconstruction. This can be a breakthrough in the therapeutic management of ocular opacification and can potentially reduce the reliance on donor cornea supply as well as the risk of graft rejection.

F-1171

CONVERSION OF MAMMALIAN PHOTORECEPTORS INTO GLAST-POSITIVE PROGENITOR CELLS DURING DEGENERATION

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Several studies investigated whether Muller glial (MG) cells of the retina have a regenerative potential to replace lost photoreceptors during retinal degeneration. In *Zebrafish*, MG cells can replace degenerating photoreceptors, whereas in mouse some MG cells start a dedifferentiation program but do not complete the acquisition of a photoreceptor fate. To date, few and unconfirmed studies addressed the photoreceptor behavior during retinal degeneration. To that aim, we crossed the *Crx-GFP* with the *Glast-DsRed* mouse lines expressing the green and red reporter genes in post-mitotic photoreceptors and in adult MG cells respectively. Time-lapse video, cell sorting, RT-PCR and immunohistochemical studies were performed. We notably observed that during retinal development the Glast-DsRed t transgene is expressed in retinal progenitors starting from embryonic day (E) 14, attested by staining and RT-PCRCell sorting study revealed the presence of double positive cells suggesting that some Glast-positive cells generate photoreceptors. Photoreceptor degeneration was induced by injecting the neurotoxic MNU compound in 2 month old mice. Retinas of treated mice were collected after 1 day of treatment and embedded in culture to follow the expression of Crx and Glast transgenes by time lapse video. Using a fully automated cell tracking software, we traced migrating green and red fluorescent cells in time and space up to 2 days. Surprisingly, we observed that after treatment several GFP-positive cells, post-mitotic photoreceptors, started translating the Glast-DsRed transgene. FACS analysis revealed that a low percentage of Crx-Glast-positive reentered the cell cycle. So far, research focused prominently on the transition of glia towards a neuronal fate during retinal degeneration and little attention was reported on photoreceptor behavior. The present data depicts a new situation in which photoreceptors lose their specific identity and start expressing progenitor-like markers in the attempt to re-enter the cell cycle rendering the interpretation of regeneration, in certain situation, challenging. This cell conversion needs also to be analyzed in light of our recent data demonstrating the important role of cell cycle proteins during the photoreceptor death process.

F-1172

CHARACTERIZATION OF A CONE-SPECIFIC MOUSE TRANSGENIC LINE AND SUBSEQUENT CONE TRANSPLANTATION IN A MOUSE MODEL OF CONE-ROD DYSTROPHY

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Cone-rod dystrophies, affecting 1 in 40'000 people, are inherited retinal dystrophies leading to the loss of central, daylight and color vision due to primary cone photoreceptor degeneration. Noteworthy, rod dystrophies due to a specific rod gene mutation are often followed by cone loss. Because the human vision relies mostly on cone-mediated vision it is of prime importance to replace cones in degenerated retinas. Here, we describe the characterization of a new transgenic mouse line, namely the Chrnb4-EGFP line, expressing the reporter gene exclusively in mature Gnat2- OPN1MW/LW- OPN1SW- positive cones of adult retinas. At early embryonic (E) stages, the EGFP co-localized with retinal progenitors positive for the proliferation markers ki67 and EdU as well as the cone-specific retinoic acid receptor RXRy. From E18 the EGFP-positive cells were observed at the apical side of the retina where the post mitotic RxRy-positive cone photoreceptors are confined. The ability of different cell populations collected from embryonic and postnatal retinas to integrate and replace dying cones was assessed by transplantation in retinas of different mouse models of cone-rod dystrophy or retinitis pigmentosa such as in CNGA3$^{-/-}$and rd10 respectively as well as in NOD-SCID immunodeficient mice. The grafting efficiency is severely influenced by the donor age, subpopulation of selected cells as well as disease type and progression, underlying the need to collect cones at a specific ontogenetic stage and to treat retinas before a critical degeneration time point. After transplantation many cells accumulate in the subretinal space of recipient retinas and express immature progenitor markers or proteins of the phototransduction pathway depending on the age of donor cells and on the type of disease treated. Few cones morphologically integrated the retina displaying a mature morphology. Nonetheless, the number of incorporated transplanted cones markedly exceeded the number of cone integration of previous published studies. In conclusion, the Chrnb4-EGFP mouse line analyzed in this work provides a tool to investigate the potential of cones to integrate adult degenerating retinas after transplantation. More efforts are still needed to reveal factors limiting cone integration and maturation in adult grafted retinas.

F-1173

TRANSPLANTATION OF HUMAN IPS-DERIVED CORNEAL ENDOTHELIAL CELL SUBSTITUTE SPHERES INTO RABBIT CORNEA

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Corneal endothelial dysfunction remains common indication for keratoplasty, accounting for half of the total number of such procedures. Corneal endothelial cell substitute from iPS cells (CECSi) may become promising source for regenerative medicine of corneal endothelial dysfunction, however, one of the major obstacles is that adequate carriers for clinical use are not available. In this presentation, we report corneal endothelial cell substitute spheres derived from human iPS cells, and trial of sphere transplantation using rabbit cornea to solve this problem. We derived CD271/CD49d double-positive neural crest cells from human iPS cells (iNCC) and purified by cell-sorter using these markers. We derived CECSi from iNCC by stimulation with retinoic acid and the GSK-3b inhibitor BIO, and obtained CECSi-cell-spheres by culturing the cells in low-cell-binding plates. For the evaluation of cell surface expression of ZO-1, N-cadherin, Na, K-ATPase, the spheres were plated and cultured on plastic culture dishes, and immunocytochemistry of migrating cells from spheres were performed. In vitro pump function of CECSi cells were measured by Ussing chamber system. Next, rabbit corneal endothelial cells were mechanically scraped, and CECSi-cell-spheres were injected into rabbit anterior chamber and attached to the posterior surface of the cornea. After two days, rabbits were sacrificed and cell morphology and attached area of transplanted CECSi cells were evaluated by immunocytochemistry of ZO-1 and human-nucleus. When CECSi-cell-spheres were attached on plastic dishes, CECSi cells showed collective cell migration maintaining a cobble stone morphology. Migrating CECSi cells maintained cell surface expressions of ZO-1, N-cadherin and Na, K-ATPase. CECSi cells had sufficient in vitro pump function compared with human corneal endothelial cell-line B4G12 cells. In vivo transplanted 4×10^6 CECSi cells covered 4×4.5 mm of posterior surface of rabbit cornea, with cobble stone appearance and cell surface expression of ZO-1. We successfully transplanted CECSi cells maintaining cell morphology and tight junction to the posterior surface of the cornea without the use of carriers.

F-1174

SUBRETINAL BIOCOMPATIBILITY OF ULTRATHIN POLYIMIDE MEMBRANES SEEDED WITH RETINAL PIGMENT EPITHELIUM CELLS DIFFERENTIATED FROM HUMAN EMBRYONIC STEM CELLS

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Retinal pigment epithelium (RPE) is a cell layer at the back of the eye essential for vision. RPE degeneration has a major role in pathogenesis of retinal diseases such as age-related macular degeneration (AMD). Transplantation of RPE differentiated from human pluripotent stem cells (hPSC) offers a promising treatment for AMD. In recent clinical trials, hPSC-RPE cells are implanted either as cell suspensions or sheets without an artificial scaffold. In many transplantation studies, including our own, survival and integration of suspended RPE cells are poor. A scaffold would ideally facilitate implantation and integration of the graft. Previously, we have shown that polyimide (PI) membrane is suitable for growth of RPE differentiated from human embryonic stem cells (hESC). In this study, we investigated the suitability of PI for subretinal implantation of hESC-RPE in rabbits. The surface properties of PI membranes were studied with scanning electron microscopy (SEM) and atomic force microscopy (AFM). PI with and without hESC-RPE were implanted in the subretinal space of New Zealand White rabbits. Animals received oral immunosuppression the entire follow-up time. In vivo follow-up was performed with fundus photography, electroretinography (ERG), and optical coherence topography (OCT). Histological examination of the eyes was done three months after implantation.

In surface analysis, PI showed better porosity compared to polyester, a standard RPE culture substrate. After transplantation, a gradual loss of pigmentation on hESC-RPE-PI was detected, potentially due to cell death of the implanted cells. In vivo analysis showed good placement and retinal biocompatibility of the implant. There was no evidence of intraocular tumors. In the eyes with PI alone, no obvious signs of inflammation or retinal atrophy were observed. However, mononuclear cell infiltration and retinal atrophy were observed around the implants in the presence of hESC-RPE-PI. The ultrathin PI membrane was well-tolerated in the subretinal space in our rabbit model and thus suitable for RPE transplantation. The oral immunosuppression was potentially insufficient for reduction of xenograft induced inflammation and needs to be adjusted in the future.

F-1175

CHEMICALLY INDUCED PHOTORECEPTOR DEGENERATION IN 3D RETINAL AGGREGATES FROM MOUSE IPS CELLS

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Retinitis pigmentosa (RP) is characterized by inherited, slow and progressive degeneration of retinal tissue, mainly rod photoreceptors. The disease mechanisms remain largely unclear, and there is no effective treatment against deterioration of visual function. The purpose of the study is to replicate RP phenotype in vitro to gain a better understanding of the pathology and quantitative estimation of protective drugs. We generated three-dimensional (3D) retinal aggregates from iPS cells established from Nrl-GFP mice that express GFP in differentiated rod photoreceptors. The photoreceptor-specific cell death was induced by 4-hydroxytamoxifen (4-OHT), an inverse agonist for estrogen related receptor β (ERRβ) that is enriched in differentiated rod photoreceptors, and α-tocopherol was added to evaluate its protective effect. The condition of the photoreceptor cells was estimated by TUNEL and oxidative stress markers and GFP intensity. The 3D retinal aggregates derived from Nrl-GFP iPS cells expressed visible GFP co-localized with a rod-specific marker (Rhodopsin). ERRβ expression was enhanced after 27 days of differentiation (Day 27). 3D retinal aggregates exposed to 5 μM 4-OHT showed a significant decrease in rhodopsin expression, and increase of TUNEL and stress markers after Day 27. These observations were synchronized with GFP attenuation. Addition of α-tocopherol caused delay of GFP attenuation. Thus, we have developed a mouse iPS cell model for RP by chemical inhibition of ERRβ, and established fluorescent live imaging system that quantifies the activity of rod photoreceptors. This model may be useful in the elucidation of disease mechanisms and development of new drugs for RP.

F-1176

VISIONING A NEW STEM CELL MARKER IN THE MOUSE CENTRAL CORNEAL EPITHELIUM

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The corneal epithelium is a squamous epithelium that covers the ocular surface and ensures proper vision by preserving the integrity of the eye. Corneal epithelium is renewed continuously throughout life from a pool of stem cells. Evidence from earlier studies has demonstrated that the limbus, located in the periphery of the cornea, serves as the stem cell niche in adults. However, contrasting evidence from clonal analysis proposes that in early postnatal life, the renewal is fueled by stem cells located in the basal layer of the central cornea. Accordingly, corneal renewal shifts from central to limbal around the age of 5 months in mice. Here we suggest Bmi1 as a marker for these interim stem cells. Bmi1+ cells contribute to the generation of suprabasal epithelia, forming clones that do not mix with each other in the central cornea. It follows that Bmi1+ stem cells are dispersed in patches among the basal corneal epithelia. The identification of Bmi1 as marker for the proposed central corneal stem cells will provide new tools for the further investigation of age-dependent renewal in the cornea.

F-1177

INTRAPERITONEAL ADMINISTRATION OF ADIPOSE TISSUE DERIVED STEM CELLS RESCUE RETINAL DEGENERATION IN MOUSE MODEL

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Whether transplantation of mouse adipose tissue derived stem cells (mADSC) through intraperitoneal space has a rescue effect on the retinal degeneration of mouse induced by NaIO3 was evaluated. The mASCs which were third passage were used for this study. For the transplantation, mASCs were stained by BrdU or PKH67. Retinal degeneration was induced by intraperitoneal injection of NaIO3 (40 mg/kg) on 8 C57BL6 mice. At 3 days and 1 weeks after the induction, mADSCs (5x105~1x105 cells/ 100 uL) were transplanted into the intraperitoneal space (stem cell treatment group; STx) and normal saline was used in the degeneration control group (DC). 3~4 weeks after transplantation, 8 mice were sacrificed and enucleated. The area of photoreceptor outer and inner segment (IS/OS), outer nuclear layer, and inner nuclear layer were analyzed in the normal control group (NC), STx, and DC. The number of retinal pigment epithelium (RPE) degeneration was also evaluated. Immunohistochemistry of BrdU and PKH67 were used to evaluate whether the presence of mASC in the retina. Electroretinogram (ERG) was used for functional test. RT PCR were performed to compare mRNA level of VEGF, BDNF, NGF, CNTF between the groups. The surface makers for mASC were that CD 105, CD 73, and CD 90 were over 90 % positive, and CD 45, CD 34, CD31 were negative. The areas of IS/OS were 3472.67 ± 31.28 pixel2 in NC, 342 ± 2.19 pixel2 in DC, 951.00 ± 8.57 pixel2 in STx. ($p<0.05$) The numbers of ONL were 327.11 ± 23.41, 28.14 ± 19.61, 84.90 ± 26.55. ($p<0.05$) The numbers of INL were 107.00 ± 14.13, 64.90 ± 7.09, 68.33 ± 10.21. ($p=0.21$) In the STx, the area of IS/OS, ONL were significantly thicker than that of DC. The BrdU stained or PKH67 stained cells was not observed in the retina. Full field electroretinography showed that in 0.063 cds/m2 and 2.00 cds/m2, the b wave amplitudes increased in STx comparing to DC, significantly. ($p<0.05$) CNTF mRNA was significantly elevated in the STx rather than that in DC. The intraperitoneal transplantation of mADSC has rescue effect on NaIO3 induced retinal degeneration, which could be caused from CNTF elevation in degenerating retina through cross talk between the transplanted ADSC and indigenous retinal cells.

F-1178

TRANSPLANTATION OF MOUSE ESC-DERIVED PHOTORECEPTORS INTO A MOUSE MODEL OF COMPLETE BLINDNESS

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Vision is one of five senses that humans have which accounts for approximately 30% of all sensory input. Our eyesight is dependent on light sensitive cells in the retina - photoreceptors - and their degeneration is the leading cause of blindness in the developed world. To date, there is not cure available and several therapeutic approaches are being developed including gene therapy and neuroprotection but most treatments rely on the presence of endogenous photoreceptors. Thus, photoreceptor replacement presents a promising alternative to restore visual function of degenerated retinas. Most photoreceptor replacement studies used primary cells transplanted into slow retinal degeneration mouse models, which does not recapitulate an eventual clinical scenario. Additionally, the use of primary human donor cells would raise several ethical, logistic and supply concerns that would be avoided with the use of a renewable and expandable donor cell source such as embryonic- or induced pluripotent stem cells. Hence, we generated 3-dimensional retina organoids from mouse embryonic stem cells (mESC) that contain high numbers photoreceptor cells. mESC-derived photoreceptors were characterized and enriched using magnetic activated cell sorting (MACS), reaching a purity of ≈90%. Enriched photoreceptors were subsequently transplanted into the subretinal space of a mouse model of complete blindness [tg(Cpfl1; Rho-/-)] and analyzed by immunohistochemistry three weeks after transplantation. Engrafted mESC-derived photoreceptors survived in the subretinal space and expressed mature photoreceptor and synaptic markers. In conclusion, we demonstrate the possibility of mESC-derived photoreceptor enrichment and transplantation into a complete retinal degeneration mouse model. These results show as proof-of-principle, the potential of mESC-derived photoreceptors transplantation for future cell replacement therapies for retinal diseases such as Age-related Macular Degeneration and Retinitis Pigmentosa.

F-1179

THERAPEUTIC DEVELOPMENT FOR BEST DYSTROPHY USING PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

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Best vitelliform macular dystrophy, also known as Best disease (BD), is a form of inherited juvenile-onset macular degeneration. Although its pathogenesis has been linked to the mutation of retinal pigment epithelium (RPE) Bestrophin-1, effective therapeutics against BD have not been identified. In present study, we isolated BD patient-derived dental pulp 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 cell (ctrl-RPE), BD-RPEs exhibited normal RPE-specific markers but had a significant lower expression of Bestrophin-1 and tight junction protein ZO-1 as well as reduced phagocytic ability. Furthermore, we also identified STXBP2 as a regulator for differentiation of RPEs by proteomics analysis. Among several candidate drugs that were screened, curcumin was the most effective for upregulating both Bestrophin-1 and ZO-1 in BD-RPEs. In summary, the application of patient-specific iPSCs can provide a platform for drug discovery and small molecular testing. This study thereby provides a potential therapy by using curcumin to regulate the antioxidative capabilities of degenerated RPEs.

F-1180

AN ALLOGENEIC TRANSPLANTATION TRIAL OF CYNOMOLGUS MONKEY IPSC-DERIVED CORNEAL ENDOTHELIAL-LIKE CELLS

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Corneal endothelial dysfunction remains a common indication for keratoplasty, accounting for half of the total number of such procedures. Keratoplasty using donor cornea includes problems such as immunological rejection. To bypass these problems, we examined application of induced pluripotent stem cells (iPSCs) to engineer artificial corneal endothelium. Corneal endothelium as well as corneal stroma originates from cranial neural crest cells (NCCs). In the past ISSCR annual meeting, we have reported about induction of NCCs from human and cynomolgus monkey iPSCs and subsequent differentiation of the NCCs to corneal endothelial-like cells. Last year, we reported that the cynomolgus monkey iPSC-derived corneal endothelial-like cells had sufficient pump function to maintain corneal thickness through in vitro pump function and in vivo transplantation to rabbit corneal endothelial cell dysfunction model. This time, to examine the efficacy and the safety of the iPSC-derived corneal endothelial-like cells in non-human primate, we tried allogeneic transplantation of the cynomolgus monkey iPSC-derived corneal endothelial-like cells to cynomolgus monkey corneal endothelial cell dysfunction model. The host corneal endothelial cells were removed by scraping and the cells suspension was injected into anterior chamber of the monkey eye. One week and 2 weeks after transplantation, reduction of edema and engraftment of the injected cells onto endothelial side of the cornea were observed in the cell transplant model. However, the expression of alpha SMA suggesting unexpected event such as EMT was found in some of the engrafted cells. In addition, the decreased expression of tight-junction marker ZO-1 and Na/K-ATPase component ATP1A1, which are important for barrier and pump function of the corneal endothelial cells, was observed as well. On the other hand, no abnormalities such as tumor formation were found in the tissues examined. Thus, although

a certain level of the efficacy and safety of the iPSC-derived corneal endothelial-like cells was observed in non-human primate model, further improvements in both induction protocol and transplantation methods are required to apply the iPSC-derived cells to regenerative medicine of corneal endothelial cells.

NEURAL CELLS

F-1181

EZH2 CONTROLS NEURAL PROGENITOR POOL SIZE AND REGIONAL IDENTITY IN THE DEVELOPING MOUSE MID-BRAIN

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Precise temporal and spatial control of gene expression is essential for the determination of the size of the developing brain as well as for establishment of correct cell identities in different brain regions. The polycomb group protein Ezh2, enhancer of Zeste homolog 2, is the catalytic subunit of polycomb repressive complex 2 (PCR2) and is primarily responsible for trimethylation of histone H3K27 (H3K27me3). This epigenetic mark contributes to repression of many genes that are pivotal for neural development. Here we show that Ezh2 is essential for midbrain development by controlling both its size and its identity. After Wnt1-Cre-mediated ablation of Ezh2 in the midbrain we performed whole-genome transcriptome analysis of mutant and control midbrains as well as H3K27me3 ChIP. Loss of Ezh2 resulted in decreased neural progenitor (NP) proliferation due to negative regulation of Wnt/β-catenin signaling and precocious exit of NP from the cell cycle. In addition, Ezh2 ablation led to a partial loss of midbrain identity, which was reflected by reduced expression of Pax3, Pax7 and other midbrain markers. Mechanistically, this phenotype was brought about by Ezh2-dependent downregulation of Wnt/β-catenin signaling. Most intriguingly, loss of midbrain identity was accompanied by aberrant upregulation of the forebrain transcription factors FoxG1 and Pax6 due to direct de-repression in Ezh2-deficient cells. Together, our data reveal a role of Ezh2 in regulating NP fate decisions and brain area identity by direct and indirect mechanisms.

F-1182

YIN YANG 1 REGULATES MURINE CORTEX DEVELOPMENT IN A DEVELOPMENTAL STAGE-SPECIFIC MANNER

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The mammalian neocortex is a highly complex structure which is composed of a broad range of neurons and glial cells. Its organization into six neuronal layers contributes to higher cognitive functions of mammals. The development of the mammalian forebrain is driven by a complex network of signaling pathways and transcriptional regulators. Neural progenitor cell (NPC) proliferation, fate decisions and survival need to be tightly controlled to ensure proper cortex development. The transcriptional regulator Yin Yang 1 (Yy1) has been shown to have context-dependent effects on these processes during development and tissue homeostasis of vertebrates. However, the role of Yy1 during early cortical development has not been addressed yet. Conditional ablation of Yy1 before the first neurogenic waves of cortical development resulted in microencephaly. Mice lacking Yy1 exhibited decreased cortical size and thickness due to the depletion of the NPC pool at early stages of corticogenesis. Loss of Yy1 led to impaired proliferation and transient G1/S cell cycle arrest at embryonic day E12.5. In addition, Yy1 maintained survival of NPCs at the onset of neurogenesis but had no effect on survival at later stages of corticogenesis. Despite its constitutive expression during corticogenesis in NPCs and immature neurons, Yy1 is only necessary during a specific time window of early cortex development. Tamoxifen-induced ablation of Yy1 at the onset of neurogenesis did not influence cortical development. In summary, our results show that the transcriptional regulator Yy1 plays a crucial role during a specific time-window in cortical development.

F-1183

TRANSPLANTATION OF ADULT NSCS THAT LACK GEMININ EXPRESSION IN A MOUSE MODEL OF PARKINSON'S DISEASE

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Idiopathic Parkinson's disease (PD) is a widespread neurodegenerative disorder characterized by substantial degeneration of the pigmented dopaminergic cells in the ventral midbrain causing a variety of motor abnormalities. Due to pharmacological insufficiency in curing PD, cell therapy seems to be an emergent alternative therapeutic strategy which aims to restore the affected nigrostriatal pathway. This study is related to the potential use of adult neural stem cells (aNSCs) in PD therapy, examining their functional integration into the damaged neuronal circuit of the 6-OHDA mouse model. Moreover, it sheds light on the role of Geminin, a small protein with profound effects on both cell division and differentiation, in aNSCs transplantations. Our laboratory has previously shown that in the absence of Geminin cortical progenitor cells show a preference in selfrenewing divisions. In order to investigate whether genetically engineered aNSCs that lack Geminin expression can be more effective on repairing neuronal loss in the 6-OHDA mouse model, aNSCs that do not express Geminin were stereotactically transplanted in Parkinsonian mice. Efficiency of transplantations was evaluated with behavioral assays. Moreover, the ability of the genetically engineered aNSCs to selfrenew and differentiate into neurons and glia was evaluated and compared to wild type aNSCs.

F-1184

DIRECT SYNAPTIC INPUTS FROM DIVERSE HOST BRAIN AREAS TO GRAFTED HUMAN IPSC-DERIVED CORTICAL NEURONS IN STROKE-INJURED RAT CORTEX

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We have recently shown improved functional recovery after transplantation of human induced pluripotent stem cell (iPSC)-derived cortical neuronal precursors in a rat model of cortical stroke. Grafted cells give rise to mature neurons that rebuild the damaged tissue and send fibers to several host brain structures. However, whether the grafted neurons receive direct synaptic inputs from the host brain and are functionally integrated into its neuronal circuitries is unknown. Here we used a rabies virus (RV)-based strategy to explore whether host cells establish functional synaptic connections with the transplanted cells. Rabies-G Glycoprotein was replaced with RFP gene in the genome of the virus and the envelope was substituted with the foreign coat protein EnvA, generating replication incompetent virus that only can infect cells expressing TVA receptor. Human iPSC-derived long-term neuroepithelial like stem (lt-NES) cells were transduced with Rabies-G glycoprotein, avian TVA receptor and histone-GFP under the control of human Syn I promoter (HTB construction). Two or five months after intracortical transplantation of HTB-lt-NES cells in a rat stroke model, we injected RV in the location of the graft. Expression of TVA receptor in the mature neurons (syn I+) generated from grafted cells makes them suitable for infection with RV. The presence of Rabies-G glycoprotein in these cells allows the virus to infect neurons that connect to them by functional synapses. Therefore, grafted and infected cells will express nuclear GFP and cytoplasmic RFP while the ones connected to them will only present RFP. Immunohistochemical analysis of injured and transplanted brains 1 week after the infection with RV revealed the presence of RFP+ neurons in different areas, some of them located far away from the implantation site. The distribution of these cells corresponds with the proper anatomical locations of neurons projecting to the cortex in the intact brain. Comparison of 2 and 5 months time-points gives insight into the dynamics of lt-NES cell integration after transplantation in the damaged-brain.
We demonstrate for the first time that intracortical grafts of human iPSC-derived cortically fated neurons establish afferent synaptic connections with diverse areas in the stroke-injured brain.

F-1185

PROTEOLYTIC PROCESSING OF CXCL12 TRANSFORMS CXCL12 INTO A DEATH FACTOR FOR NEURAL STEM CELLS

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The neural stem cells (NSC) resident in mammalian adult central nervous system (CNS) represent an encouraging potential for neurodegenerative and acute brain diseases treatment. NSC can migrate to CNS injury answering to chemotactic factors like CXCL12. Despite the NSC capacity to generate newborn neurons to different CNS areas, the most of neural stem cells undergo apoptosis after arrive at lesion site. CXCL12 or SDF-1 (stromal cell-derived factor 1) is a chemotactic cytokine and together with its CXCR4 receptor are constitutively and broadly expressed in many tissues. Together with cytokines and chemokines, matrix proteases are also released in brain injury states and activated to contribute for breakdown of the extracellular matrix and consequent cell migration. Among those, matrix metalloproteases 2 and 9 (MMP-2 and 9), gelatinase A and B, respectively, are secreted locally and could cleave CXCL12 removing the first four N-terminal aminoacids generating CXCL12(5-67). We produced CXCL12 and CXCL12(5-67) recombinant proteins, and evaluated its effect on mice astrocytes and NSC in vitro. We identified that CXCL12(5-67) impairs neural stem cell migration, reduces astrocytes viability and induces apoptosis through caspases 9 and 3 activation in adult neural stem cell. A possible CXCL12(5-67) involvement in a low CNS regeneration capacity could became it a target for trauma and neurodegenerative disease treatment.

F-1186

TGF-BETA2 REGULATES TEMPORAL NEURONAL PATTERNING AND NEURAL PROGENITOR POTENCY IN VIVO AND IN PLURIPOTENT STEM CELL CULTURES IN VITRO

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The functional and anatomical organization of the vertebrate central nervous system (CNS) critically relies on integrated developmental programs that regulate the generation of distinct neuronal subtypes from multipotent neural progenitors in space and over time. How the sequential specification of neuronal subtypes is regulated over time remains poorly resolved, but extrinsic cues have been implicated in this process and it is known that ageing neural progenitors undergo a progressive restriction in potency during the temporal patterning process. In the ventral hindbrain, a pool of neural progenitors defined by expression of the homeodomain protein Nkx2.2 produces visceral motor neurons (MNs), serotonergic neurons (5HTNs) and oligodendrocyte precursors (OLPs) in a temporally regulated fashion, and we have recently identified Tgfß2 as a key temporal switch signal that terminates the production of MNs and triggers the induction of 5HTN in vivo. To examine the role of Tgfß2 in temporal patterning and the regulation of progenitor cell potential in further details, we have established a synchronized and highly robust differentiation paradigm in which the sequential specification of MNs, 5HTNs and OLPs by neural progenitors is recapitulated in embryonic stem cell (ESC) cultures in vitro. By analyzing the differentiation potential of Nkx2.2+ progenitors isolated at different stages, we provide evidence that young progenitors initially are tri-potent, but that become progressively restricted in their potential by losing competence to generate early-born cell types over time. We show that transient exposure of young progenitors to Tgfß2 is sufficient to suppress MNs and triggers premature induction of 5HTNs, and that the timing of Tgfß2 application to cells is associated with an enduring loss of potential to generate early born MNs. 5HTNs are implicated in numerous neurological and psychiatric disorders, and early application of Tgfß2 in stem cell cultures results in a highly enriched generation of 5HTNs which should facilitate large-scale production of functional 5HTNs in culture. Our study thereby provides a proof-of-concept that the identification of signaling molecules regulating the temporal patterning of CNS can be utilized to effectively produce late-born neurons from stem cells in vitro.

F-1187

DETERMINISTIC HOX PATTERNING AND MICROPATTERNED CULTURE SUBSTRATES ENABLE HIGHLY EFFICIENT DERIVATION OF A SPECTRUM OF REGIONAL HUMAN MOTOR NEURON POPULATIONS

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During posterior CNS development, rostrocaudal regional identity is specified by the combinatorial expression of 39 *HOX* genes, which are critical determinants of neural cell fate and circuit organization. Patterning of *HOX* expression in hindbrain and spinal cord tissues is collectively regulated by Wnt/β-catenin, fibroblast growth factor (FGF), growth differentiation factor (GDF), and retinoic acid (RA) signaling. However, a complete understanding of how to implement these morphogens during human pluripotent stem cell (hPSC) differentiation to enable deterministic *HOX* patterning in neuroectoderm and subsequent motor neuron populations has remained elusive. We recently published a chemically defined culture system that can differentiate hPSCs into >90% $Pax6^+/Sox2^+$ neuroectoderm within 6 days and without dual SMAD inhibition. Using this system, we discovered how to modulate FGF8b, Wnt/β-catenin, and GDF11 signaling to differentiate hPSCs into a 97% ± 2% $Sox2^+/Brachyury^+$ neuromesodermal culture that is stable for 7 days and exhibits full colinear *HOX* activation. At any point during this period, a transition to media containing RA halted *HOX* activation and converted neuromesoderm to >83% $Pax6^+/Sox2^+$ neuroectoderm possessing Hox transcription factor (TF) profiles indicative of discrete regions in the hindbrain (e.g. rhombomere 4, 5, and 6-8) and spinal cord (e.g. cervical, thoracic, and lumbar). Furthermore, we differentiated such neuroectoderm into motor neurons that exhibit Hox TF profiles and phenotypes indicating regionalization to 6 distinct hindbrain and spinal cord domains. Thus, we are the first to demonstrate (1) maintenance of a stable neuromesodermal state for 7 days, providing access to a spectrum of CNS regional phenotypes, and (2) the role of RA as a 'stop' signal for *HOX* progression to enable deterministic patterning. Finally, based on our discovery that efficient derivation of $Olig2^+$ motor neuron progenitors requires a cell density-dependent conversion of neuroectoderm to polarized neuroepithelium, we engineered micropatterned culture substrates that force high cell density in 2-D culture to consistently enable derivation of regional cultures with >75% $Isl1^+$ or $Hb9^+/SMI\text{-}32^+$ motor neurons.

F-1188

INCREASED MITOCHONDRIAL FISSION CONTRIBUTES TO THE PROPOFOL-INDUCED STEM CELL-DERIVED HUMAN NEURON DEATH THROUGH CDK1/DRP1 PATHWAY

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Studies in developing animals have shown that when anesthetic agents are administered during brain growth spurt, it can lead to neuronal cell death and learning disabilities, raising significant safety concerns regarding the use of anesthetics (e.g., propofol) in pediatric patients. Development of human embryonic stem cell (hESC)-derived neurons has provided a valuable tool for understanding effects of anesthetics on developing human neurons. Mitochondria continuously undergo cycles of fusion and fission. Unbalanced fusion/fission leads to various pathological conditions including neurodegeneration. The aim of this study was to dissect the role of mitochondrial dynamics and the underlying mechanism in propofol-induced neurotoxicity. hESCs were differentiated into developing neurons following a four step differentiation protocol. Following exposure of two-week-old neurons to 20 μg/mL propofol for 6 hours, cell death was assessed using TUNEL staining. Changes in mitochondrial dynamics (fusion and fission) were assessed by immunofluorescence staining of TOM20 expressed in mitochondrial outer member. Expression of proteins of interest [e.g., dynamin-related protein 1 (Drp1, a key regulator in mitochondrial fission) and cyclin-dependent kinase 1 (CDK1)] was assessed by Western blot. Mdivi-1 (a selective inhibitor of Drp1) and Roscovitine (an inhibitor of CDK1) were administered 1 hour prior to propofol exposure in order to study functional roles of mitochondrial fission and CDK1 in propofol-induced neuron death.

Results: Propofol induced significant death of hESC-derived neurons and led to detrimental increases in mitochondrial fission that was accompanied by the increased expression of activated Drp1 and CDK1. Pretreatment of the neurons with mdivi-1 rescued the propofol-induced toxicity and mitochondrial fission. Inhibition of CDK1 with Roscovitine attenuated the increases in cell death, activated Drp1 expression, and mitochondrial fission conferred by propofol. These data demonstrate for the first time that propofol-induced toxicity in human neurons occurs through a CDK1/Drp1/mitochondrial fission-mediated pathway. Inhibition of this pathway attenuates propofol-induced neurotoxicity and is a promising neuroprotective strategy.

F-1189

JAGGED1 AS A PIVOTAL REGULATOR OF NEURAL STEM CELL DIFFERENTIATION IN THE FOREBRAIN NEUROGENIC NICHE

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During mammalian brain formation, neural stem cells (NSCs) transit through sequential periods of expansion, neurogenesis and gliogenesis. Notch signaling is required for maintenance of NSCs and inhibits differentiation by blocking transcription of pro-neurogenic factors. Notch ligands are expressed by differentiating progenitors

and activate lateral inhibition signals through Notch receptors on opposing progenitor cells. However, whether individual Notch ligands regulate NSC differentiation into specific cell types is unclear. Type B precursor cells can give rise to intermediate progenitors, which can produce both neuronal and glial cell populations. To study the role of Jagged1 (Jag1) ligand in regulating neural precursors fate commitment we used a gain-of-function approach, transducing proliferating progenitors lining the subventricular zone (SVZ) with retroviruses that express full-length Jag1 (Jag1FL). Surprisingly, Jag1FL induced a fate switch to Sox10+ oligodendrocyte precursors at the expense of astrocytes. Furthermore, Jag1FL oligodendrocytes become mature myelinating oligodendrocytes more quickly, as well as expressing the paranodal marker Caspr suggesting a functional interaction with the axon in vivo. This phenotype was lost when Jag1 was conditionally ablated from SVZ progenitors. Overexpression experiments in NSCs in vitro recapitulated this phenotype to some degree. In line with our in vivo and in vitro data, RNA-Sequencing analysis of Jag1FL transduced NSCs revealed an induction of key genes involved in oligodendrocyte maturation and myelination. Our data reveals a novel role for Jag1 in regulating fate switch to an oligodendrocytic lineage.

F-1190

CDK4/CYCLIN D1 OVEREXPRESSION IN THE SVZ EXPANDS NEURAL STEM CELLS AND INCREASES THE NUMBER OF NEURONS IN THE OLFACTORY BULB

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Neural stem cells (NSC) in the adult mammalian brain generate neurons and glia throughout life within two restricted areas: the subventricular zone (SVZ) of the lateral ventricles and the dentate gyrus of the hippocampus. Although the physiological role of adult neurogenesis remains controversial, current strategies aim to manipulate this process in vivo as a promising approach towards therapy. Unfortunately, and similarly to other somatic stem cells, controlling the proliferation versus differentiation of NSC remains a challenge. Our group has previously shown that the expansion of endogenous NSC can be controlled during embryonic development and adulthood by manipulating the expression of Cdk4/cyclin D1 (4D). In this study, we generated a multiple transgenic mouse line to temporarily control the proliferation of NSC in the adult SVZ thereby increasing the final output of neurons whose survival, connectivity and function was investigated. Importantly, our approach may allow the expansion of somatic stem cells in, virtually, any tissue depending on the transgenic line chosen for driving 4D expression. This may provide an excellent tool towards understanding the physiology and role of other stem cells and the development of new regenerative therapies.

F-1191

X-LINKED SEVERE COMBINED IMMUNODEFICIENCY (X-SCID) RAT FOR XENO-TRANSPLANTATION AND FUNCTIONAL EVALUATION

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Pluripotent stem cells are one of the promising approaches for Parkinson's disease (PD). Recently, an efficient differentiation method for dopaminergic (DA) neurons has been established using dual SMAD inhibition strategy. While many studies focus on validation of ESC/iPSC-derived DA neurons as donor, few studies have been reported about host animals as recipient. PD model rats that could be produced by unilateral injection of 6-hydroxy-dopamine (6-OHDA) have often been used to evaluate the degree of severity by rotational movement. In the case of xeno-transplantation, administration of immunosuppressive drugs is required to prevent a host immune reaction. These drugs, however, can suppress activation and proliferation of lymphocytes but it has also been reported organ failure by prolonged use in vivo study. It is difficult to explain that symptomatic improvement of PD model rats is directly correlated with graft-induced effect because immune reaction associated with xeno-graft and side effects of immunosuppressive drugs have complicated understanding of the effect. For these reasons, we focused on validation of host animals in different immune states, namely F344 (Wild), F344 with cyclosporine A (CsA), F344/NJcl-rnu/rnu (Nude) and F344-Il2rgem2Kyo (X-SCID). When we injected human ESC-derived DA neurons into the brain of these rats, few immune cells such as MHC class II- and CD45-positive cells were observed in the graft of Nude and X-SCID rats. Total numbers of survived DA neurons, however, were not differences between these rats. Furthermore, we produced PD model rats by unilateral injection of 6-OHDA and then grafted human iPSC-derived DA neurons into the brain of these rats. Although X-SCID PD model rats showed behavioral recovery and good survival of DA neurons, but Nude PD model rats showed variable rotation and some of them showed spontaneous recovery. These results provide a more accessible and reliable method to evaluate the in vivo function of human-derived DA neurons, potentially offering a pre-clinical study for the application of pluripotent stem cells.

F-1192

MTORC1 CONTROLS NEURODEVELOPMENT AND SYNAPTIC PLASTICITY IN A TIME-DEPENDENT MANNER IN HUMAN STEM CELL MODELS OF TUBEROUS SCLEROSIS

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Tuberous sclerosis is a monogenic disorder associated with a high rate of autism and is caused by loss of function mutations in TSC1/2, negative regulators of mTORC1. It remains largely unknown how developmental processes and biochemical signaling affected by mTORC1 dysregulation contribute to human neuronal dysfunction. Here, we have combined genome engineering and human stem cell differentiation approaches to analyze the trajectory of onset of neuronal alterations caused by loss of function mutations in TSC2. TSC2 deletion leads to mTORC1 overactivation at specific neurodevelopmental stages in a gene dosage-dependent manner and this correlates with distinct kinetic and severity of cellular alterations. Homozygous TSC2 deletion causes severe neurodevelopmental abnormalities which recapitulate pathological hallmarks of cortical malformations in patients. Both TSC2+/- and TSC2-/- neurons display altered excitatory synapse transmission and deficits in mGluR-dependent synaptic plasticity. Finally,

pharmacological inhibition of mTORC1 corrects developmental abnormalities and synapse dysfunction in a defined temporal order. In conclusion, our results dissect stage-specific roles of mTORC1 in human neuronal development and demonstrate the potential to revert deficits in synaptic plasticity independently of developmental alterations. Ultimately, this work contributes to better understanding of the onset of neuronal pathophysiology in tuberous sclerosis.

F-1193

LONG-TERM HUMAN NEUROEPITHELIAL-LIKE STEM (LT-NES) CELLS CAN BE STABLY PASSAGED WITH EITHER OF THE GROWTH FACTORS EGF OR FGF2 TO PROMOTE DIFFERENTIATION OF SPECIFIC SUBTYPES

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Neural progenitor cells provide a platform for drug screening, regenerative medicine, disease modelling and studies of cellular processes in neurodevelopment. For instance, lt-NES cells, derived from iPS, ES or foetal cells are stably propagated in media containing growth factors EGF and FGF2 with maintained stem cell properties. Growth factor removal induces spontaneous differentiation and expression of early neuronal markers, e.g. Tuj1 and DCX dominates the culture while a smaller fraction of cells express the glial marker GFAP after a few weeks. Although spontaneous differentiation of lt-NES cells produces various neuronal and glial populations, improved and directed differentiation schemes are sought for. Here we compare culturing with both EGF and FGF2 (E+F), only EGF (-F) or only FGF2 (-E) and analyse proliferation, multipotency and subsequent differentiation potential. We find that lt-NES cells can be continuously passaged in all tested conditions with maintained stem cell characteristics. Further, cell growth and proliferation analysis reveals that (-E) initially led to a minor growth rate reduction not observed at later passages. The (-F) condition initially lowered growth rate with 50% but cultures adapted and at later passages growth rate was around 70% of control. Further, differentiation following (-E) or (-F) passaging, promotes an earlier exit from the proliferative state. This was seen by a 2-fold reduction of ki67+ cells compared to control along with reduced CD133 expression. In addition, at early stage differentiation, the portion of cells expressing the neuronal marker Doublecortin (DCX) more than doubled in cultures previously passaged with only one factor. Interestingly, differentiation following (-F) induced a 2,5-fold increase of DCX+ cells (26%) within 1 week. However, the DCX+ fraction did not increase further during the following 2 weeks and along with reduced CD133 and ki67 expression, this suggests an altered, less neuronal, population. In differentiation following initial EGF-removal, the fraction of DCX+ cells increased to 58% compared to 25% in control after 2 weeks. Thus, multipotent lt-NES cells can be stably propagated in media supplemented with only one of the growth factors EGF or FGF2 in order to prime the cells for distinctive and enhanced differentiation routes.

F-1194

COXSACKIEVIRUS TARGETS THE PROGENITOR CELL POOL DURING JUVENILE INFECTION LEADING TO LASTING MEMORY DEFICITS IN THE CENTRAL NERVOUS SYSTEM AND IMPAIRED REGENERATIVE CAPACITY IN THE HEART

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Recent studies have identified a number of pathogenic viruses which infect progenitor cells in host tissues. Coxsackievirus B3 (CVB3), a member of the picornavirus family and enterovirus genus, preferentially targets progenitor cells in the juvenile host. CVB3 might target progenitor cells for a variety of reasons, including their increased expansion / proliferative capacity, their exceptional autophagic activity (particular during differentiation), their migratory nature and ability to distribute infectious virus, and their distinctive antiviral response - all factors which might aid in virus replication and dissemination. We recently observed that CVB3 infection of progenitor cells stimulated the ejection of intracellular autophagosomes harboring infectious virus, and that these virus-associated microvesicles may represent a novel route of virus dissemination within the host. By scanning electron microscopy, three morphologically distinct types of microvesicles were observed on the surface of progenitor cells at 4 days post-infection, an effect not seen in mock-infected control cells. We found that infection of progenitor cells in the juvenile mouse heart predisposed the host to pathologic remodeling later in adult life. Also, depletion of neural progenitor cell reservoirs in the neurogenic regions of the central nervous system (CNS) following juvenile CVB3 infection contributed to memory deficits in persistently-infected mice. The establishment of a carrier-state infection in neural progenitor cells grown in culture revealed coevolution of the viral genome in parallel with permanent alterations in autophagic flux and differentiation in the surviving host cell. Hence, the exhausted cardiac progenitor cell pool following juvenile CVB3 infection may impair the heart's ability to increase capillary density to adapt to increased load. Similarly, a reduction of neurogenesis and permanent alterations in progenitor cell function in the CNS following CVB3 infection may lead to lasting memory deficits in the surviving host.

F-1195

SIRT1-MEDIATED SELECTIVE PROTECTION OF NEURAL STEM CELLS FROM DNA DAMAGE DURING AGING

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It is long presumed that DNA damage accumulates in adult stem cells and hence their ability to withstand accumulating DNA

damage and maintain tissue homeostasis is a principal mechanism modulating aging. Nonetheless, few studies have determined whether DNA damage accumulates in adult stem cells and whether such accumulation contributes to physiological stem cell aging. Surprisingly, our study has uncovered a reduced DNA damage accumulation and apoptosis in neural stem cells (NSCs) and a preferential protection of NSCs with age. This is achieved partially through selective up-regulation of Sirt1 and enhancement of non-homologous end joining (NHEJ) DNA repair in aged NSCs. Sirt1 deficiency abolishes this selective advantage, leading to stem cell exhaustion and accelerated aging. The declining DNA damage accumulation and the up-regulation of Sirt1 in stem cells with age may represent one compensatory mechanism evolved to selectively protect stem cell pool from excessive DNA damage and other stresses encountered during aging. Our work poses a major challenge for the wide-spread presumption that DNA damage accumulation is a determining factor in tissue aging, and highlights the fact that aging is multi-factorial, context-dependent and tissue-specific. Separating these related but different events represents logical step-stones for a complete understanding of DNA damage and its impact on adult stem cells and aging, which requires development of novel techniques and strategies.

F-1196

NO RECRUITMENT OF PERIPHERAL MONOCYTES TO THE BRAIN AFTER CRANIAL IRRADIATION

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Cranial irradiation (IR) induces loss of neural stem and progenitor cells and is followed by progressive cognitive deterioration in both young and adult patients. Earlier studies have suggested that neuroinflammation contributes to the development of common adverse defects, including a shift from neurogenesis to gliogenesis. Given the difficulties in distinguishing resident microglia from macrophages derived from blood-borne, peripheral monocytes, the relative contribution of these two evolutionarily different groups of macrophages and antigen-presenting cells is not known. In this study, we utilized a unique reporter mouse strain (CX3CR1$^{GFP/+}$CCR2$^{RFP/+}$) to delineate the resident (CX3CR1^{+}; GFP-labeled) versus peripheral (CCR2^{+}; RFP-labeled) innate immune response in the brain following IR. Our results demonstrate that no RFP-labeled peripheral monocyte-derived macrophage infiltrated the brain at any experimental time point after receiving a single dose of 8 Gy IR at postnatal day 10 (PND10) or 90. In the juvenile brain (PND10), the number of resident microglia in the granular cell layer (GCL) increased 6h after IR, peaked at 24h, decreased thereafter back to the baseline at 1 month after IR. In the adult brain (PND90), however, the number of microglia peaked already at 6h after IR and returned to the baseline after 24h. Concurrently, there was a decrease of microglia in the molecular layer of the dentate gyrus in both groups after IR. The microglia increase in the GCL appears to be mainly due to the migration from the molecular layer since microglia proliferation is rarely observed in the brain. Microglia exhibited round phagocytic and amoeboid morphology and expressed the macrophage marker CD68 6h after IR and this microglia activation lasted for 1w in the juvenile brain but only 1d in the adult brain, indicating a more transiently up-regulated inflammatory profile in the adult brain after IR. The expression of chemokine CCL2 was strongly up-regulated 6h after IR in both PND10 and PND90 mice and still elevated 1 month after IR. Cumulatively, we show that IR induces transient microglial activation, enduring CCL2 up-regulation and monocyte does not appear to contribute to the inflammatory response.

F-1197

ASSESSMENT OF TUMORIGENIC POTENTIAL OF INDUCED PLURIPOTENT STEM CELL DERIVED NEURAL STEM/PROGENITOR CELLS BY CNV AND DNA METHYLATION ANALYSIS

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Recently, we have demonstrated the therapeutic potential of transplanting human induced pluripotent stem cell-derived neural stem/progenitor cells (iPSC-NS/PCs) into injured spinal cord of rodents and non-human primates. However, some iPSC-NS/PC 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 copy number variation (CNV) and DNA methylation analyses. Samples are prepared as follows: four iPSCs cell lines established in the Center for iPS Cell Research and Application (Kyoto University), two groups of iPS-NS/PCs (tumorigenic cell lines (TC); 253G1 and 836B3, non-tumorigenic cell lines (Non-TC); 201B7 and 414C2). Illumina Infinium® Omni Express-24 and Methylation 450 Bead Chip were used to evaluate genome wide CNV and DNA methylation analyses of these iPSC-NS/PCs. Some CNVs related to cancer pathogenesis were detected in the TC lines. Furthermore, different DNA methylation patterns were observed between the TC and Non-TC lines. Interestingly, the CNVs and DNA methylation patterns were closely associated with the difference in cell lines and the passage numbers. Although we previously demonstrated a difference in the expression levels of specific genes between TC and Non-TC lines using transcriptome analyses, it was difficult to set a cut-off value of the gene expression level between these cell lines. Here, we revealed a difference in their CNVs and DNA methylation pattern, which enables us to establish criteria for quality control of iPS-NS/PCs in terms of their tumorigenicity.

F-1198

DEVELOPMENT OF LESS INVASIVE AND STABLE TRANSIENT MIDDLE CEREBRAL ARTERY OCCLUSION MODEL ON COMMON MARMOSET

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Ischemic brain stroke is one of the leading causes of death all over the world. Stem cell-based therapy may become a valuable strategy for functional restoration after stoke. However, there is no clinical relevant model on primates to assess the safety and therapeutic effect before the clinical trial. Here we have developed a less invasive transient middle cerebral artery occlusion (MCAO) model on primate, common marmoset. Female laboratory-bred common marmosets were used. Under general anesthesia, wire thread was inserted into right MCA via carotid artery and occluded for 3 hours in a low O2 concentration followed by magnetic resonance imaging (MRI) and angiography (MRA). Animals were assessed behaviorally, radiologically such as MRI and positron-emission tomography (PET), and immunohistochemically after surgery. Many of animals were alive and stable after surgery. MRA had shown the occlusion of right MCA in all animals immediately after surgery. T2 weighted image on MRI showed the high intensity area, indicating the brain infarction, on right hemisphere including basal ganglia at 7 days. PET study using BCPP-EF, which is ligand to the complex1 of mitochondria, showed the comparable result. The immunohistochemical study also showed the defect of neural cells on the same lesion as MRI and PET study. Animals exhibited neurological deficits with left hemiparesis at 4 days after surgery and thereafter. Its neurological function improved gradually, however, motor paresis such as ham hand was remained in food retrieval test. Our data were mimicking the findings in human. We have now developed the less invasive and stable marmoset MCAO model, which are clinically relevant to ischemic brain stroke. This model may be useful to evaluate the effect and safety of the cell therapy for the cerebral infarction as pre-clinical study.

F-1199

GLYPICAN-2 IS A CSF BIOMARKER OF ADULT HIPPOCAMPAL NEUROGENESIS AND DECREASES IN HUMAN AGING

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Adult hippocampal neurogenesis (AHN) is a remarkable form of human brain structural plasticity by which new functional neurons are generated from adult neural stem cells/precursors. Although the precise role of this process remains elusive, AHN is important for learning and memory and it is affected in disease conditions associated with cognitive impairment, depression and anxiety. Moreover it is modulated by neuropsychiatric compounds. The inability to detect adult neurogenesis in living humans has hampered the translational and human mechanistic understanding of this process. To this end, we performed a unbiased proteomic screen using a model of in vitro human neurogenesis, which recapitulates key aspects of adult neurogenesis. We identified the proteoglycan Glypican-2 (Gpc2) whose temporal protein expression was similar to immature neuronal markers such as DCX, GPR56, TUC-4 and TUJ-1. Notably, several modulators of hippocampal neurogenesis including running or fluoxetine administration, increased the abundance of Gpc2 in cerebrospinal fluid (CSF) of rodents, while aging led to a marked reduction, in all cases correlating with the degree modulation of hippocampal neurogenesis. To address specificity, using two models to genetically ablate adult neurogenesis, we could demonstrate a marked reduction Gpc2 expression in brain concomitant with a reduction of protein abundance in CSF. Using IP of Gpc2, we demonstrate that it binds to both FGF2 and its receptor, furthermore endogenous application of GPC2 could inhibit FGF2 induce neural stem cell mitogenic activity, suggesting the proteoglycan sequesters FGF2 and modulates the transition from proliferation to differentiation. Notably, Gpc2 is abundant in human cerebrospinal fluid and markedly decreases in a longitudinal cohort of aging CSF. Collectively, these results suggest that Gpc2 acts as a niche factor involved in regulation of adult neurogenesis and offers the potential to assess in a non-invasive manner the degree of neurogenesis in in human physiology and disease.

F-1200

REGULATORY FACTOR X TRANSCRIPTION FACTORS CONTROL MUSASHI1 TRANSCRIPTION IN MOUSE NEURAL STEM/PROGENITOR CELLS

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The transcriptional regulation of neural stem/progenitor cells (NS/PCs) is of great interest in neural development and stem cell biology. The RNA-binding protein Musashi1 (Msi1), which is often employed as a marker for NS/PCs, regulates Notch signaling to maintain NS/PCs in undifferentiated states by the translational repression of Numb expression. Considering these critical roles of Msi1 in the maintenance of NS/PCs, it is extremely important to elucidate the regulatory mechanisms by which Msi1 is selectively expressed in these cells. However, the mechanism regulating Msi1 transcription is unclear. We previously reported that the transcriptional regulatory region of Msi1 is located in the sixth intron of the Msi1 locus in NS/PCs, based on in vitro experiments. In the present study, we generated reporter transgenic mice for the sixth intronic Msi1 enhancer (Msi1- 6IE), which show the reporter expression corresponding with endogenous Msi1-positive cells in developing and adult NS/PCs. We found that the core element responsible for this reporter gene activity includes palindromic Regulatory factor X (Rfx) binding sites and that Msi1-6IE was activated by Rfx. Rfx4, which was highly expressed in NS/PCs positive for the Msi1-6IE reporter, bound to this region, and both of the palindromic Rfx binding sites were required for the transactivation of Msi1-6IE. Furthermore, ectopic Rfx4 expression in the developing mouse cerebral cortex transactivates Msi1 expression in the intermediate zone. This study suggests that ciliogenic Rfx transcription factors regulate Msi1 expression through Msi1-6IE in NS/PCs.

F-1201

TRANSPLANTATION OF LONG TERM SELF RENEWABLE FEEDER FREE HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURAL PROGENITORS INTO A CONTUSION SPINAL CORD INJURY MODEL IN RHESUS MACAQUE MONKEYS

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Human induced pluripotent stem cells (hiPSCs) have led to an important revolution in stem cell research and regenerative medicine. To create patient-specific neural progenitors (NPs), we have established a homogenous, expandable, and self-renewable population of multipotent NPs from hiPSCs, using an adherent system and defined medium supplemented with a combination of factors. The established hiPSC-NPs were continuously propagated for 1 year without losing their potential to generate astrocytes, oligodendrocytes, and functional neurons and maintained a stable chromosome number. The characteristics of our generated hiPSC-NPs provide the opportunity to use patient-specific or ready-to-use hiPSC-NPs in future biomedical applications. For approving our claim, we have tried to use the hiPSC-NPs as asuitable source for cell transplantation in primate spinal cord injury model. In this experimental study, hiPSC-NPs were analyzed by flow cytometry, immunocytochemistry, and RT-PCR. Next, BrdU-labeled cells were transplanted into a SCI model. The SCI animal model was confirmed by magnetic resonance imaging (MRI) and histological analysis. Animals were clinically observed for 6 months. Analysis confirmed homing of hiPSC-NPs into the injury site. Transplanted cells expressed neuronal markers. Hind limb performance improved in transplanted animals based on Tarlov's scale and our established behavioral tests for monkeys. Our findings have indicated that hiPSC-NPs can facilitate recovery in contusion SCI models in rhesus macaque monkeys. Additional studies are necessary to determine the improvement mechanisms after cell transplantation.

F-1202

INVOLVEMENT OF IKK-BETA-NFκB IN ADULT HIPPOCAMPAL NEUROGENESIS THROUGH WNT/BETA-CATENIN PATHWAY

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GFAP-positive neural stem cells (NSCs) are self-renewing to maintain undifferentiated state and differentiating to neurons and glia cells in the subgranular zone of the dentate gyrus. The local niches and environments are the important factors in the fate determination of adult NSCs. Wnt/β-catenin and NF-κB pathway have been known to play important roles in adult neurogenesis of the hippocampus. However, the role of IKKβ in mediating the phosphorylation of IκB and a convergence point of most signal transduction pathways to NF-κB, has been not understood in regulating adult neurogenesis of the hippocampus yet. For elucidating the role of IKKβ, we ablated IKKβ by tamoxifen-induced IKKβ knock-down in GFAP positive cells (*CreER*^{T2}/*IKK* $^{F/F}$) of the mouse hippocampal dentate gyrus. The knockdown of IKKβ had the enhanced BrdU^{+} cells with increased expression of cell cycle related molecules, cyclinD1 and cyclin E, but decreased in CDK2 and CDK4. Moreover, the knockdown of IKKβ induced the improved cell survival and the neural differentiation of the NSCs with up-regulating Wnt3a ligand, β-catenin and NeuroD1. The results demonstrate that IKKβ suppresses the NSC proliferation, cell survival by NF-κB and the neural differentiation of the NSCs by Wnt/β-catenin signaling respectively.

F-1203

HUMAN STEM CELLS BASED PLATFORM TO STUDY THE INTERACTIONS BETWEEN KERATINOCYTES AND NERVE FIBERS AT THE EPIDERMIS

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The skin is the largest organ in the body and it forms a physical barrier that separates the organism from the external world. It plays a fundamental role maintaining homeostasis and is innervated by nerve fibers which allow it to transduce external stimuli such as heat, touch, etc. The most peripheral region of the skin, the epidermis, is innervated by polymodal C-fiber sensory neurons, which can sense various chemical and physical (e.g. mechanical, thermal, electromagnetic waves) stimuli. There is growing evidence that keratinocytes, the primary cells within the epidermis, possess sensory properties and have functional interactions with nerve endings in modulating sensation by forming a sensory unit. However, the validity of this claim and the exact molecular mechanism of their interactions, especially in the human system, are yet to be experimentally shown. While it is relatively easy to get human epidermal keratinocytes from human skin biopsies, the derivation of functional primary human sensory neurons is practically impossible and thus alternative methods need to be utilized. During development, sensory neurons are derived from the trunk section of the neural crest. Embryonic stem (ES) cells or induced pluripotent stem (iPS) cells were thus differentiated into neural crest cells (NCCs), which were then differentiated, via a previously developed neuronal differentiation protocol, into sensory neurons so as to elucidate their interactions with keratinocytes. The PGP 9.5 and peripherin expressing neurons were co-cultured with human primary adult keratinocytes and human primary adult fibroblasts. Results indicate that neurons send neurite protrusions which make dynamic physical interactions with particular keratinocytes, but not with fibroblasts, thus suggesting a specific interaction. High resolution imaging show that the nerve fibers make physical interactions with the keratinocytes in different ways, some fusing with the keratinocyte membrane, some penetrating the keratinocytes, while in some cases, the actin filaments of the nerve fibers merge with the actin filaments of the keratinocytes. 3D human epidermal biopsies similarly showed that nerve fibers formed very intimate physical interactions with keratinocytes, thus validating the findings of this model.

F-1204

IN VITRO APPROACHES TO UNDERSTANDING HUMAN CORTICAL NEUROGENESIS AND LINEAGE USING HUMAN EMBRYONIC STEM CELLS

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In vivo human brain development remains one of the greatest challenges of scientific understanding, and while previous efforts at the Allen Institute for Brain Science have focused on the adult human brain, the In Vitro Human Cell Types program takes a complementary approach by studying differentiation and development in vitro using human pluripotent stem cells. To this end, we have established a robust in vitro differentiation platform based on a previously published protocol, yielding neuronal cells of various cortical identities. In addition, we leverage single-cell methodologies such as flow cytometry, immunocytochemistry, Fluidigm qRT-PCR, and RNAseq (CelSeq) to profiles cells at various time points during in vitro differentiation. Single-cell collection and fractionation of these cells based on cell surface markers and/or engineered reporter human embryonic stem cell lines result in a preliminary separation of cells into potentially biologically relevant populations. Subsequent profiling of these cells and computational analyses of the resulting data predict the presence of multiple, robust types of cells generated in vitro. Further, our computational analyses also predict lineage relationships among these cell types. Verification of these cell types and lineages is then performed by perturbation studies of genes that are candidates for causation or involvement in lineage selection, comparison of our in vitro data to that collected from post-mortem in vivo human tissue, and the use of clonal differentiation assays and barcoded viral lineage tracing assays. Altogether, these approaches provide us and the community with tools to further our understanding of human cortical development.

F-1205

CHARACTERIZATION OF LOCAL VASCULARIZATION OF TRANSPLANTED HUMAN NEURAL STEM CELL GRAFTS IN THE PORCINE SPINAL CORD

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Transplantation of stem cell-based therapeutics into the spinal cord for treatment of neurological diseases, such as traumatic Spinal Cord Injury, Amyotrophic Lateral Sclerosis, and Multiple Sclerosis, is under pre-clinical and clinical investigation. However, little is known about the immunological factors impacting graft survival and the post-transplantation fate of cell grafts. Clinical trial subjects are typically given an immunosuppression regimen adapted from the experience with solid organ transplantation. Understanding the post-transplantation fate of grafted cells and the immunological mechanisms is essential to widespread translation of stem cell-based therapeutics. In pre-clinical studies conducted by our group, we observed a heterogeneous pattern of graft survival and vascularization. We hypothesized that increased vascular infiltration correlated with graft rejection. To assess this, human fetal-cortex derived neural progenitor cells were transplanted into the thoracolumbar spinal cord of Göttingen minipigs (n=3). Twelve discrete 2.5e5 cell grafts were transplanted with non-traumatic intraparenchymal microinjection in each pig. The pigs were maintained on IV tacrolimus (0.005 mg/kg/day) for the duration of the study and sacrificed after six weeks. The spinal cords were harvested and every 6th section was stained for the human nucleus. All transplanted cell grafts (36) were identified and graft-specific quantitative stereology was performed (mean 22.0% cell engraftment, range of 0.0 - 65.7%). The center of each graft was co-stained for CD31 and glial fibrillary acidic protein to identify the vasculature in the transplanted graft. Five high-powered fields with the most vessels were acquired for each graft and average micro-vascular density (MVD) was calculated (mean 18.3 infiltrating vessels per graft, range 3 - 46). Linear regression showed a statistically significant inverse correlation ($r = -0.60$, $p = 0.0002$) between MVD and cell engraftment. Furthermore, preliminary qualitative staining showed increased vascular endothelial growth factor expression and Ki67+ endothelial cells. In conclusion, we observed an inverse correlation between MVD and engraftment in our xenograft model of stem cell transplantation in the spinal cord.

F-1206

COMPARISON OF SYN1 AND CAMKII PROMOTERS IN THE GENERATION OF OPTOGENETICALLY MODIFIED IPSC-DERIVED HUMAN NEURAL NETWORKS

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Development of optogenetically controllable human neural network models can provide an investigative system that is relevant to the human brain. Light-sensitive neurons can be generated by transducing Channel Rhodopsin-2 (ChR2) into human iPSC derived neural progenitors. Using lentiviral transduction, we compared the use of the promoters for Synapsin-1 (SYN1) and Calcium-calmodulin Kinase II (CaMKII) in regulating neuronal specific ChR2-YFP expression in a mixed population of human iPSC-derived cortical neurons, astrocytes and progenitor cells. Viability of the cells at 7 day-post-infection achieved 80-97% in all conditions tested. In line with the literature, transduction efficiency of neurons at day 14, using flow cytometry, was between 3% and 6% for SYN1 and between 2 and 5% for CaMKII. ChR2-YFP was expressed over 28 days as the neural sub-population matured, and continued to be stably expressed through-out higher passages (≥ P10) and for periods up to several months. A similar level of ChR2-YFP expression was found in GABAergic neurons under the SYN1 and also the CaMKII promoter. Under the CaMKII promoter, expression in Glutamatergic neurons was higher than GABAergic neurons. GABAergic ChR2 expressing neurons were low in number but not eliminated. In conclusion, both neural specific promoters SYN1 and CaMKII effectively targeted the expression of ChR2 in the neuronal population of a mixed cortical culture derived from human iPSC progenitors; however, some expression of ChR2 within GABAergic neurons was also found under the CaMKII promoter, suggesting that glutamatergic neuronal targeting using lentivirus containing the CaMKII may be less specific than previously thought.

F-1207

DOMINANT-NEGATIVE EFFECTS OF ADULT ONSET MUTANT HUNTINGTIN LEADS TO AN ALTERED DIVISION OF HUMAN EMBRYONIC STEM CELLS DERIVED NEURAL CELLS

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Mutations in the huntingtin protein (HTT) underlie both the adult onset form and the juvenile form of the neurodegenerative disorder, Huntington disease (HD). HTT is essential to control mitosis with important impact on asymmetric division and cell fate of cortical progenitors of the ventricular zone. Using Human embryonic stem cells (hESC) characterized as adult onset mutant-gene carriers during a pre-implantation genetic diagnosis procedure, we qualified the role of human HTT in mitotic spindle orientation in human neural cells and the effect of the mutation of patient with adult onset HD. RNAi-mediated silencing of both allele of huntingtin in neural stem cells derived from wild type hESC disrupts spindle orientation and mislocalizes dynein, the P150Glued subunit of dynactin and the large nuclear mitotic apparatus NUMA protein. We then addressed the effect of adult onset HD mutation on the role of HTT during spindle orientation in neural cells derived from HD-specific hESC. Combining SNP-targeting allele-specific silencing and gain of function approaches we show that a 46 glutamine expansion in human HTT is sufficient to induce a dominant negative effect on spindle orientation and alteration of the localization of dynein, P150Glued and NUMA in neural cells. These findings reveal that neural derivatives of disease-specific human pluripotent stem cells constitute a relevant biological resource for exploring the impacts of adult onset HTT mutations on neural progenitors division with potential application in HD drug discovery targeting HTT-dynein-P150Glued complex functions.

F-1208

GENOME-WIDE FUNCTIONAL SCREENING IDENTIFIES MIRNA REGULATORY NETWORK OF OLIGODENDROCYTE PROGENITOR CELL DIFFERENTIATION

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Oligodendrocyte progenitor cells (OPCs) represent the largest stem cell population in the central nervous system and are the principal source of myelinating oligodendrocytes. In demyelinating disorders such as multiple sclerosis (MS), enhancing the generation of new oligodendrocytes from native OPCs is a highly sought after treatment strategy to regenerate myelin. However, precise control of OPC differentiation in vivo remains a challenge. Towards this goal, we recently developed a method to generate pure populations of OPCs in large numbers from mouse pluripotent stem cells. Here, we leveraged this scalable resource to perform a high-throughput phenotypic screen using high content imaging analysis to evaluate the ability of all known microRNAs (miRNAs) to modulate the differentiation of OPCs. Testing of a miRNA mimic and a miRNA inhibitor for all 1,309 miRNAs (as of miRBase 19) enabled discovery of a novel set of miRNAs that function to control the differentiation of OPCs into mature oligodendrocytes. Combining small-RNA and mRNA sequencing with the functional screening data, we define the global miRNA-mRNA interaction network that regulates oligodendroglial cell fate and function. Given the ability of miRNA mimics and inhibitors to be delivered to the CNS, top hits from our screen provide promising candidates to be tested as RNA therapeutics in demyelinating diseases like MS.

F-1209

PROSPECTIVE DELINEATION OF HUMAN FETAL NEURAL PRECURSOR HIERARCHY UNCOVERS A TWO STAGE GLIOGENIC FATE TRANSITION

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The understanding of the human fetal brain development, has been severely restricted due to the lack of identifying markers which can distinguish between human central nervous system (CNS) neural stem cells (huNSC) and CNS multi-potent progenitor cells (huCNS-MPPs or NPCs). The specific identification of live huNSC has been compounded not only due to the lack of known surface markers but also selective reporters for these cells. Furthermore our understanding of developmental processes at various stages of gestation and their effect on intrinsic lineage commitment is at present incomplete hence the need to reproducible identify these cells becomes ever more important. Here we present our results on identifying and characterizing the surface markers to prospectively isolate and separate huNSCs and huCNS-MPPs from various gestational stages of human fetal brain. After screening a panel of 334 antibodies to prospectively isolate neurospheres forming cells from human fetal brain tissue we narrowed down to a permutation of four antibodies (i.e CD15, Notch1, EGFR, and CD90) which marked the NSC/NPCs cells. Subpopulations of cells derived after FACS were analyzed for single cell lineage potency, self renewal and clonogenic characteristics. Using an in-vitro limiting dilution assay, we show that double-positive (DP; CD15+ Notch1+ EGFR- CD90-) populations have the highest frequency of self-renewing cells, followed by quadruple-positive (QP) populations (CD15+ Notch1+ EGFR- CD90-). qPCR analysis and In-vitro differentiation assays reveal both DP and QP to be multipotent in nature however serial passaging showed that only the DP cells have a consistent self-renewing capacity, whereas QP derived cells were unable self renew after 5-6 passages. Further more single cell differentiation assays from sorted fetal brain cells at various stages of gestation (9-22 weeks) shows a switch between neurogenic to astrogenic lineage bias at 14 to 16 week of gestation with the first evidence of oligodendrocytes at approximately 18 weeks. We further characterize the role of Notch, BMP4 and JAK/STAT signaling pathways in regulating the developmental lineage bias of human neural precursor cells.

F-1210

CULTURE OF HUMAN NEURAL STEM CELLS ON RLAMININ-521 PRECOATED CULTUREWARE

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Human neural stem cells (hNSCs) are multipotent stem cells of the nervous system that have tremendous potential in basic neuroscience research, tissue engineering and cell-based therapies for neurodegenerative diseases. These applications require robust culture conditions that allow for efficient large-scale expansion and differentiation of hNSCs. Laminin protein is important for the support of hNSCs in vivo and is a key regulator of hNSC behavior. In vitro, hNSCs have been cultured on a variety of surfaces, ranging from tissue-culture treated to extracellular matrix proteins coatings, with the most commonly used surface as Laminin with poly-L-ornithine. However, these animal-derived proteins contain undefined components which might give rise to lot to lot variability and self-coating can be time consuming. Here, we developed rLaminin-521 precoated ready-to-use cultureware and demonstrated culture of hNSCs (H9 hESCs-derived) in serum-free media for three passages. During culture, cells exhibited normal hNSC morphology and remained undifferentiated, as demonstrated by the expression of hNSC-specific markers nestin, SOX2 and proliferation marker Ki67 using flow cytometry. Furthermore, hNSCs maintained their differentiation potential which was shown by successfully differentiating them 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 and large-scale expansion hNSCs.

F-1211

ROLE OF ASTROCYTES IN FORMATION OF FUNCTIONAL NEURONAL NETWORKS IN VITRO

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Astrocytes are the most numerous cell type in the CNS involved in normal development as well as dysfunctions of neuronal networks. Recent studies have indicated that besides providing metabolic support for neurons, astrocytes have also more active roles in the brain function such as participation in dendrite and axon outgrowth, formation synapses and removal of neurotransmitters from the synaptic cleft. Although still controversial, astrocytes have been even shown to involve in synaptic transmission and plasticity. Further, astrocytes are being recognized as contributors to several nervous system dysfunctions including neurodevelopmental diseases. Thus, understanding the roles of astrocytes in human neuronal network formation and functionality is a key step towards development of disease models for astrocyte contributory diseases and revealing mechanisms of astrocyte dysfunction facilitating development of novel therapeutic approaches, perhaps targeting astrocytes. Here, we differentiated human pluripotent stem cells into 1) neuron enriched and 2) astrocyte enriched cultures. Properties of these cultures were compared with gene and protein expression studies, with electrophysiology (microelectrode array measurements (MEA)), and calcium imaging. The main focus of the study was in the MEA measurements. The results show that the amount of astrocytes affects the functional development of neuronal networks. The detailed analysis of vast data sets is currently ongoing. These results suggest that astrocytes should be taken into account when developing in vitro disease models as they are part of normal neuronal network development.

F-1212

RAPID DERIVATION OF A DISEASE-RELEVANT CORTICAL NEURONAL SUBTYPE

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Brain imaging and post-mortem studies suggest that structural and functional abnormalities of the human cerebral cortex are a prominent feature of schizophrenia. Specific changes have been observed at the genetic, morphologic, and physiologic levels in the excitatory projection neurons of the cerebral cortex, in particular in the pyramidal projection neurons of the upper layers. While previous studies have described the use of patient-derived human pluripotent stem cells (hiPSC) to model neuropsychiatric diseases in vitro, a full understanding of the pathophysiology of schizophrenia will only be possible if the human neuron classes specifically affected are generated. Here, we have developed a protocol that combines conditional expression of the Ngn2 transcription factor with progenitor patterning via a DUAL SMAD inhibition protocol, to generate cortical neurons from human pluripotent stem cells in two weeks. Molecular characterization by both immunohistochemistry and single-cell transcriptional profiling indicate that the neurons generated by these methods acquire a molecular signature of upper layer projection neurons and do not express markers of deep layer neurons. This molecular signature was validated by population-level RNA sequencing. Furthermore, multielectrode array (MEA) and Calcium Imaging analysis by Fluorescent Imaging Plate Reader (FLIPR) reveal that hiPSC-derived neurons begin to be electrophysiologically active as early as two weeks after Ngn2 induced expression. In agreement, whole-cell patch clamp recording detects functional expression of glutamate receptors (both AMPARs and NMDARs) and large synaptic transmission events by 3-4 weeks after Ngn2 induction. In summary, we have established an efficient in vitro platform for the derivation and functional characterization of cortical projection neuron subtypes of clinical relevance. This will enable interrogation of the cellular, molecular, and physiologic effects of specific genetic variants associated with schizophrenia and other psychiatric diseases.

F-1213

OPTIMIZATION OF FREEZING AND THAWING METHOD OF HUMAN IPS CELL-DERIVED NEURAL STEM / PROGENITOR CELLS FOR CLINICAL APPLICATION IN SPINAL CORD INJURY

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Recently, we have reported the effectiveness of transplanting human iPS cell-derived neural stem/progenitor cells (iPS-NSPCs) for subacute spinal cord injury (SCI) in mice as well as common marmosets. Because it takes about 6 months to establish iPS-NSPCs derived from SCI patient's somatic cells, at present, it is impossible to perform auto-graft of iPS-NSPCs within an optimal therapeutic time window for subacute SCI. To extend our results into clinical application, allogeneic transplantation of iPS-NSPCs is a realizable goal. There are still some concerns to overcome, such as iPS-NSPCs storage and supply. Especially, it is critical to determine whether freezing and thawing affects the viability and proliferation ability of iPS-NSPCs. The purpose of this study is to assess the effects of cryopreservation on the characters of iPS-NSPCs. 201B7 iPS-NSPCs, which are consider safe as reported previously, were used in the present study. The iPS-NSPCs were cryopreserved in STEM-CELLBANKER by slow-freezing method. To determine the timing of freezing (3 or 6 culture days after the last passage), the number of frozen cells (2 or 5 million/ml) and freezing method (program freezer or freezing container), survival, proliferation and differentiation assays were performed under the different conditions. The cell viability was highest when the iPS-NSPCs were frozen on 6 days after the last passage at the concentration of 2 million cells/ml. Compared to the freezing container, the program freezer significantly increased the cell survival after thawing. In addition, there were no significant differences in proliferation and differentiation ability between frozen-thawed cells and non-frozen cells. Towards clinical application of cell transplantation for subacute SCI, cryopreservation of iPS-NSPCs is essential in terms of cell viability after thawing. In this study, we succeeded in improving the viability of the iPS-NSPCs by means of the program freezer. Furthermore, frozen-thawed cells showed similar proliferation and differentiation ability to non-frozen cells. Further study of transplantation of the frozen-thawed iPS-NSPCs into injured spinal cord of mice should be performed to determine their effectiveness and safety.

F-1214

CHD7 PROMOTES PROLIFERATION OF NEURAL STEM CELLS AND GLIOMA STEM CELLS

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Macrophage migration inhibitory factor (MIF) plays an important role in supporting the proliferation and/or survival of murine neural stem/progenitor cells (NSPCs), yet the downstream effectors of this factor remain unknown. Here, we show that MIF increases the expression of Pax6 and Chd7 in NSPCs in vitro. During neural development, Chd7 is expressed in the ventricular zone of the telencephalon of mouse brains at embryonic day 14.5, as well as in cultured NSPCs. Retroviral overexpression of Pax6 in NSPCs increased Chd7 gene expression. Lentivirally-expressed Chd7 shRNA suppressed cell proliferation and neurosphere formation, and inhibited neurogenesis in vitro, while decreasing gene expression of Hes5 and N-Myc. In addition, Chd7 overexpression increased cell proliferation in human ES cell-derived NSPCs. In Chd7 mutant fetal mouse brains, there were fewer Tbr2/Ki67 double-positive cells compared to wild type brains, indicating that Chd7 contributes to neurogenesis in the early developmental mouse brain. Furthermore, database analysis showed high expression of CHD7 in gliomas among the CHD family and the prognosis of patients with high CHD7 expression was worse than that of patients with intermediate expression of the gene. We observed high levels of CHD7 expression in glioma stem cells compared to astrocytes, and CHD7 was shown to contribute to glioma stem cell proliferation and apoptosis. Collectively, our data demonstrate that CHD7 is an important factor in the proliferation and stemness maintenance of NSPCs and CHD7 is a promising therapeutic target for the treatment of gliomas.

F-1215

MODIFICATIONS IN CULTURE CONDITIONS OF NEUROSPHERE FROM MGE INFLUENCE THE EXPRESSION OF DLX1 AND THE NUMBER OF INHIBITORY INTERNEURONS

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Neural stem cells (NSC) from the medial ganglionic eminence (MGE) originate most of inhibitory interneurons in the cortex and hippocampus in the adult brain. During development, specific transcription factors such as Dlx1 are essential for the development of inhibitory interneurons. In order to increase the yield of NSC derived from MGE embryonic tissue, we tested whether modifications in culture conditions of MGE-derived NSC influence differentiation into inhibitory neurons in vitro. MGE from rat embryos (E14) were dissected and cells were cultured as neurospheres in distinct conditions: in the presence of epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2) (group E/F); and with Retinoic Acid (RA) in the absence of EGF/FGF-2 (RA). In both conditions DMEM/F12, L-glutamine, N2 supplement and antibiotic were present. RNA was extracted from neurospheres after 7 days and from the MGE (E14) as a positive control. The cDNA was synthesized and Dlx1 gene expression was analyzed by qPCR. For differentiation, neurospheres were plated on an adherent substrate for 10 days. The pattern of differentiation was analyzed by immunofluorescence (IF) and flow cytometry, using specific markers for inhibitory interneurons (GABA, Neuropeptide Y - NPY and Parvalbumine- PV). Flow cytometry and IF suggested that RA group showed a significant increased percentage of GABAergic interneurons (30,88% of GABA+ cells, 15% of PV+ cells and 15,7% of NPY+ cells) compared with the E/F group (23,55% of GABA+

cells, 7,5% of PV+ cells and 1,4% of NPY+ cells). In accordance with the IF data, qPCR indicates that the RA group showed a significant increase in Dlx1 expression (25 times) compared with the E/F group. However, embryonic MGE cells have 7 to 160 fold more Dlx1 transcript levels than neurosphere cultures.Our data suggest that variations in culture composition were able to modify the pattern of neuronal differentiation and to increase the expression of Dlx1 gene. The absence of growth factors EGF/FGF-2 and the addition of RA affected the fate of neurospheres from MGE by increasing the population of inhibitory neurons. Our results also show that expression of Dlx1 gene by MGE cells has an important role in the differentiation of inhibitory interneurons, not only in the developing brain but also in vitro.

F-1216

ZAC1 CONTROLS CELL CYCLE EXIT OF NEURAL PROGENITORS THROUGH DIRECT REGULATION OF CYCLIN-DEPENDENT KINASE INHIBITOR EXPRESSION ALONG THE ENTIRE ROSTROCAUDAL AXIS OF THE DEVELOPING CENTRAL NERVOUS SYSTEM

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The central nervous system (CNS) is characterized by a sophisticated architecture where different cell types support neuronal functions. During CNS development neurons, astrocytes and oligodendrocytes are generated from a pool of neural stem cells located in the ventricular zone (VZ) and subventricular zone (SVZ). Proper development and functionality of CNS is achieved via regulatory mechanisms that dictate when neural progenitors should proliferate or exit the cell cycle. Despite the crucial importance of coordination between cell cycle exit and differentiation, such mechanisms remain poorly understood. Here we show that the zinc finger transcription factor Zac1, which expression in the developing cortex and spinal cord is restricted to the dividing neural progenitors in the VZ and SVZ, is co-expressed with SOX2 transcription factor known to block neuronal differentiation and maintain progenitor properties. Our data showed that SOX2 binds to Zac1 gene in a conserved region and it negatively regulates Zac1 expression strengthening the idea of a role for Zac1 during neurogenesis. Zac1 overexpression, in cortex and spinal cord, elicits cell cycle exit and expulsion from the germinal zones. In the cortex this is accompanied by upregulation of the CKI Cdkn1c, whereas in the spinal cord Cdkn1b expression is induced. The premature cessation of proliferation is, however, not accompanied by precocious acquisition of differentiated neuronal characteristics. In vitro, we show that, Zac1 directly binds to the promoter region of the Cdkn1c gene and forcefully induces its expression. Our results show that Zac1 is a key regulator of cell cycle exit in cycling neural progenitors through the induction of CKIs.

F-1217

EFFECT OF METFORMIN TREATMENT ON CELLULAR AND COGNITIVE OUTCOMES IN TWO MOUSE MODELS OF JUVENILE ACQUIRED BRAIN INJURY

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Childhood brain injury can lead to significant and debilitating cognitive impairment throughout development and into adulthood. One potential treatment option is to use an endogenous repair strategy to activate resident neural stem and progenitor cells to promote tissue regeneration and functional recovery after injury. Based on recent work demonstrating enhanced neurogenesis and cognitive function in adult mice following treatment with the drug Metformin, we sought to determine it's potential benefits in models of early postnatal acquired brain injury. We have demonstrated that Metformin, a commonly used drug to treat type 2 diabetes, activates neural precursor cells in newborn mice leading to expansion of the neural precursor pool. In addition, we performed lineage tracing and found that metformin leads to significant increases in neuronal and oligodendrocyte formation in the brain parenchyma in a model of neonatal hypoxia-ischemia (H/I). Strikingly, one week of metformin treatment also leads to a reversal of sensorimotor deficits observed post-H/I. Herein we asked whether metformin treatment was able to promote long term cognitive recovery in two different injury models. First, in the neonatal H/I model and second, in a juvenile cranial irradiation mouse model. We have shown that long term metformin treatment leads to enhanced executive functioning compared to controls using the puzzle box task. The cellular basis for this cognitive repair in the H/I model is currently being explored using a transgenic mouse model to permit neural precursor cell tracking. We chose the cranial irradiation model to mimic cognitive impairments in children that are observed following brain irradiation to treat medulloblastoma. Mice received cranial irradiation on postnatal day 17 and the size of the neural precursor pool in the neurogenic regions of the forebrain was examined. We observed a significant reduction in the neural stem cell pool in the subventricular zone and hippocampus and observed that neural stem cells in the hippocampus were responsive to metformin. We predict that any cognitive deficits observed following cranial irradiation will be improved following metformin treatment. Our findings support the hypothesis that endogenous repair is a promising strategy to promote brain repair.

F-1218

TRANSPLANTATION OF ADULT SVZ DERIVED NEURAL PROGENITOR CELLS TO IMMUNE COMPETENT RATS IMPROVES LOCOMOTOR FUNCTION AFTER SPINAL CORD INJURY

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Spinal cord injuries (SCI) are caused by trauma and leads to devastating and irreversible consequences like loss of sensory and motor functions below the injury. Adult NSCs have capacity to differentiate into cells of the CNS and constitute a potential source for therapy for SCI. Here we transplanted NSCs isolated from the SVZs of ubiquitin:EGFP transgenic Lewis rats into SCI of immune competent wild type litter mates 10 days after injury. Spinal contusion injury was made on the dorsal side of thoracic T11 level using IH impactor. A total of 230,000 neurospheres (≤40μm) in 5μl of medium were transplanted in two injection sites of the spinal cord at the epicenter of the injury. We assessed recovery of rats after NSC cell transplant with BBB score and kinematic like body height and angle, base of support, linear displacement, toe clearance, paw potion and rotation, step cycle, ratio of hind limb and forelimbs in walking, horizontal grid walking and foot prints. The majority of the NSCs survived after 15weeks of transplantation and differentiated into oligodendrocytes (APC), astrocytes (GFAP) and neurons (Tuj-1). The NSCs filled the cyst and migrated 200um from the injection site. The majority of the cells differentiated into functional oligodendrocytes (MBP) and some migrated into the white matter of the spinal cord. On group level, the recovery of transplanted animals started to be apparent after 28 days. We conclude that transplanted NSCs from SVZ improved recovery after SCI.

F-1219

NSUN2 KNOCK OUT IMPAIRS DIFFERENTIATION IN THE MOUSE EMBRYONIC BRAIN AND HUMAN NEURAL STEM CELLS

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Loss-of-function mutations in the cytosine 5-RNA methyltrasferase Nsun2 are associated with microcephaly and neuronal abnormalities in both mouse and human. In vivo data showed that the absence of Nsun2 causes hypo-methylation of RNAs, especially tRNAs, which in turn triggers the activation of stress pathways leading to cellular dysfunction. However, it is currently unknown how 5-cytosine methylation of RNAs contributes to the correct development of the brain. Here, we use a combination of system-wide approaches, mouse models and in vitro differentiation assays to analyze the regulatory roles of ctyosine-5 RNA methylation during neural stem cell differentiation. In the developing mouse brain Nsun2 is highest expressed in the ventricular zone and the cortex. Total deletion of NSun2 led to a reduced weight of the brain, decreased cortical thickness and lower number of neurons in the upper cortical layers compared wild-type littermates. RNA bisulfite sequencing using the whole forebrain revealed a set of mRNAs that showed NSun2-dependent methylation. Those Nsun2 targets are involved in stress response and are usually methylated near 3' splice junctions. In the human embryo, NSUN2 is expressed in the forebrain in regions overlapping with stem and progenitor cells. Furthermore, NSUN2 knockdown in human embryonic neuroepithelial cells caused delayed differentiation into neurons. Thus, our data demonstrate a role for Nsun2 in promoting neural differentiation. We further identify mRNAs that are cytosine-5 methylated at 3' splice sites, indicating that cytosine-5 methylation induces the translation of distinct protein isoforms. Further studies are needed to confirm that cytosine-5 methylation indeed alters RNA splicing of key factors regulating neural development.

F-1220

CHEMICAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO NEURAL CREST STEM CELLS

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Neural crest cells are a multipotent population that arise from the border of the neural plate and epidermal ectoderm, migrate extensively and differentiate into diverse cell types during vertebrate embryogenesis. Defects in neural crest development cause a number of human birth defects and syndromes known collectively as neurocristopathies; these include Hirschsprung disease, Treacher Collins, and Waardenburb syndrome. Medical therapy for Hirschsrung disease is limited, and surgical intervention may incur significant morbidity. Alternatively, transplantation of NCSCs may regenerate enteric ganglia and provide therapeutic benefit. Before NCSCs can be administered in therapeutic modalities, however, a reliable source and culture conditions must be developed that allows for the generation and expansion of neural crest stem cells. Here we report a novel chemically culture method that can be used to generate and expand homogenous populations of human pluripotent stem cell derived neural crest stem cells (hpNCSCs). The majority of the hpNCSCs (>70%) derived using this culture method express the typical neural crest markers including Sox10, Nestin, p75 and HNK1 as determined by Immunofluorescence staining and FACS analysis. Additionally, the chemically defined medium (without added exogenous growth factors) described herein is capable of proliferating hpNCSCs in an undifferentiated state for >10 passages and remain karyotypically normal. The expanded hpNSCs can be subsequently differentiated towards peripheral nervous system and mesenchymal lineages. Our novel differentiation method provides a tool for investigating neural crest development and permits the generation of expandable homogenous populations of NCSCs that could potentially be used in cell-based therapies for Neurocristopathies.

F-1221

ABNORMAL MICROGLIAL ACTIVATION LEADS TO OLFACTORY DYSFUNCTION IN NIEMANN-PICK DISEASE TYPE C1 MICE THROUGH SUPPRESSION OF NEUROGENESIS IN THE OLFACTORY BULB

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Progressive olfactory impairment is one of the earliest markers of neurodegeneration. However, the underlying mechanism for this dysfunction remains unclear. The present study investigated the possible role of microgliosis in olfactory deficits using a mouse model of Niemann-Pick disease type C1 (NPC1), which is an incurable neurodegenerative disorder with disrupted lipid trafficking. At 7 weeks of age, NPC1 mutants showed a distinct olfactory impairment in an olfactory test compared with age-matched wild-type controls (WT). The marked loss of olfactory sensory neurons within the NPC1 affected olfactory bulb (NPC1-OB) suggests that NPC1 dysfunction impairs olfactory structure. Furthermore, the pool of neuroblasts in the OB was diminished in NPC1 mice despite the intact proliferative capacity of neural stem/progenitor cells in the subventricular zone. Instead, pro-inflammatory proliferating microglia accumulated extensively in the NPC1-OB as the disease progressed. To evaluate the impact of abnormal microglial activation on olfaction in NPC1 mice, a microglial inhibition study was performed using the anti-inflammatory agent Cyclosporin A (CsA). Importantly, long-term CsA treatment in NPC1 mice reduced reactive microgliosis, restored the survival of newly generated neurons in the OB and improved overall performance on the olfactory test. Therefore, our study highlights the possible role of microglia in the regulation of neuronal turnover in the OB and provides insight into the possible therapeutic applications of microglial inhibition in the attenuation or reversal of olfactory impairment.

F-1222

HUMAN NEURAL PROGENITOR CELLS FROM NEURAL TISSUES AND THOSE FROM IPS CELLS SHOW DIFFERENT CHEMOSENSITIVITY AGAINST ALKYLATING AGENTS

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Conventional neurotoxicity assay has been carried out using experimental animals and human cell lines, although that is not necessarily optimal evaluation system to predict in vivo toxicity. Human neural tissue-derived primary cells are coherent materials, but those are very difficult to obtain enough cells for high throughput screening. Recently, it was demonstrated that neural cells can be differentiated from either human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs), and those are adaptable and powerful alternatives for tissue-derived primary cells in both regenerative medicine and neurotoxicological assays. In this study, we generated hiPS-derived neural progenitor cells (hiPS-NPCs) with self-renewal potential and differentiation capacity restricted to neural lineage (neurons and astrocytes). iPS-NPCs were expanded as free-floating spheroids (neurospheres) and verified the usefulness by comparison with human neural tissue derived NPCs (hN-NPCs). The hiPS-NPCs were almost identical in phenotype to hN-NPCs, in both cell-surface marker expression and their ability to differentiate into neuronal cells, although gene expression profiles showed that the hiPS-NPCs had higher neural and lower glial gene expression, along with mid-hindbrain-like regional specificity. We also examined chemo-sensitivities of hiPS- and hN-NPCs against anticancer agents, like nimustine (ACNU) and temozolomide (TMZ). These neurotoxicity tests revealed the difference between hiPS- and hN-NPCs in sensitivity to those agents and expression pattern of O-6-methylguanine-DNA methyltransferase (MGMT) and mismatch repair-related genes. Our present findings will indicate some clues to establish more accurate assay system.

F-1223

CEREBROSPINAL FLUID REGULATION OF ADULT NEURAL STEM CELLS

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Neural stem cells in the adult ventricular-subventricular zone (V-SVZ), adjacent to the lateral ventricles (LV), continuously generate new neurons. Quiescent neural stem cells become activated and divide to give rise to rapidly dividing transit amplifying cells that in turn generate new neurons that migrate to the olfactory bulb. In vivo, proliferation and neurogenesis decline dramatically with aging. An underappreciated component of the V-SVZ stem cell niche is the cerebrospinal fluid (CSF). The main source of the CSF is the choroid plexus (CP), a mini-organ that floats in the ventricles, but it also contains factors from other sources. V-SVZ stem cells extend a process between ependymal cells to contact the LV, and are continuously bathed by the CSF. The adult CSF compartment is largely thought to maintain stem cell quiescence. Here we show that adult CSF and CP conditioned medium also promote the robust proliferation in vivo and in vitro of quiescent and activated V-SVZ stem cells, as well as transit amplifying cells. In addition, more cells are activated in vitro to form colonies than with EGF alone controls. Thus, the CSF compartment is an important reservoir of factors for V-SVZ stem cell lineage regulation. Strikingly, we find that the functional effect of the CP secretome changes with aging. In vitro experiments and in vivo infusions using young (2 month) or old (18 month) SVZ cells and age-matched or swapped CP conditioned medium, reveal that activated neural stem cells are uniquely sensitive to factors in the young and aged CP secretome. Together these data highlight that the CSF and CP are a key niche compartment that dynamically changes with aging.

F-1224

MICRORNA-145 REGULATES NEURAL STEM CELL DIFFERENTIATION THROUGH THE SOX2/LIN28 SIGNALING PATHWAY

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MicroRNAs (miRNAs or miRs) regulate several biological functions, including cell fate determination and differentiation. In this respect, we and others have recently demonstrated that apoptosis-associated

miRNAs have a functional role during neural differentiation through mechanisms independent of cell death. MiR-145 was already shown to repress sex-determining region-box 2 (Sox2), a key transcription factor for self-renewal, to inhibit pluripotency in human embryonic stem cells. Recently, a role for the Sox2/LIN28/let-7 signaling pathway in regulating proliferation and neurogenesis of neural precursors has been reported. In the present study, we aimed to investigate the precise role of pro-apoptotic miR-145 in neural stem cell (NSC) fate decision, and the possible involvement of the Sox2/LIN28/let-7 signaling pathway in the miR-145-regulatory network. For that, NSCs derived from 14.5-dpc mouse fetal forebrain were grown in monolayer and induced to differentiate. Our results showed that miR-145 significantly increased after induction of neural differentiation, reaching an ~ 11-fold peak at day 3, when compared to undifferentiated cells. miR-145 expression remained elevated throughout NSC differentiation, while protein levels of Sox2 and LIN28, a well known suppressor of let-7 miR biogenesis, decreased. Of note, NSC differentiation also resulted in let-7a upregulation. The transfection of NSCs with anti-miR-145, in turn, increased both Sox2 and LIN28 while decreasing let-7a and neuronal markers, including βIII-tubulin. More importantly, Sox2 silencing partially rescued the impairment of neuronal differentiation by anti-miR-145. In conclusion, our results demonstrate a novel role for miR-145 regulation of NSC differentiation, where miR-145 upregulation, and subsequent decrease of Sox2 and LIN28, appear to be crucial for neurogenesis progression.

F-1225

THE INFLUENCE OF ELECTROSPUN POLY-LACTIC CO-GLYCOLIC ACID FIBERS ON NEURAL DIFFERENTIATON OF MUS MUSCULUS EMBRYONIC STEM CELLS

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Neural stem cells offer a promising approach for cell therapy in neurodegenerative diseases. However, cell transplantation in spinal cord injury (SCI) presents some challenges in terms of maintaining the cells at the injury site and offering spatial cues for the regenerating axons. Biodegradable polymer scaffolds provide an excellent approach for both delivering cells and offering growth substrates in SCI. In this study, new lineages of mouse embryonic stem cells (mESCs) were isolated and differentiated towards neural precursor cells and their differentiation on poly-lactic-co-glycolic acid (PLGA) scaffolds was compared with the differentiation on culture plates. mESC lineages were derived from the C57BL/6 strain and characterized in the laboratory following standard protocols. mESCs were induced to differentiate into neuronal lineage by the formation of embryoid bodies (EBs), followed by treatment of embryoid bodies with retinoic acid (RA), EC-23 or retinol for 2 days and a selection of neural precursors in a serum free medium containing insulin, transferrin, selenium and fibronectin. The media was changed every other day. In the first part of the study, the neurogenic effect of the three afore mentioned retinoids was compared. Similar results for the nestin expression were obtained with RA and EC-23, with EC-23 accelerating the differentiation of neural precursors into neurons, as seen by the β3-tubulin staining. Lower counts of nestin-positive cells were obtained with the retinol treatment. In a second part of the study, the topographic cues offered by a scaffold with either aligned or random fibers were investigated. Fibers with an average diameter of 3 μm were produced by electrospinning with an 18% solution of PLGA. The cells cultivated on the fibers migrated less than the cells cultivated on the plates, remaining localized near the region of the main embryoid body. They were also found on the top and inside the scaffold, as seen by scanning electron microscopy. mESCs cultivated on PLGA nanofibers were able to differentiate into neural precursors and neurons, similarly to the control groups, as revealed by immunostaining with nestin and beta 3 tubulin. These results indicate that a combination of biodegradable scaffolds and cells could provide better treatment for spinal cord and peripheric nerve injury.

F-1226

ADULT HUMAN BRAIN PERIVASCULAR MESENCHYMAL STEM CELLS SHOW NO INTRINSIC NEUROECTODERMAL BUT HIGH MESODERMAL DIFFERENTIATION POTENTIAL

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Mesenchymal stem cells (MSCs) are promising source for autologous cell therapy of neurological diseases. These cells are able to migrate to injury sites and promote survival and regeneration of host cells. MSCs have been proposed to be of perivascular origin. Such perivascular cells have been identified in adult human brain. Understanding of these MSCs might help their efficient implementation in treatment of neurological diseases. Here, we systematically characterized adult brain perivascular MSCs (aBPMSCs) isolated from subcortical white matter and hippocampus and compared their neuroectodermal and mesodermal differentiation potential with human adult neuroprogenitor cells (aNPCs). Both aBPMSCs were positive for Nestin (over 50%) but showed no OLIG2 and A2B25 expression. When stained for pericyte marker, 73 ± 8% of aBPMSCWM and 76 ± 2% of aBPMSCHIP were positive for PDGFR-β, both similar to bone-marrow derived MSCs. These aBPMSCs were unable to differentiate into immature or mature neurons but could efficiently differentiate into cells of mesodermal lineage. aBPMSCs were positive for lipid

formation when induced towards adipogenic lineage, although no droplet formation was observed as in mesodermal-derived stromal cells. Whole-transcriptome profiling showed that aBPMSCWM and aBPMSCHIP differed only in 42 transcripts and shared more commonality to MSCs than aNPCs and human fetal neural stem cell. Next, we transplanted aBPMSCs into brains of E10.5 mouse to assess if these cells harbour innate neuroectodermal differentiation potential. Engrafts were analyzed 7 days post transplantation. Cellular implants formed spheres within the ventricular system but did not migrate into the host brain. We could not detect neural stem cell (SOX2+), immature (DCX+) or mature (MAP2+) neurons originating from grafted cells. We could show here that aBPMSCs retain mesodermal lineage commitment and do not harbour intrinsic neuroectodermal differentiation potential. However, due to their similarities to MSCs, which are known for the trophic support or modulatory effects of diseased neurons, aBPMSCs might attract attention as a potential endogenous cell therapeutic source.

F-1227

YY1 IS REQUIRED FOR NEURAL CREST DEVELOPMENT

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The neural crest (NC) is a transient embryonic stem cell population unique to vertebrate embryos that can give rise to a multitude of cell types including neurons and glial cells of the peripheral nervous system, melanocytes, bone and cartilage of the facial elements. Aberrant NC development is at the onset of many congenital diseases including craniofacial and cardiovascular syndromes. Therefore, understanding the transcriptional network that regulates NC stemness is highly relevant. To address this question we have performed a microarray analysis of migratory NC stem cells and NC cells primed to the mesenchymal, neuronal, and glial lineages. 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 by using ISMARA. ISMARA models genome-wide expression data with regard to local occurrence of predicted regulatory sites for transcription factors. ISMARA analysis revealed high Yin Yang (Yy1) motif activity in undifferentiated NC stem cells when compared with their differentiating counterparts. The transcription factor Yy1 is a broadly expressed, multifunctional protein, able to activate or repress gene expression depending on the cellular context. Yy1 can interact with a variety of other factors, including Mitf in melanoblasts and epigenetic regulators of the polycomb-group proteins. Depending on the cell type, its role includes control of proliferation, differentiation, and apoptosis. To dissect the in vivo role of Yy1 during NC cell development, we conditionally ablated Yy1 in the premigratory NC by crossing Wnt1-Cre mice with mice carrying Yy1 floxed alleles. Strikingly, loss of Yy1 in the NC impaired the formation of all NC derivatives analyzed: Virtually all NC-derived craniofacial structures were missing in Yy1 cko embryos. Additionally, Yy1 ablation resulted in loss of the enteric nervous system and reduced size of dorsal root ganglia (DRG). Of note, mutant DRG contained sensory neurons and satellite glial cells, suggesting that differentiation as such is not impaired by Yy1 inactivation. Likewise, Yy1 cko embryos displayed reduced numbers of melanocytes. Taken together our results suggest that the transcription factor Yy1 plays a crucial role in NC stem cell biology.

F-1228

EVIDENCE FOR PHENOTYPE INTERCONVERSION OF HUMAN NEURAL PRECURSOR CELLS

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A number of cell types have been suggested as potentially useful for cell therapy in spinal cord injured patients. We and others have shown that acute-subacute transplantation of human fetal neural precursor cells (hNPCs) results in functional improvement in animal models of spinal cord injury (SCI). Similar cells are currently studied in two clinical SCI trials, one using sorting of CD133+/CD24lo NPCs, suggested to be human neural stem cells (hNSC). To evaluate the phenotype of sub-populations of hNPCs, we have assayed more than 25 neurosphere lines for ten cell surface antigens, expressed in 10-85% of spinal cord-derived NPCs. To study the functional relevance of these markers, we used some - i.e. CD15, CD133, CD24, CD29, A2B5 and PSA-NCAM - for cell sorting with FACS, evaluating the stability of marker expression and phenotype of the sorted cells during continued culture. For all antigens tested we unexpectedly found that the proportions of cells expressing any of these antigens before sorting were reestablished to the proportions seen prior to sorting within two passages (4-6 weeks), after negative or positive sorting. This shows that phenotypic identity of individual cells, as displayed by cell surface antigens, is inherently unstable in cultured hNPCs as a consequence of interconversion. We have previously found that several pluripotency genes are transcribed in these non-pluripotent hNPCs. Using viral reporter genes and sorting of hNPCs expressing these genes, we showed that this feature is also subject to interconversion. At present, we do not know to what extent the mitogens (bFGF, EGF and CNTF) present in the culture media are responsible for the interconversion, or if this phenomenon will also occur in vivo. Our data suggest that hNPCs in vitro attain a default phenotype marker expression pattern irrespective of previous sorting, implying that lineage-restricted hNPCs can not be maintained in vitro. The hierarchical model of phenotype specification must also be questioned for these cells.

F-1229

OSVZ PROGENITOR CELL IN THE TREE SHREW EMBRYONIC NEOCORTEX

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Recent studies revealed a new class of radial glia - like cells in primates referred to as oRG (OSVZ radial glia) cells with characteristic monopolar morphology that populate the OSVZ. oRG cells have also been discovered in the developing cortex of non-primates, including rodents and carnivores. In the primate brain, the outer region of the SVZ (OSVZ) is a massively expanded and cytoarchitecturally distinct layer. The expansion of the subventricular zone may have been an essential evolutionary step leading to gyrencephalic neocortical expansion, however, the mechanisms underlying the expansion of the OSVZ and of the progenitor cells

that underlie evolutionary cortical expansion are unclear. Here we show that the Chinese tree shrew, a member of the order Scandentia, closely related to primates, is an ideal species to explore cytoarchitecture layer formation in the developing cortex. We find that a subtype of progenitor cell that resembles the oRG cell in human fetal brain is present in tree shrew embryonic neocortex within a cytoarchitecturally distinct layer, which is similar to the OSVZ region in primates. oRG cells in this area exhibit high proliferation activity. With the technique of in-utero electroporation, three shrews will be a favorable model to explore the cellular and molecular mechanism of cytoarchitecture formation and neocortical expansion.

F-1230

STUDY NEURONAL DIVERSITY AND FUNCTIONAL MATURATION USING HUMAN PLURIPOTENT STEM CELLS

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Cortical projection neurons are highly diverse due to their dendritic morphology, laminar position, project pattern, gene expression and firing properties. However, a more comprehensive understanding of neuronal diversity requires integration of these phenotypic data modalities. To understand human cortical excitatory neuronal diversity and function, we have developed a highly efficient and reproducible monolayer-based method to differentiate human embryonic stem cells (hESC) into different subtypes of cortical excitatory neurons. In order to enrich and isolate the cortical progenitor and neuronal populations, we generated H1 hESC lines stably expressing Citrine fused to either SOX2 or DCX genes. Using single cell profiling at various stages of human stem cell-derived cortical neurogenesis, we observed different cell type clusters in SOX2-Citrine+ progenitors and DCX-Citrine+ maturing neurons. We further analyzed ion channel and receptor expression patterns and distribution among these clusters and our results indicate that neurons start acquiring their functional ion channels and receptors as they gain their cell identities and some of these ion channels and receptors are specifically expressed among cell subtypes. Finally, our calcium imaging and single cell patch-clamp recording showed that hESC derived cortical neurons mature at a slow rate in vitro. Time series transcriptome analysis over the course of maturation informed us the key genes and networks that regulate the neuronal maturation.

F-1231

ADULT DERIVED PRIMITIVE NEURAL STEM CELLS THAT EXPRESS OCT4 AND ARE GFAP NEGATIVE, GIVE RISE TO GFAP EXPRESSING DEFINITIVE NEURAL STEM CELLS IN THE ADULT MAMMALIAN BRAIN

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GFAP+ "definitive"neural stem cells(dNSCs) reside in the periventricular region of adult mammalian CNS and proliferate to give rise to neuronal progenitors that migrate along the rostral migratory stream(RMS) generating interneurons in the olfactory bulb(OB). In vitro, dNSCs proliferate in EGF and FGF supplemented media to give rise to clonally derived neurospheres which display cardinal stem cell properties of self-renewal and multipotency. Recently, we discovered a novel population of adult NSCs that express the pluripotency marker Oct4 and form neurospheres in leukemia inhibitory factor(LIF) alone. These "primitive"NSCs(pNSCs) are found throughout development and persist into adulthood. Herein we will delineate the relationship between dNSCs and pNSCs in the adult brain. We labeled a cohort of GFAP+dNSCs in GFAP-CreERT2 x ROSAyfp$^{fl/fl}$ x GFAPtk mice using tamoxifen followed by ablation of GFAP+dNSCs using cytosine arabinoside(AraC) and ganciclovir(GCV). 30days post-kill, we found that neither migrating cells along the RMS nor neurospheres grown in either LIF or EGF/FGF were YFP+ after repopulation. Thus, GFAP+/YFP+/dNSCs are not the source of repopulation after the kill and further, pNSC are not GFAP+. Next, dNSCs are ablated in ROSAyfp$^{fl/fl}$xGFAPtk mice while concurrently labeling proliferating cells using retrovirus delivering Cre-recombinase, resulting in a population of GFAP-/YFP+/pNSCs. As predicted, there was no dNSC neurospheres immediately following ablation while ~15% of pNSC neurospheres were YFP+ showing that GFAP-/pNSCs were actively proliferating during repopulation. At 28 days post-ablation, ~40% of dNSC neurospheres are YFP+, revealing that pNSCs are lineally related to dNSCs. Similarly in vivo, we observed very rare YFP+ cells in the periventricular region immediately following ablation. However, by 28 days, YFP+ cells can be found along the RMS as well as the OB. Finally, we have shown that 10% of pNSCs express YFP in Oct4-CreERT2 x ROSAyfp$^{fl/fl}$ x GFAPtk adult mice following tamoxifen treatment. We hypothesize that following ablation of dNSCs, labeled YFP+ pNSCs will repopulate the dNSC population and generate YFP+ progeny on the RMS and in the OB. Taken together these findings demonstrate the lineage relationship between the pNSCs and dNSCs in the adult forebrain.

F-1232

IDENTIFICATION AND ACTIVATION OF QUIESCENT NEURAL STEM CELLS IN THE ADULT MOUSE BRAIN

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We have identified primitive neural stem cells (pNSCs) as a novel population of adult NSCs that are multipotent, self-renewing, *Oct4*-expressing, GFAP-, and upstream of the GFAP+ NSCs that form neurospheres in EGF and FGF2. PNSCs form clonogenic neurospheres in LIF that can be derived from the developing mouse embryo as early as embryonic day 5.5, 2 days earlier than FGF2-dependent neurospheres can be derived. PNSC-derived neurospheres can be isolated from brain germinal regions throughout the lifespan of a mouse, with a peak in abundance postnatally and dramatic decrease into adulthood. We posited this decrease in adult LIF neurospheres is due to a gradual transition to quiescence rather than a loss of pNSCs in vivo. We performed a long-term label retention experiment using doxycycline-inducible histone 2B-GFP mice to test whether pNSCs are indeed the resident quiescent cells of the adult subependymal (SE) zone. A 1-year chase period allowed us to calculate the cell cycle time of adult pNSCs as 3-5 months, while GFAP+ NSCs have a cell cycle time of 2-4 weeks.

Further, we report that pNSCs can exit quiescence in response to ablation of downstream cells by intraventricular infusion of the anti-mitotic drug, AraC. To elucidate the kinetics of pNSC activation after AraC, we performed a time course of AraC exposure. Surprisingly, pNSC-derived LIF neurospheres increased immediately following 1 day of AraC, and continually over 7 days of treatment. We hypothesized that this increase was not due to an increase in pNSCs in vivo, but rather an increase in the proportion of pNSCs that have been primed to exit quiescence, and thus that generate neurospheres in LIF. This suggests that under baseline conditions, a minority of pNSCs in the adult brain form neurospheres in LIF due to quiescence. To test this, SE cells from untreated adult mice were co-cultured with early postnatal SE cells. In the presence of the postnatal niche, there were significantly more primary adult pNSC-derived neurospheres, supporting the idea that the adult niche induces quiescence of pNSCs and limits formation of pNSC-derived neurospheres. In conclusion, we report that adult pNSCs are quiescent with a cell cycle time of 3-5 months, but can be released rapidly from this quiescent state in response to either ablation of their downstream progeny or exposure to a young niche.

F-1233

SINGLE TRANSCRIPTION FACTOR CAN DIRECTLY REPROGRAM HUMAN SOMATIC CELLS INTO OLIGODENDROCYTE PROGENITOR CELLS

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Generation of human oligodendrocyte precursor cells (OPCs) and oligodendrocytes, the myelinating glia in the central nervous system (CNS), from the brain or embryonic stem cells (ESCs) in vitro could provide new insights into human demyelinating diseases such as multiple sclerosis (MS), as well as other neurological disorders. However, natural human oligodendrocyte lineage cells in brain are limited and differentiation from ESC is time consuming. Advance in cellular reprogramming provide the fastest route to the production of patient-specific OPCs. Here we report direct reprogramming of human somatic cells to induced oligodendrocyte progenitor cells (iOPCs) using combinations of single transcription factor and cocktails of small molecule. Induced OPCs exhibit a bipolar morphology and marker gene expression, such as CD140a, A2B5, Sox10, consistent with OPCs and can be expanded in vitro for at least five passage in response to PDGF-AA and bFGF while retaining the ability to differentiate into MBP positive mature oligodendrocytes when co-culture with rat primary neurons. Importantly, PAX6, SOX1, SOX2, known as Neural Stem Cells markers, were undetectable during the entire reprogramming process. In conclusion, we identified that combination of small molecules with single transcription factor can induce oligodendrial fate and thus may lead to find therapeutic approaches in neurological disorders.

F-1234

ROLE OF BFGF AND EGF IN NEURAL ROSETTE FORMATION - MODELING HUMAN NEUROGENESIS IN THE DISH

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Human induced Pluripotent Stem Cells (hiPSC) allow the generation of patient-specific neurons, which offer a platform for in vitro investigation of central nervous system diseases. For these studies hiPSCs are induced to become neural precursor cells (NPCs). The most critical milestone for the successful neural induction in vitro is the formation of neural rosettes. Rosette NPCs are valuable intermediate cells to obtain neuronal and glial cells in culture. These cells offer a platform for in vitro drug screening and testing assays. NPCs and their derivatives serve as an unlimited source of immune compatible cells for cell replacement therapies to treat neurodegenerative diseases such as Parkinson's or Alzheimer's disease. Proliferation of NPCs can be stimulated by addition of basic Fibroblast Growth Factor (bFGF) and Epidermal Growth Factor (EGF). Here, we hypothesized that rosette neural progenitor cells are dosage sensitive to bFGF and EGF. To study the dosage sensitiveness, we used 2 hiPSC lines generated by lentiviral transduction and 2 hiPSCs lines derived from the non-integrative plasmid-based technology. We compared whether low (10 ng/ml) or high (25 ng/ml) concentrations of bFGF and EGF could affect neural rosettes formation. All examined lines were immunocytochemically positive for NPC markers (N-cadherin, Pax6, and Nestin) independently of the applied mitogen concentrations. However, high concentrations of bFGF and EGF significantly reduced the area of the lumen of neural rosettes ($P < 0.01$). At high concentrations of bFGF and EGF the proportion of large neural rosettes was significantly reduced in all studied cell lines ($P < 0.05$). After 14 days of terminal differentiation neurons were analyzed by immunocytochemistry and quantitative real-time PCR. Obtained results show that at low concentrations of bFGF and EGF NPCs exhibit a default anterior regional identity that mainly results into the generation of cortical neurons. In contrast, high concentrations of both mitogens lead to the down-regulation of cortical marker mRNA (Pax6, Tbr2 and Tbr1) with a simultaneous up-regulation of hindbrain marker (Hox2A and Hox2B). Our studies indicate that bFGF and EGF control the positional identity of NPCs along the anterior-posterior axis in a concentration-dependent manner. SZ and KS equal contribution

F-1235

PU.1 SILENCING TO REPROGRAM MONOCYTES INTO ERYTHROCYTES

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In development of hematopoietic system, the antagonistic interplay between lineage-determining transcription factors (TF), PU.1 and GATA.1 ascertain gene expression programs of monocytic and erythroid cells from common myeloid progenitors, respectively. This concept in transdifferentiation approaches has not been well considered yet, especially in intra-lineage conversion systems. To demonstrate whether PU.1 suppression induces monocyte lineage conversion into red blood cells, a combination of three PU.1 specific siRNAs were implemented to knocked-down PU.1 gene expression and generate the balance in favor of GATA1 expression to induce erythroid differentiation program and force monocytes cells to change their mind. In order to achieve this goal we isolated monocytes from peripheral blood of a healthy donor (n=3) and PU.1 gene expression was silenced. Our results have shown that after 72h the rate of PU.1 expression was reduced which resulted in significantly over expression of GATA1 and hemoglobin α, β and γ as erythroid cell specific genes, while reduced expression of monocyte CD14 gene was observed in qRT-PCR and flowcytometry results. We also detected hemoglobin protein by western blot. Our results suggest that it may be possible to change the fate of cells by manipulating the ratio of the two TFs in bifurcation differentiation pathways via applying siRNA technology as a safe way for therapeutics application.

REPROGRAMMING

F-1236

SMALL COMPOUNDS FACILITATE RAPID AND SYNCHRONOUS REPROGRAMMING OF SOMATIC CELLS INTO IPS CELLS BY DEFINED FACTORS

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Reprogramming differentiated cells to pluripotency by transcription factors such as OCT4, KLF4, SOX2 and c-MYC (OKSM) is an inefficient and lengthy process. Recently, several groups reported increased reprogramming efficiency with reduced latency following knockdown of MBD3 or with transient activation of C/EBPα in B cells prior to OKSM expression. These findings suggest that OKSM-induced iPSC formation can be drastically improved upon genetic manipulation. Given the obvious challenges surrounding genetic manipulation, we sought to develop an efficient and synchronous method to reprogram somatic cells to iPSCs without genome modification. We tested a select set of small molecules in a secondary OKSM reprogramming system and found that ascorbic acid (AA) and the GSK3-beta inhibitor CHIR-99021 strongly increased reprogramming efficiency. Combing AA and GSK3-beta inhibitor (termed "AGi") we observed a synergistic enhancement of reprogramming using different cell types (GMPs, proB cells, IgM+ B cells and fibroblasts). Moreover, AGi exposure reduced iPSC latency to little as 48 hours of factor expression using GMPs from our secondary system. To ensure accurate quantification of reprogramming efficiency and study reprogramming kinetics, we performed single cell experiments in a 96-well format. Remarkably, AGi treatment during reprogramming yielded over 95% Oct4-GFP^{+} wells in just 5 days. Previous studies have shown that GSK3-beta inhibitors stabilize c-MYC. To determine whether the effect of AGi is mediated by c-MYC, we tested reprogramming efficiency in secondary system that included only OCT4, SOX2, and KLF4. Even in the absence of ectopically expressed c-MYC, we noted a strong increase in reprogramming efficiency, suggesting that c-MYC is not solely responsible for AGi's reprogramming effect. In this study, we demonstrate that AGi in combination with OKSM induces nearly homogeneous reprogramming of a variety of cell-types at efficiencies and kinetics that have so far only been achieved with genetic manipulation. The synchronous nature of reprogramming in the presence of AGi should allow more detailed analysis of cell identity transition in bulk cultures and greatly aid reprogramming systems that have thus far been hampered by low efficiencies.

F-1237

KLF4 N-TERMINAL VARIANCE MODULATES POLYCISTRONIC STOICHIOMETRY AND INDUCED REPROGRAMMING TO PLURIPOTENCY

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As the quintessential reprogramming model, OCT3/4, SOX2, KLF4, and c-MYC re-wire somatic cells to achieve induced pluripotency. Yet, subtle differences in methodology confound comparative studies of reprogramming mechanisms. With a specific focus on the design of polycistronic reprogramming constructs, we employed a standardized *piggyBac* transposon vector to systematically assess the hallmarks of mouse somatic cell reprogramming elicited by various polycistronic cassettes. Responses varied overtly in the extent of reprogramming initiation and stabilization of pluripotency. Notably, cassettes were found to employ one of two KLF4 variants ($KLF4_S$ and $KLF4_L$; Genbank Accession No's: AAC52939.1 and AAC04892.1), differing only by nine N-terminal amino acids. In a polycistronic context, these two variants generated dissimilar protein stoichiometry, where $Klf4_L$ vectors produced more protein than those encoding $Klf4_S$. The two Klf4 variants associated with observed differences in reprogramming hallmarks, including colony formation frequencies, SSEA-1 cell surface presentation, and the stabilization of pluripotency as indicated by transgene-independence and activation of a Nanog-GFP reporter. Extension of the shorter variant by nine N-terminal amino acids, or augmenting stoichiometry by $Klf4_{S/L}$ supplementation, rescued both protein levels and phenotypic disparities, implicating a threshold in determining reprogramming outcomes. Strikingly, global gene expression patterns elicited by published polycistronic cassettes diverged according to each KLF4 variant, underpinning the importance of factor stoichiometry in determining reprogramming outcomes. Our data exposes a Klf4 reference cDNA variation that alters polycistronic factor stoichiometry and predicts key aspects of reprogramming. We hope this study will help to guide the comparison of compatible public data sets.

F-1238

THE M3 REVERS TRANSCRIPTIONAL TRANSACTIVATOR ENHANCES THE REPROGRAMMING EFFICIENCY OF THE OKSM MOUSE MODEL

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Mature cells can be reprogrammed to a pluripotent state equivalent to embryonic stem cells. These so called induced pluripotent stem (iPS) cells are able to give rise to all cell types of the body and consequently have vast potential for regenerative medicine applications. In order to study the reprogramming process and optimize it for clinical applications, we need to find an efficient reprogramming system to understand the mechanisms of reprogramming. A major breakthrough in the reprogramming field was the introduction of a reprogrammable mouse model harbouring the transcription factors the Oct-4, Klf-4, Sox-2, and c-Myc (OKSM) cassette, under control of a doxycyline inducible promoter at the Col1α1 locus and expressing the m2 reverse transcriptional transactivator (rtTA) protein from the ubiquitously active ROSA26 locus. We previously have shown that mouse embryonic fibroblasts (MEFs) homozygous for both loci, reprogram at much higher efficiency compared to MEFs heterozygous for both loci. However it is still challenging to isolate sufficient numbers of reprogramming intermediates for mechanistic studies even from a double homozygous background. Recently, an improved, more active version of the M2 rtTA, the so called M3 rtTA has become available. We hypothesised that the efficiency of the OKSM mouse models with the M3 rtTA cassete would be superior compare with the traditional M2 version. Our results showed that OKSM MEFs harbouring the M3 rtTA reprogram more efficiently than the M2 rtTA without any changes to the reprogramming kinetics. Consequently, the OKSM mouse model with the M3 rtTA does permit isolation of the rare reprogramming intermediates at high numbers to study the underlying mechanism of iPS cells generation.

F-1239

DIRECT CONVERSION OF HUMAN EPIDERMAL STEM CELLS INTO FUNCTIONAL NEURAL CREST FATE UNDER DEFINED CONDITIONS

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During embryonic development, neural crest stem cells (NCSC) migrate laterally along the length of developing notochord and give rise to diverse cell types (e.g. peripheral neurons, Schwann cells, melanocytes and skeletal and connective tissue). In this work, we have shown, for the first time, that human epidermal stem cells (EpSC) derived from neonatal and adult skin can be coaxed to acquire functional NCSC fate under defined culture conditions, without any transgene overexpression. To this end, we isolated KRT14+ EpSC from both glabrous (lacking hair follicles) and non-glabrous skin and induced them into NC fate by treating them with cocktail of growth factors and chemicals. After induction, resulting EpSC derived NCSC-like cells (EpSC-NCSC) were found to be uniformly positive for NC genes i.e. SOX10, FOXD3, PAX3, KIT, NGFR, NES and lacked KRT14/KRT5 (basal EpSC markers) as evidenced by transcriptional and translational analysis. In addition, EpSC-NCSC induction was characterized by epithelial to mesenchymal transition (EMT) as confirmed by transcriptional and translational upregulation of key EMT genes SNAI1, SNAI2, TWIST, FOXC2, VIM, CDH2 and downregulation of CDH1. Illumina based RNA sequencing analysis showed that in contrast to EpSC, EpSC-NCSC possess global transcriptional profile similar to native NC. To confirm that only bonafide EpSC give rise to NCSC, we established clonal cultures of EpSC and induced into NC fate. Resulting clonal EpSC-NCSC expressed characteristic NC genes confirming clonal NC potential of EpSC. Under appropriate differentiation conditions, EpSC-NCSC differentiated and matured along functional neuronal, Schwann cell, melanocyte and mesenchymal (osteocytes, myocyte, chondrocytes, adipocytes) fate as confirmed by mRNA and protein analysis as well as functional tests for each lineage. Finally, we tested EpSC-NCSC ability to migrate and contribute into NC lineages in-vivo. Upon implantation into 10-13 somite stage chick embryos, EpSC-NCSC migrated extensively and differentiated into multiple NC derivatives including neurons, glia, myocytes and melanocytes. In summary, this work establishes NC plasticity of EpSC and identifies EpSC-NCSC as a novel multipotent stem cell source, which has implications for tissue engineering and regenerative medicine applications.

F-1240

INHIBITION OF MIR-145 IN PRIMARY HUMAN FIBROBLASTS FACILITATES REPROGRAMMING TO INDUCED PLURIPOTENT STEM CELLS

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Cellular reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) requires comprehensive rearrangement of cellular signalling pathways and molecular profiles, including the microRNA profile. MicroRNA (miRNA) molecules are short, non-coding RNA molecules (~22 nucleotides in length) that play crucial active role in many cellular processes by post-transcriptional regulation of specific messenger RNA (mRNA). Although some miRNAs have been shown to play an important role in various stages of reprogramming, our understanding of how miRNAs regulate this process remains elusive. We undertook a miRNA expression profile to identify candidate miRNAs whose expression is significantly changed during reprogramming of fibroblasts to iPSCs. This analysis indicated that miR-145 is highly expressed in human fibroblasts (hFs) and its expression is rapidly down-regulated at early stage of the reprogramming process to iPSCs. In order to study the role of miR-145 in detail, we inhibited miR-145 in hFs. Inhibition of miR-145 led to the induction of "cellular plasticity" demonstrated by: I) change of cellular morphology from long-spindled fibroblast shape to a short-spindle epithelial morphology; II) upregulation of pluripotency genes including KLF4, SOX2, c-MYC, III) down-regulation of miRNA Let-7b, and IV) induction of mesenchymal-to-epithelial transition (MET). Furthermore, inhibition of miR-145 led also to significantly higher reprogramming efficiency of hFs to iPSCs in the presence of reprogramming factors, indicating an important role of miR-145 in this process, moreover our data suggest a likely role for miR-145 in induction of MET, which represents a crucial initial step for successful reprogramming to iPSCs.

F-1241

DERIVATION AND CHARACTERIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FROM CLINICALLY DISCARDED CUMULUS CELLS

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Human cumulus cells are normally discarded in routine human intracytoplasmic sperm injection (ICSI) practices. These cells represent a valuable source of biological materials. While induced pluripotent stem cells (iPSCs) can be derived from a wide spectrum of cell types nowadays and cumulus cells were among the first cell type used for animal cloning and have been wildly used for somatic nuclear transfer (SCNT) based reprogramming studies, no group has reported derivation of human iPSCs using clinically discarded cumulus cells to date. In the present work, we obtained cumulus cells separated from human metaphase II oocytes that were used for ICSI in the human IVF clinic at Mackay Memorial Hospital (Taipei, Taiwan). The work is approved by Institutional Review Boards (IRB) at Mackay Memorial Hospital. Human cumulus cells were expanded and cultured. Cultured cumulus cells at P2 were induced into pluripotent stem cells by the lentiviral protocol of reprogramming factors (OCT4, SOX2, NANOG, and LIN28). The derivation efficiency is 0.04%. These cumulus cells derived iPSCs (hc-iPSCs) showed typical characteristics of pluripotency, such as alkaline phosphatase activity, expression of OCT4, SOX2, TRA-1-60 and SSEA4. In addition, MEG3 and MEG8, two imprinted genes whose expression levels positively correlate with full developmental potential, were highly expressed in the hc-iPSCs. Embryoid body (EB) assay showed that these hc-iPSCs are capable of differentiating into all three germ layers in vitro. The present work for the first time demonstrated that human cumulus cells, an otherwise discarded material in human IVF practices, can be efficiently induced to pluripotent stem cells, expanding the horizon of available biomaterials for regenerative medicine.

F-1242

REGENERATION OF INSULIN-PRODUCING BETA-CELLS BY ISLET CELL TYPE LINEAGE REPROGRAMMING IN DIFFERENT MOUSE MODELS OF EXPERIMENTAL DIABETES

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We previously described that massive loss of pancreatic islet insulin-producing β-cells results in the spontaneous engagement of a small fraction of α- and δ-cells, which normally express the hormones glucagon and somatostatin, respectively, into insulin production. These unexpected α-to-β and δ-to-β cell fate conversions account for the regeneration and diabetes recovery observed in transgenic mice bearing on their β-cells the human receptor for diphtheria toxin (DT), several months after diabetes induction with DT treatment. This islet cell plasticity was never reported before in other common experimental diabetes models, most likely because α- and δ-cells were never lineage-traced in these situations. Two drugs, streptozotocin (STZ) and alloxan, are glucose analogues efficiently entering β-cells, hepatocytes and kidney cells through the Glut2-type glucose transporter. Both compounds trigger diabetes because they destroy on average 70% of the β-cell mass: STZ is an alkylating agent inducing DNA breaks, and alloxan generates ROS free radicals. Here, we investigated the ability of the adult pancreas to regenerate β-cells in these two models of drug-induced diabetes after partial β-cell loss, through α- and δ-cell reprogramming. We combined STZ or alloxan treatment with fate tracking of α- and δ-cells. One month after incomplete β-cell destruction we found that some α-cells expressed β-cell-specific transcription factors, and a fraction of them also expressed insulin. α-cell conversion was noticed thanks to the genetic labeling of reprogrammed α-cells, half of which maintained glucagon expression. This could explain why α-to-β conversion has been unappreciated in these models. The extent of α-to-β and δ-to-β cell conversion in STZ- and alloxan-treated mice was slightly lower than that observed after near-total β-cell loss (99%) in the DT model, thus confirming that the severity of β-cell ablation determines whether α- and δ-cells do reprogram to produce insulin. Interestingly, we observed that the proportion of α- and δ-cells that started making insulin after partial β-cell injury was increased upon intra-islet insulin signaling blockade. Conversely, insulin administration was sufficient to completely abolish the reprogramming ability of these islet non-β-cells.

F-1243

LOGICAL MODELLING OF IMMUNE CELL SPECIFICATION AND REPROGRAMMING

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Blood cells arise from a common set of hematopoietic stem cells that differentiate into more specific progenitors, ultimately leading to different functional lineages. This process relies on the activation and repression of different genes modules, controlled by transcription factors (TFs). Novel high-throughput technologies allow the characterisation of cell-specific regulatory elements by studying chromatin state and TFs binding sites, in conjunction with gene expression. Proper integration and analysis of these data enable the delineation of novel regulatory interactions, which can be modelled and analysed using formal methods, thereby fostering our understanding of the mechanisms controlling cell fate at a system level, and enabling the prediction of the effects of molecular perturbations in silico.

To reconstruct the regulatory network controlling hematopoietic specification, we combined information extracted from the literature with data from ChIP-seq experiments targeting TFs involved in myeloid and lymphoid specification. Additionally, we used histone modifications ChIP-seq data (H3K4me1/2/3, K3K27ac, H3K27me3) to identify others regulatory elements, along with sequence analysis (motif discovery and pattern matching) to predict TF recruitments. These experiments were performed at different stages of hematopoietic development (stem cells, restricted progenitors and differentiated cells), and during reprogramming of B lymphocytes into macrophage. Using a multilevel logical framework, we built a dynamical model of the resulting network and fitted it to gene expression data. Dynamical simulations of this model further enabled

us to predict the effect of perturbations of specific regulatory components or interactions (gain/loss-of-function, mutation of binding sites) at specific stages of development. These prediction are currently being assessed experimentally. This study has already contributed to the identification of novel regulatory interactions, and ongoing experiments should further help us to refine our model. This will ultimately allow us to generate more precise predictions, in particular regarding efficient cell reprogramming protocols.

F-1244

MIRNA-FACILITATED SELF-REPLICATIVE RNA (SRRNA) REPROGRAMMING OF ENDOTHELIAL PROGENITOR CELLS (EPCS) DERIVED FROM HUMAN PERIPHERAL BLOOD AND CORD BLOOD

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In 2010, it was first published that repeated transfection of fibroblasts with a cocktail of reprogramming mRNAs resulted in the generation of stable, integration-free human iPS cells. While many advancements have been made to refine this process on fibroblasts, to date no group has been able to apply this same technology to reprogram 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 both fresh and frozen human peripheral blood and cord blood. The EPCs adherent nature and high proliferative capacity while maintaining their cell identity makes them highly desirable for transfection with RNA. 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 blood derived EPCs, cord blood derived EPCs, as well as adult fibroblasts. The incorporation of reprogramming associated microRNAs into a two transfection, no-split protocol on extracellular matrix without the need for conditioned medium has resulted in a simple, reproducible and robust reprogramming protocol applicable to multiple target cell types. Integration-free EPC-derived iPS cells exhibit unique genetic stability, making them an exceptional choice for applications requiring clinical grade cells. Lastly, those clinical grade EPC-iPS cells generated using this novel srRNA reprogramming technology present a therapeutic opportunity to treat myeloproliferative disorders in which the disease-causing somatic mutations are restricted to cells in the hematopoietic lineage. Here we present data demonstrating the unique combined application of microRNA and srRNA for the cellular reprogramming of human EPC lines derived from peripheral blood and cord blood as well as adult fibroblasts into stable, pluripotent and clinically relevant iPS cells.

F-1245

DIRECT REPROGRAMMING OF HUMAN FIBROBLASTS TO NEURAL STEM CELLS BY SMALL MOLECULES

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Here we report a safer and more efficient method to induce human fibroblasts into neural stem cells using only small molecules. The small molecule-induced neural stem cells closely resemble neural stem cells, in morphology, gene expression patterns, self-renewal and multipotency. Furthermore, the SMINS cells are able to differentiate into astrocytes, functional neurons and oligodendrocytes in vitro and in vivo. Thus, we have established a novel and safe way to efficiently induce neural stem cells from human fibroblasts using only small molecules. Such chemical induction removes the risks associated with current techniques such as the use of viral vectors or the induction of oncogenic factors. This technique may therefore enable NS cells to be utilized in various applications within clinical medicine.

F-1246

MODELING ALS USING PATIENT-DERIVED INDUCED ASTROCYTES AND MOTOR NEURONS

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Astrocyte dysfunction during development has profound non-cell autonomous effects on the surrounding tissues in brain, chiefly neurons, leading to disease pathology. This is critical for understanding a range of neurodevelopmental diseases which includes neuropsychiatric ailments and neurodegenerative diseases were notable astrocytic defects has been detected. Our lab is studying the role of astrocytes in amyotrophic lateral sclerosis (ALS), a neurodegenerative disease affecting motor cortex and spinal cord motor neurons. Expanded GGGGCC repeat in intronic region of the gene C9orf72 is the most common genetic cause of ALS. We have made motor neurons from C9orf72 ALS patients by forced expression of transcription factors (TFs), an established protocol in our lab. Preliminary data shows that the C9orf72 ALS patient-derived iMNs successfully recapitulated the severe survival defects of this form of the disease in vitro. We used automated imaging to measure survival of iMNs from 3 C9orf72 ALS patients and 3 healthy controls. We found that iMNs from all 3 C9orf72 patients consistently degenerated faster than control iMNs after withdrawal of neurotrophic factors ($p<0.001$). We further generated iPSC lines from one of the C9orf72 patients and used CRISPR-Cas9 genome-editing to specifically correct the repeat expansion to the normal length (2 repeats). This completely restored survival in these iMNs to healthy control level, demonstrating that the neurodegeneration witnessed in vitro is specifically due to the C9orf72 repeat expansion. It has already been shown that astrocytes seem to mediate non-cell autonomous toxicity to surrounding motor neurons in familial cases of ALS harboring SOD1 mutation. There is a need for a feasible and efficient process to generate astrocytes for developing a 'disease-on-a-chip' model with patient-specific neurons

and astrocytes in high-throughput from other familial and sporadic forms of ALS mutations. We have now developed a TF-mediated direct reprogramming strategy to make induced astrocytes (iAs) from mouse and human fibroblasts. We have used this TF cocktail to successfully generate C9orf72 ALS patient-derived iAs. We intend to use this to analyze toxicity and survival of large cohorts of patient-derived iMNs-iAs in vitro to model disease susceptibility and drug efficacy.

F-1247

DIRECT CONVERSION OF CORD MATRIX DERIVED MESENCHYMAL STROMAL CELLS TO ENDOTHELIAL CELLS BY NON-ONCOGENIC TRANSCRIPTION FACTORS

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Mesenchymal stromal/stem cells derived from human cord matrix, Wharton's jelly (WJ-MSCs) have emerged as a favorable source for autologous and allogenic cell therapy, because these cells were young and less immunogenic. The generation of endothelial cells (ECs) is necessary to transplant ECs for ischemic diseases and provide vascular structure to regenerated organs in tissue engineering. Here, we attempted to directly convert WJ-MSCs to ECs by introducing non-oncogenic transcription factors. We selected transcription factors that are related to vascular endothelial development, and examined their expression levels in WJ-MSCs. We selected two transcription factors that were not expressed or expressed at low levels, and further selected two non-oncogenic transcription factors Oct4 and Nanog that are related to reprogramming. WJ-MSCs were transduced with lentiviral vectors carrying Oct4, Nanog, Tal1, or LMO2 gene, incubated under antibiotic selection pressure, and induced under endothelial induction condition for 2-4 weeks. CD31 and CD144 positive cells (2% ~ 7%) were isolated by FACS and the purified induced ECs (iECs) were expanded in EC growth media. The iECs formed a typical cobblestone monolayer and continued to express endothelial makers (CD31, VE-cadherin and VEGFR2). These iECs also showed uptake of acetylated LDL and formation of networks of tubular structures on Matrigel. These results indicate that WJ-MSCs can be converted to ECs by these four transcription factors

F-1248

ESRP1 AS A POSITIVE REGULATOR FOR PLURIPOTENCY MAINTENANCE AND REGAINING

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Alternative splicing is a key mechanism for molecular repertoire diversity and represents a widely acting mode of gene regulation. However, its role in regulating pluripotency and differentiation is largely unknown. Here, we showed the role of ESRP1 (epithelial splicing regulatory protein 1) in the pluripotency control as a positive regulator. We found the enriched expression of ESRP1 mRNAs in pluripotent embryonic stem cells (ESCs), and their expression was conversely down-regulated upon differentiation. Five splice variants of ESRP1 were detected in undifferentiated ESCs and their expression levels were decreased upon differentiation. During human somatic cell reprogramming, forced expression of those ESRP1 variants promote human induced pluripotent stem cell (hiPSC) generation under virus free reprogramming condition. Conversely, ESRP1 shRNA treatment blocked the hiPSC generation. We also determined ESRP1 impact on mesenchymal-epithelial transition (MET) and confirmed down-regulation of the expression of MET related genes. Our results suggest that ESRP1 has a positive role in the maintenance and acquisition of pluripotency, and its functional roles may relate to the suppression of MET related gene expression.

F-1249

DYNAMIC TRANSCRIPTOMIC AND EPIGENETIC CHANGES THAT DICTATE HEPATIC SPECIFICATION OF DONOR BONE MARROW PROGENITOR CELLS DURING LIVER REGENERATION IN MICE

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The strictly regulated unidirectional differentiation program in somatic cells has been shown to be modified in regenerating conditions where the lineage barrier can be overcome by either direct differentiation or heterotypic cell fusion. Here we have examined the molecular changes during reprogramming of bone marrow progenitor cells (Lin^- BMCs) to hepatocyte-like cells during liver regeneration. Lin^- BMCs from C57BL6J-GFP (syngeneic) female mice were transplanted in FVIII knockout male mice after inducing liver injury with acetaminophen. After 4 months of transplantation we identified donor bone marrow-derived (BMD) hepatocytes in the liver parenchyma which expressed hepatic markers. Donor-derived eGFP-expressing hepatic cells were isolated by flow cytometry from crude single cell preparation of hepatocytes. XY-interphase FISH and real time PCR-based absolute quantification of SRY gene indicated fusion in only 1.8% of the BMD hepatocytes. Genome-wide expression analysis with BMD hepatocytes isolated after 1 month and 5 months of transplantation showed induction of hepatic transcriptional program and decline in donor signatures over time. However they also exhibited some dissimilarity in expression profile with respect to primary hepatocytes and retained expression of a few donor hematopoietic genes. We further investigated the epigenetic mechanisms that co-ordinate these transcriptional changes. Enrichment of activating histone marks (H3K4me3and H3K9Ac) were found at promoters of crucial hepatic transcription factors whereas repressive marks (H3K27me3 and H3K9me3) were lost from these loci after reprogramming, among which loss of H3K27me3 mark was most striking. The loss of H3K27me3 mark in BMD hepatocytes was found to be mediated by the binding of JMJD3 at these loci along with loss of EZH2, both of which are chromatin modifying enzymes that antagonistically regulate the methylation status of H3K27. Bisulfite sequencing analysis revealed that DNA at promoter regions of HNF1α was hypomethylated whereas that of GATA2 was hypermethylated in BMD hepatocytes as compared to Lin^- BMCs. In summary, regulation of epigenetic marks by chromatin modifying enzymes orchestrate stable transcriptional reprogramming during differentiation of BM progenitor cells to hepatocyte-like cells.

F-1250

REPROGRAMMING OF HUMAN FIBROBLASTS TO ECTODERMAL AND MESODERMAL PRECURSOR CELLS WITH PLANT STEM CELL EXTRACT

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We have previously induced pluripotent stem cells by delivering embryonic stem cell-derived proteins into adult mouse fibroblast. However, it was not optimized in human because difficulty to prepare proper amount of human ES extracts. To overcome this problem, we hypothesize whether plant stem cell-derived proteins could reprogram human fibroblasts. The plant callus, a dedifferentiated plant cell mass, can regenerate itself and differentiate into many tissues of whole plant body. In this study, based on the dedifferentiation feature of plant callus, we observed the reprogramming activities of plant callus extract on human skin and dermal fibroblast. Here, we demonstrate that major components of plant callus, reprogrammed somatic fibroblast to ectodermal and mesodermal precursor cells. These cells grew as spheres and expressed stem cell specific genes including Sox2 through demethylation of its promoter. And they showed endodermal and mesodermal differentiation potential. Furthermore, we elucidated that they inhibited cellular senescence by decreasing SA-β-galactosidase expression and elongating telomere length mediated through the expression TERT. And they assesse the tissue remodeling activities through increasing type1 procollagen and decreasing MMP-1. Resultantly, the restoration of skin equivalent tissue was accelerated after CO2-laser puncturing. These newly investigated results indicated that the SG plant callus could be means for improving tissue regeneration and prevent aging.

F-1251

LARGE SCALE GENERATION OF HUMAN IN WITH TWO IN ONE VECTOR SYSTEM

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Human fibroblast can be directly reprogrammed to induced neurons (iNs) using lineage specific transcription factors. However, since the resulting cells are post mitotic, a limited number of iNs are available from each conversion, which could limit their use in biomedical applications and regenerative medicine. In this study we have designed a dual promoter system that allows for the expression of two reprogramming factors from the same vector. The resulting iNs exhibit functional properties of neurons in vitro and in slice cultures. The two-factor iN cells survive transplantation to the adult rat brain better than iNs what have been generated using multiple single-gene vectors. Interestingly, neural conversion of human fibroblasts using the dual promotor vector only takes place when the cells are cultured in neuronal medium, which allows for passaging, expansion and banking of transduced cells. Cells up to passage three can readily be converted to neurons by a simple medium switch, albeit at lower efficiency compared to when fibroblasts are switched to neuronal medium at the same time as vectors are delivered. We report that by changing the order of transcription factors in the constructs, conversion efficiency was greatly alternated. When studying this phenomenon we realized that the orientation of factors has a great impact on the ratio of protein expression and that stoichiometry of conversion factors thus have an important impact on reprogramming efficiency. The high conversion efficiency, in combination with the possibility to passage and bank cells expressing the dual promoter construct, points to the possibility that human iNs can be used for large scale studies such as drug screening and disease modeling.

F-1252

DIRECT CONVERSION OF MOUSE AND INDUCED HUMAN FIBROBLASTS TO NICOTINE RESPONSIVE NEURONS

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Nicotine addiction - despite the existence of variety of smoking cessation therapies - remains one of the leading causes of death worldwide. The point of access nicotine has to our nervous system is a class of cationic channels defined as nicotinic acetylcholine receptors (nAchRs). The regional distribution profile of nAchR subtypes in the CNS is distinct, where unique combinations of nine α (α2- α10) and three β (β2- β4) subunits label defined populations of neurons. Among these subunits, GWAS studies complemented by mouse behavioral studies suggest that predisposition to nicotine addiction is most strongly correlated to genetic variations at a loci that includes three nAchR subunits (α3, β4 and α5). In the CNS, these subunits are highly expressed in the medial habenula (mH) and co-expression of nAChR α3 and α4 is nearly exclusive to a subset of mH neurons. However, the regional complexity of the mouse medial habenula coupled with the lack of accessibility to human neuronal cells has hindered efforts to delineate the contribution of these cells to addiction. Being able to generate this particular habenular subtype in a dish for both mouse and human may offer a window into more accurately understanding the cellular mechanisms that influence predisposition to nicotine addiction. Here we show that a novel combination of virally transduced transcription factors can directly reprogram mouse embryonic fibroblasts (MEFs) into nicotine-responsive induced neurons that express the relevant nAChRα3, β4 and α5 genes. Further characterization of induced human neurons generated from iPSCs derived from different individuals using the same transcription factor code will be presented. Through comparison with both endogenous and induced neuronal mouse counterparts, we hope to will establish a new method to produce a specific human neuronal subtype that bear important consequence for human health.

F-1253

DNMT3L-KNOCKOUT DONOR CELLS IMPROVE SOMATIC CELL NUCLER TRANSFER REPROGRAMMING EFFICIENCY

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Nuclear transfer is a technique to investigate the development and reprogramming potential of a single cell. DNA methyltransferase-3-like (DNMT3L), which has been characterized as a transcriptionally repressive regulator, is expressed in naturally fertilized eggs and blastocysts at the pre-implantation stage. In this study, we demonstrated that Dnmt3l-knockout donor cells synergize with Trichostatin A to improve the developmental efficiency and quality of cloned embryos. Compared with wild-type group, Dnmt3l-knockout donor cell-derived cloned embryos exhibited increased cell numbers associated with restricted OCT4 expression in the inner cell mass and proper silencing of transposable elements at the blastocyst stage. However, our results indicated that zygotic Dnmt3l was dispensable for cloned embryo development and retrotransposon silencing at the pre-implantation stage. Reduced nuclear localization of HDAC1 and decreased accumulation of the repressive histone modification mark H3K9me3 in the Dnmt3l-KO donor cells may be responsible for improved SCNT reprogramming efficiency. As Dnmt3l-KO cells cannot be reprogrammed properly through induced pluripotent stem cell approach due to inappropriate retrotransposon silencing, the successful generations of cloned blastocysts using Dnmt3l-KO donor cells suggest that nuclear transfer followed by embryonic stem cell derivation may be a potential strategy to generate human DNMT3L-deficiency cell line for studying DNMT3L-assoicated disease and pharmaceutical development.

F-1254

FATTY ACID METABOLISM ASSOCIATED SMALL MOLECULAR COMPOUNDS ENHANCE REPROGRAMMING EFFICIENCY VIA HISTONE ACETYLATION

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Despite several advances in the field of induced pluripotent stem cells (iPSCs), reprogramming safety and efficiency are still need improve. Modification of the metabolic pathways is an important step in the reprogramming process. Upregulation of glycolysis can promote induction of iPSCs. However, the roles of other metabolic pathways in the reprogramming process are unclear. Comparison of gene expression profiles of somatic cells, reprogrammed intermediate phase cells, and iPSCs showed that Cpt1b, a rate-limiting enzyme in fatty acid metabolism, is upregulated during the reprogramming process. Furthermore, overexpression of Cpt1b or addition of palmitoylcarnitine and acetyl-CoA, the primary and final products, respectively, of Cpt1b-mediated fatty acid oxidation, increases reprogramming efficiency. This study showed that small molecular compounds can increase reprogramming efficiency by regulating fatty acid metabolism. Furthermore, the study showed that the main downstream pathways of these compounds include upregulation of histone acetylation.

F-1255

ACTIN REGULATES CELL REPROGRAMMING

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Induction of pluripotency is normally achieved through transgene expression, while in some reports partial induction is obtained by extracellular stimuli, of which signals, though largely unknown, are presumably transduced in part through cytoskeleton. Here we show that β–actin dynamics regulates induction of pluripotency. Using shRNA screening in neural progenitors, we found that induced pluripotent stem cell (iPSC) induction was prominently enhanced by repression of *Actb* (encoding β-actin). *Actb* repression downregulated cell type-specific genes whereas upregulated targets of the serum response factor (Srf). Manipulation of actin polymerizing/depolymerizing dynamics indicated that *Actb* repression mimicked actin polymerization (i.e. reduction of G-actin) which leads to Srf activation. Overexpression and repression of Srf enhanced and suppressed iPSC induction, respectively, through regulation of cell type-specific genes. Additionally, in Srf-knockout neural progenitors, *Actb* repression still enhanced iPSC induction through downregulation of cell type-specific genes, indicating that an Srf-independent pathway(s) also regulates reprogramming. Moreover, enhanced iPSC induction by *Actb* repression was also observed in another cell type, hepatoblasts, suggesting generality of its function. In conclusion, in each cell type, induction of pluripotency is suppressed by cell type-specific genes that are maintained in part by β–actin dynamics through multiple pathways including Srf. Extracellular stimuli that disrupt its homeostasis will thus result in accelerated dedifferentiation. Our data contribute to understand cell fate determination and to efficiently regulate it in vitro and possibly in vivo.

F-1256

NANOG, OCT4 AND TET1 INTERPLAY IN ESTABLISHING PLURIPOTENCY

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The core circuitry of the master transcription factors inside embryonic stem (ES) cells and induced pluripotent stem (iPS) cells is thought to control the cells' pluripotency. Several molecular characterizations of the ES and iPS cells' pluripotent state were put forward both on the transcription factor and epigenetic levels. Whereas the core players have been identified, it is desirable to map out gene regulatory networks that describe the molecular dynamics, which govern the reprogramming of somatic cells into iPS cells and the ES and iPS cells' decisions to self-renew or commit towards various lineages. Towards this goal, we propose a computational model where Oct4, Nanog and Tet1 genes regulatory network is dominated by multiple positive feedback loops. We put forward a mechanistic understanding of the molecular dynamics which account for i) Oct4 overexpression is sufficient to induce pluripotency in somatic cell types expressing the other Yamanka reprogramming factors endogenously; ii) Tet1 can replace Oct4 in reprogramming cocktail; iii) Nanog is not necessary for reprogramming however its over-expression leads to enhanced self-renewal; iv) DNA methylation is the key to the regulation of pluripotency genes.

F-1257

SMALL MOLECULES INCREASE DIRECT NEURAL CONVERSION OF HUMAN FIBROBLASTS AND PROVIDE MECHANISTIC INSIGHT INTO THE REPROGRAMMING PROCESS

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The ability to generate functional induced neurons represents a novel technique with great potential for brain repair, disease modeling as well as drug screening. By screening a series of annotated compound libraries, we have identified 6 small molecules that greatly enhance direct neuronal conversion of human fibroblasts. When these compounds are added during the initial conversion phase, conversion efficiency of >600% and neuronal purity of >50% are reached. In order to gain mechanistic insight on how these small molecules promote direct neuronal conversion, transcriptional profiles of induced neurons treated with these small molecules alone or in combination will be obtained and analyzed using the newly established platform CellNet. This will furthermore allow for a better understanding of the signaling pathways crucial during the early stages of direct neuronal lineage reprogramming of human cells. Taken together, this work presents several compounds that dramatically increase conversion efficiency, and provides insight into the mechanism of direct neuronal conversion.

F-1258

REPROGRAMMING OF HUMAN MULTIPOTENT ADULT PROGENITORS INTO DEFINITIVE ENDODERM PROGENITORS BY DEFINED TRANSCRIPTION FACTORS

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Large-scale application of islet transplantation is hampered by immunological challenges as well as by donor shortage. An alternative source for human cadaveric islets is generating insulin-producing β cells from stem cells. A challenge in this area is to find adequate stem or progenitor cells and to identify the mechanisms by which functional insulin-producing β cells can be produced from such stem / progenitor cells. The development of techniques to reprogram somatic cells to stem cells, specifically the description of induced pluripotent stem cells, has provided the basis for our hypothesis that, using defined transcription factors (TFs), it should be possible to reprogram human bone marrow (BM) Multipotent Adult Progenitors (MAPC) into endoderm progenitors. To test this hypothesis we selected 16 transcription factors, known to play a key role during (pancreatic) endoderm development combined with transcription factors expressed in pluripotent stem cells. Here we demonstrated that endodermal progenitors (termed iEndo) can be generated from human BM derived MAPC cells by transduction of 16TFs and culture in endoderm conditions. Following transduction and culture in endoderm conditions, hMAPC underwent mesenchyme to epithelium transition (MET), forming cluster of epithelioid cells that express endodermal marker genes as demonstrated by qRT-PCR and FACS. By selectively removing 1 or more TFs from the combination of 16 TFs, we have shown that at least 2 TFs are essential while the other 14 may not be essential, for induction of MET, or induction of endogenous endodermal marker expression. Based on these studies, we are currently transducing MAPCs with a cocktail of 6 TFs to induce iEndo cells that are not yet committed to more mature endodermal cells. We will then differentiate iEndo cells to pancreatic β cells, those function will be tested in vitro and in vivo. Our results provide a novel approach for generating insulin producing β cells for cell-based therapy.

F-1259

CD24, A NOVEL MARKER FOR DIVERGENT PLURIPOTENT STATES

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Reprogramming is a dynamic process that can result in multiple pluripotent cell types emerging from divergent paths. Cell surface protein expression is a particularly desirable tool to categorize reprogramming and pluripotency as it enables robust quantification and enrichment of live cells. As part of the international Project Grandiose initiative, we used cell surface proteomics to interrogate reprogramming dynamics and discovered CD24 as a novel marker that tracks transgene expression levels during early reprogramming. Furthermore, CD24 enables the analysis and enrichment of transgene-dependent and -independent cells at later stages of reprogramming. Finally, CD24 can be used to delineate naïve and primed pluripotent states in the mouse system: EpiSCs and ESCs. Importantly, regulated CD24 expression is conserved in human pluripotent stem cells, tracking the conversion of human ESCs to more naïve-like pluripotent states and enabling enrichment for naive-like human ESCs following induction with recently reported strategies. Overall, CD24 is a tool for benchmarking reprogramming systems by providing a means of delineating transgene-dependent and -independent states while also separating different states in pluripotent culture, thus providing mechanistic insight into cell fate transitions in transient (reprogramming) and stable (pluripotent) culture.

F-1260

IDENTIFICATION OF WHSC1 AS AN EPIGENETIC REGULATOR IN B CELL TRANSDIFFERENTIATION AND EMBRYONIC STEM CELL DIFFERENTIATION

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Accumulated evidence has shown that differentiated cells are remarkably plastic and can be converted from one cell type to another. Although epigenetic changes are known to be crucial for cell fate conversions, much remains to be learned about epigenetic regulators that play a role in these processes. Our group has described the conversion of mouse and human pre-B/B cells into macrophages following forced expression of CCAAT/enhancer binding protein-α (C/EBPα); these fast and highly efficient models give us a unique opportunity for identifying epigenetic facilitators and barriers during transdifferentiation. We compiled a list of 633 potential epigenetic regulators and found that the expression of 78 of them decreases during mouse and human B to M transdifferentiation, suggesting that they act as potential cell fate conversion barriers. We also performed an shRNA screen of 464 chromatin related proteins during human B to M transdifferentiation. One downregulated gene, namely Wolf-Hirschhorn Syndrome Candidate 1(WHSC1, NSD2 or MMSET), was also among the top hits in the shRNA screen. Depletion of this gene, which encodes a histone-lysine N-methyltransferase is able to reproducibly accelerate B to M transdifferentiation of human and mouse B cells. In addition, we addressed the role of WHSC1 during differentiation of mouse embryonic stem cells (ESCs) into embryoid bodies. Surprisingly, WHSC1 deficiency in ESCs was found to impair their differentiation under permissive culture conditions and to increase their pluripotency. Performing in vivo and in vitro differentiation assays we found that WHSC1 is necessary specifically for the commitment of cells from the endodermal and mesodermal but not ectodermal lineages. We are now focusing on possible targets within the mesodermal and endodermal lineages regulated by WHSC1.

F-1261

DONOR-DEPENDENT CHARACTERISTICS OVERRIDE THE PARENTAL CELL EPIGENETIC FEATURES IN ISOGENIC IPSC LINES

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Epigenetic memory from the cell of origin is a potential problem for the pluripotent differentiation capacity of induced pluripotent stem (iPSC). In this study we addressed the issue of optimal donor cell-type for generation of iPSC biobanks for disease modelling. We compared transcriptomic, epigenetic, and in vitro differentiation propensities of isogenic human iPSC derived from fibroblasts (F-iPSC) and peripheral blood mononuclear cells (B-iPSC) of several donors. Isogenic iPSC lines had highly similar transcriptional and global DNA methylation profiles and most importantly, very similar differentiation potential despite of different tissue of origin. However, marked differences were identified in the transcriptional and DNA methylation profiles as well as in hematopoietic differentiation potential between iPSC lines derived from different donors. These interindividual differences were enriched in expression of genes controlling early embryonic developmental programs. We conclude that donor-specific genetic differences are the main determinants of iPSC differentiation propensity, overriding the epigenetic memory of the parental cell type. Based on this, we suggest that iPSC biobanks can be established by combining good quality iPSC lines from variable tissues. In order to observe the impact of specific genetic variants, biobanks should rather involve iPSC lines from a large number of donors than several isogenic clones from a limited number of donors.

F-1262

EFFICIENT REPROGRAMMING OF CENTENARIANS SOMATIC CELLS, PERSONALIZED MEDICINE IN GERIATRIC MEDICINE

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Since 2006, following successful reprogramming of somatic cells to pluripotency, induced pluripotent stem (iPS) cell has become a valuable tool for regenerative medicine, disease modeling, drug discovery and basic research in biology. The need for regenerative medicine and cellular therapies is perhaps greatest in the geriatric population. To address this issue, patient-specific iPS cells and ultimately organs could be required. However, reprogramming somatic cells from geriatric patients faces additional hurdles not encountered with the younger cells normally used for such studies. We report methods for efficient reprogramming of dermal fibroblasts isolated from subjects 104-109 years of age. Standard conditions were those recommended by the manufacturer for the CytoTune reprogramming Kit that uses three Sendai virus (SeV) vectors for delivering and expressing four reprogramming factors, OCT4, SOX2, KLF4, and c-MYC. Fibroblasts from three centenarians and cells from neonatal foreskin (HFF) and young adult dermal fibroblasts (HDF) served as controls. Preliminary transduction experiments with SeV-based GFP vector showed all cells are amenable to SeV transduction, however the percentage of cells expressing GFP in centenarian fibroblasts were significantly lower than control cells. The percent of GFP positive cells 48 hours after transduction was 14-26% for HFF and 10-14% for HDF. In the same experiment (MOI=3), the results for centenarian fibroblasts are 3-7%. Culture conditions for viral exposure were altered in attempts to improve vector transduction. Maintaining the same MOI per cell, the volume of media used for exposure was reduced from 300μL to 200μL and hydrodynamic pressure was applied by centrifuging culture plates at 300 g for 15 minutes to facilitate viral integration. These combined procedures significantly increased viral transduction and consequently GFP expression 19.02 and 27.19 fold in HFF and HDF cells respectively. A full reprogramming protocol was applied to all cell types. While the standard procedure recommended by

the manufacturer efficiently reprogrammed fibroblasts from young individuals, no reprogrammed cells were observed in cultures of centenarian fibroblasts. However, the use of the optimized protocol resulted in efficient reprogramming of centenarian cells.

F-1263

GREATER CHROMOSOMAL STABILITY IN NEURAL CELLS DERIVED BY TRANSDIFFERENTIATION THAN THOSE DERIVED FROM STEM CELLS

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Differentiation into neurons can be achieved from either pluripotent stem cells or adult stem cells, but also by direct cellular reprogramming mediated by tissue master regulators. Here, we have compared the chromosomal aberrations in neural cells differentiated from the different sources. In our analysis, we have examined the chromosomal integrity of over 550 samples of neural cells from 84 studies, by examining microarray datasets using e-karyotyping methodology. Chromosomal aberrations were documented in about half of the studies with human or mouse pluripotent stem cells, and in about third of the studies with adult stem cells. Interestingly, analyzing neural cells derived by direct reprogramming showed much lower incidence of aberrations, where only one out of 7 or 9 studies in either human or mouse showed any chromosomal aberrations. Among the chromosomal aberrations detected in the neural cells differentiated from pluripotent cells were those known to be selected during derivation and culture adaptation of pluripotent cells. However, we also detected distinct recurrent aberrations positively selected during differentiation of neural cells, either from pluripotent or adult stem cells. Amazingly, aberrations detected in the direct reprogramming samples also included the signature chromosomal aberrations of the pluripotent state. Careful analysis of the direct reprogramming methodologies in these cases revealed that in addition to brain specific transcription factors, pluripotent genes were also utilized, suggesting that the cells dedifferentiated into a pluripotent stage prior to their final neural differentiation. Recently, we have adopted the e-karyotyping methodology for RNA-sequencing data. In this platform, we could also detect neural samples with chromosomal aberration derived from their pluripotent origin. Overall, our analysis revealed surprising high levels of genomic instability in neuronal cells, with the exception of those differentiated by direct reprogramming. Our analysis sheds light on the mechanism of direct reprogramming, and thus to the potential value of trans-differentiated cells in future regenerative medicine. The initial part of the work was recently published in Cell Stem Cell.

F-1264

MAGNETIG BEAD SORTING OF GLYCAN MARKER POSITIVE REPROGRAMMED CELLS FOR IMPROVED GENERATION OF IPS CELL LINES FROM HUMAN DONORS

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More effective, robust and affordable methods are needed to facilitate large-scale generation of iPS cell lines for e.g. biobanking. We have developed a robust technique for isolation of iPS cells and generation of new iPS cell lines using surface marker based isolation of reprogrammed cells by magnetic bead sorting. We have previously identified type 1 Lewis glycans as novel markers of pluripotent stem cells. Live cell imaging and fluorescence-assisted cell sorting with anti-type 1 Lewis glycan antibodies during reprogramming of human fibroblasts showed that marker-positive cells emerge early in the process and can be identified and isolated based on their surface marker expression even before they start to form the characteristic stem cell colonies. Based on this knowledge, we started developing a method for efficient isolation of new iPS cell lines using magnetic bead isolation of the reprogrammed cells. Using this method, the newly formed pluripotent cells could be purified from the contaminating cells in one magnetic bead isolation step. Subsequent expansion of the cells led to a pure iPS cell line in drastically reduced time compared to the traditional approach of cell line maturation by repeated passaging and culturing. After 2-3 passages, there was enough cell mass for both cell line banking and characterization. In addition to reduced time from somatic cell sample to iPS cell line (down to 4-5 weeks compared to at least 10 weeks without sorting), the benefits of the method included lowered needs for sample cells, transfection vectors, media, plates, coating substrate, incubator space, and expert time for passaging the cells. In conclusion, using magnetic beads coupled with the glycan marker antibodies significantly reduce the time and cost required for iPS cell line generation.

IPS CELLS

F-1265

COMPARISON BETWEEN FEEDER FREE AND FEEDER DEPENDENT SYSTEMS FOR LARGE SCALE PRODUCTION OF HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS)

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Human induced pluripotent stem cells (hiPSCs) are ever developing tool for disease modelling and drug discovery. The aim of the HipSci project is to derive hiPSCs from 1000 donors (500 normal and 500 rare genetic disease samples) to compare inter and intra donor variability between the lines and to offer a highly characterised hiPSCs bank for research purposes. To achieve this goal, high throughput generation of good quality hiPSCs is vital. In this study, we have compared feeder dependent and feeder free approaches. Primary fibroblast lines were derived from dissected 2mm human skin biopsies and successfully reprogrammed with Sendai virus encoding Oct3/4, Sox2, Klf4, and C-myc to produce iPSC colonies. The feeder free system comprised of Essential 8 (E8) medium and Vitronectin coated plates, whereas feeder dependent system consisted of co-culture of iPSCs with mouse embryonic fibroblasts (MEFs) and DMEM F12 medium with 20% knockout serum replacement (KSR). Subsequently, hiPSCs lines were cultured in parallel in both conditions for comparison. Established hiPSCs in each system were expanded for quality control experiments such as expression of pluripotency markers, directed differentiation into

three germ layers, genomic stability (genotyping and karyotyping), expression microarrays for stem cells markers, freezing capacity and recovery of cells post thaw. The hiPSCs cultured in feeder free system were passaged and cryopreserved at up to 1:6 split ratios. In contrast, the hiPSCs cultured in the feeder dependent system were passaged up to 1:3 split ratios and cryopreserved at 1:1 split ratio. The hiPSCs cultured in the feeder free system grew faster than their corresponding feeder dependent equivalent and also showed better recovery post thaw. Genomic stability was unaffected by culturing cells in the absence of feeders. Finally, whereas the feeder dependent system contains multiple undefined components, the feeder free system has the desirable property of being completely chemically well defined. Overall, the feeder free system appeared advantageous and is here demonstrated to be more amenable for large scale hiPSCs production.

F-1266

GENERATION OF EDITED INDUCED PLURIPOTENT STEM CELLS AS CELL MODELS

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The ability to generate patient-specific induced pluripotent stem cells (iPSCs) offers a powerful platform for the generation of physiologically relevant cell models for dissecting basic biology and for use in drug screening. A critical requirement for such models will be generation of homogeneous cell populations that can be characterized and confirmed to be the cell type of interest prior to use in downstream studies. Reporters driven by lineage-specific promoters are powerful tools to identify and enrich specific cell types in a heterogeneous mixture of cells. In addition, they offer the advantage of enabling live monitoring of cells during complex biological processes. We have generated stable human embryonic stem cell (ESC) lines using non-integrational episomal vectors or site-specific insertion of the reporters into safe-harbor genomic sites. Both methods achieve context-specific expression with measurable GFP in the pluripotent state and absence of GFP after differentiation. While the hOCT4-GFP episomal ESC line is free of genomic effects, reporter expression level is heterogeneous and nonclonal. The hOCT4-GFP ESC line generated using site-specific genomic insertion exhibits a more homogeneous and uniform reporter expression but is not a true indicator of regulated expression by the endogenous promoter. Therefore, the endogenous genomic site was targeted to create lineage-specific reporter knock-in lines. Human iPSC and ESC were edited for the insertion of a GFP-Neomycin fusion expression cassette at the endogenous OCT4 locus using GeneArt® Precision TALs and CRISPR/Cas-9 based gene editing technologies. Clones with confirmed editing and no detectable off-target insertions, expanded and assessed in parallel with existing engineered lines for sensitivity and context- specific expression of the GFP reporter, will be further used as cell models.

F-1267

ELECTROMAGNETIC FIELDS MEDIATE EFFICIENT CELL REPROGRAMMING INTO A PLURIPOTENT STATE

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Life on Earth is constantly exposed to natural electromagnetic fields (EMFs), and it is generally accepted that EMFs 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 EMFs influence these processes remain unclear. Here we show 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 an efficient tool for epigenetic reprogramming including the acquisition of pluripotency.

F-1268

FEEDER-FREE REPROGRAMMING OF SOMATIC CELLS TO INDUCED PLURIPOTENT STEM CELLS USING A SYNTHETIC SELF-REPLICATING RNA VECTOR SYSTEM

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Integration of reprogramming factors into the genome of the somatic cells used during induced pluripotent stem cell (iPSC) generation is undesirable because it can potentially affect differentiation capacity or lead to oncogenic transformation. Synthetic mRNA circumvents these integration risks but typically requires repeated transfections. We are currently developing a self-replicating RNA reprogramming vector ReproRNA-OKSGM, which expresses reprogramming factors Oct4, Sox2, Klf4, c-Myc, Glis1, and a puromycin selection cassette, for reprogramming of fibroblasts with just a single transfection. To generate iPSCs, 4 X 10□ neonatal human foreskin fibroblasts (HFFs) or adult human dermal fibroblasts (HDFs) were transfected with 2 ug of ReproRNA-OKSGM. Transfected cells were plated and selected by culture in medium containing puromycin for 8 days, after which they were harvested, dissociated and re-plated on either inactivated mouse embryonic fibroblasts (iMEFs) in standard hES medium (DMEM/F12, 20% KOSR, bFGF, NEAA, L-Glutamine), or on Matrigel™ in ReproTeSR™ Medium. B18R protein was added throughout the selection/induction phases to suppress the interferon response to the RNA vector. Embryonic stem (ES) cell-like colonies were counted to determine reprogramming efficiencies and colonies were selected for further characterization. Reprogramming efficiencies on iMEFs, for HFFs and HDFs, were 0.07 ± 0.02% (n=2) and 0.12 ± 0.05% (n=6), respectively. Reprogramming efficiencies in feeder-free conditions in ReproTeSR™ was similar, at 0.047 ± 0.004% (n=1) and 0.13 ± 0.07% (n=6) for HFFs and HDFs. ES-like colonies arose within 2 weeks after transfection of ReproRNA-OKSGM with cells displaying high nucleus to cytoplasm ratio and by 3-4 weeks were large enough to be manually selected for subculture. Individual clones were subcultured in mTeSR™1 and expressed pluripotency-associated markers Oct4, SSEA-3, and Tra-1-60. Moreover, subclones exhibited pluripotent capacity as demonstrated by differentiation to cells of the 3 germ-layers using STEMdiff™ differentiation kits. In summary, our results demonstrate the ease of reprogramming fibroblasts to iPSCs

with a single transfection of ReproRNA-OKSGM non-integrating, self-replicating RNA vector under feeder-free and defined conditions.

F-1269

A SYSTEMATIC MULTIPLEX ANALYSIS OF CHROMATIN FACTORS IDENTIFIES NOVEL ROADBLOCKS OF REPROGRAMMING

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Reprogramming to pluripotency is governed by a set of four transcription factors, however the majority of cells remains refractory to induction of this cell fate switch. To identify molecules that safeguard somatic cell fate, we performed a pooled RNAi screen targeting all known chromatin regulators during transcription factor-mediated reprogramming of mouse fibroblasts to induced pluripotent stem cells (iPSCs). In addition to known reprogramming barriers that control DNA methylation and heterochromatin maintenance, we uncovered two novel repressor pathways at unprecedented clarity in our primary screen. We validated, that inhibition of each dramatically enhances reprogramming. Inhibiting both pathways is further synergistic. Additionally we dissected the kinetics of reprogramming and noticed both accelerated reprogramming as well as an increase in incidence. Together, these findings reveal two novel gatekeeper of somatic cell identity and provide a potential strategy to modulate cellular plasticity in a regenerative setting.

F-1270

SCALABLE XENO-FREE CULTURE SYSTEM FOR HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human iPSC culture using Essential 8® Medium and Vitronectin successfully enables iPSC cultures under adherent monolayer conditions. Vitronectin supports the expansion of human iPSCs either in coated plates or on polystyrene-coated microcarriers, while maintaining cell functionality and pluripotency. The scale-up of the microcarrier-based system was accomplished using a 50 mL spinner flask, under dynamic conditions. A three-level factorial design experiment was carried out to identify the optimal conditions in terms of a) initial cell density and b) agitation speed, to achieve the highest cell yield in a spinner flask culture and c) to minimize the shear force. A maximum cell expansion of 3.5-fold was achieved by inoculating 55,000 cells/cm^2 and using an agitation speed of 44 rpm, which yielded a final cell density of 1.4×10^6 cells/mL after 10 days of culture. At the end of the dynamic culture, cells maintained their typical morphology, pluripotency-associated marker expression as well as their tri-lineage differentiation capability, which was verified by inducing their spontaneous differentiation through embryoid bodies. Microcarrier expanded iPSCs were further subjected to directed differentiation to neural lineages and cardiac lineages successfully. In conclusion, a limited xeno-free scalable culture system with Essential 8® Medium was successfully developed for the large-scale production of human iPSCs. Beyond the scale of 50 mL spinner or aggregate cultures, the culture media would need modifications to achieve further high scalability such as bioreactor type of cultures.

F-1271

CHROMOSOMAL INSTABILITY AND MOLECULAR DEFECTS IN INDUCED PLURIPOTENT STEM CELLS WITH NIJMEGEN BREAKAGE SYNDROME

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Nijmegen breakage syndrome (NBS) results from the absence of the NBS1 protein, responsible for the detection of double strand breaks (DSBs). NBS syndrome is characterized by microcephaly, growth retardation, immunodeficiency, and cancer predisposition. In this study we show successful reprogramming of fibroblasts with NBS into induced pluripotent stem cells (NBS iPSCs). Our data suggests a strong selection for karyotypically normal fibroblasts to go through the reprogramming process. The derived NBS iPSCs acquire numerous sporadic chromosomal aberrations through this process and display a delayed response to the induction of double strand breaks. Furthermore, NBS iPSCs show slower growth, abnormal gene expression, mitotic inhibition and a reduced apoptotic response to stress. Our results demonstrate the importance of NBS1 in early development, shed new light on the molecular mechanisms that underlie this severe syndrome and further expand our knowledge of the genomic stress cells experience through the reprogramming process.

F-1272

DEFINING PLURIPOTENT STATES BY LONG NON-CODING RNAS

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It is generally perceived that induced pluripotency is the acquisition of an embryonic stem cell (ESC) like state through transcription factor-driven somatic cell reprogramming. However, we have recently shown using the mouse "Project Grandiose" dataset that an alternative pluripotent state, F-class, that is distinct from the well-defined ESC-like induced pluripotent stem cells (iPSCs), can be achieved through maintained high levels of reprogramming factors expression. Here, we explore the role of long non-coding RNAs (lncRNAs), a new class of genes, in defining the F-class versus ESC-like iPSC state. First, we identified several novel lncRNAs with expression specific to these two pluripotent cell types. We then narrowed our list to 16 lncRNAs that exhibited syntenic conservation between mouse and human. Interestingly, our lncRNA cohort showed distinct expression profiles during human ESC differentiation with the majority down-regulated upon differentiation, suggesting a conserved function in maintaining both mouse and human pluripotency. A subset of these lncRNAs showed binding to chromatin regulators and contained conserved sequence motifs that were present at promoters of genes involved in cell differentiation

and embryonic development. These results propose potential lncRNA-protein and lncRNA-DNA interactions responsible for controlling cell fate during reprogramming and/or differentiation. Our data and analysis approach provide a unique model to characterize the molecular structure and function of lncRNAs in acquisition of pluripotency and cell fate determination.

F-1273

GENERATION OF CILIATED AIRWAY EPITHELIAL CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Ciliated airway epithelial cells are key players of mucociliary clearance in host defense of the respiratory system and are associated with various refractory respiratory diseases, such as cystic fibrosis, primary ciliary dyskinesia, bronchial asthma, chronic obstructive lung disease (COPD), and bronchiectasis. However, it has been challenging to recapitulate human airway diseases in vitro, because of difficulty to isolate primary bronchial epithelial cells from diseased patients. In this new era of human induced pluripotent stem cell (hiPSC) technologies, we obtained the chance to establish disease-specific hiPSCs and differentiate them to disease-relevant cell types in vitro. In a previous report, we identified carboxypeptidase M (CPM) as a surface marker of NKX2.1+ "ventralized" anterior foregut endoderm cells (VAFECs) for subsequent induction of alveolar epithelial cells. In the present study, we constructed a stepwise protocol to generate ciliated airway epithelial cells via CPM+ VAFECs from hiPSCs. In brief, hiPSCs were stepwisely differentiated into NKX2.1+ FOXA2+ VAFECs during the first two weeks. We then replated isolated CPM+ cells and continued to culture to differentiate them. After several weeks of culture, FOXJ1+ NKX2.1+ cells and Acetylated-tubulin+FOXJ1+ cells were observed by immunostaining, and they looked morphologically similar to ciliated epithelial cells by electron microscopy. This method of generating ciliated airway epithelial cells via CPM-based sorting probably help the future study of human airway disease modeling that have been considered unapproachable through animal modeling.

F-1274

GENE EXPRESSION ARRAYS FOR MARKER DISCOVERY AND PLURIPOTENT STEM CELL STANDARDIZATION

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Gene expression profiling has been extensively used to define and identify pluripotent stem cells (PSCs). More recently, comparison of expression profiles between embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) has revealed subtle differences that could provide important clues on their potential impact on functionality. In this study, we explore the utility of the gene expression microarray for the discovery of unique PSC markers and a focused array for the standardized analysis of pluripotent stem cells. We had earlier reported the use of global microarray analyses to identify genes that are differentially expressed in partially reprogrammed and fully reprogrammed cells. Our studies indicated several markers that were expressed in parental fibroblasts and partially reprogrammed cells but not in fully reprogrammed iPSCs or in ESCs. Here we confirmed the differential expression of these markers using antibody staining. We then combined these markers with known pluripotent stem cell markers to monitor the progression of reprogramming via flow analysis. Simultaneously, established PSC lines used in the original microarray study were analyzed using the TaqMan hPSC Scorecard, a standardized characterization method. The TaqMan hPSC Scorecard successfully detected the expression of self-renewal markers in undifferentiated cells and the expression of trilineage markers in spontaneously differentiated cells, thereby confirming the results from cellular analyses. Based on our studies, gene expression arrays offer a powerful and accurate means to identify novel markers to track somatic reprogramming while offering a consistent and robust method to standardize pluripotent stem cells.

F-1275

A SMALL MOLECULE LIBRARY SCREEN IDENTIFIES A NOVEL SMALL MOLECULE THAT ENHANCES REPROGRAMMING EFFICIENCY AND IPSC GENERATION

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Human somatic cells can be reprogrammed into embryonic stem cell (ESC)-like induced pluripotent stem cells (iPSCs) by ectopic expression of key reprogramming factors. However, the potential application of iPSCs in regenerative medicine is still hampered by several obstacles including the low efficiency and slow kinetics of reprogramming. Here, we identified new reprogramming enhancers of iPSC generation from selected small molecule libraries (FDA-approved drug collections of 362 compounds and other 303 clinical medicines). We initially used the secondary OSKM-transgenic mouse embryonic fibroblast (MEF) induction system and then further examined 37 selected compounds in human somatic cell reprogramming. Among candidates, the compound No.149 promoted iPSC generation most efficiently in both mouse and human cell system. The pluripotency of fully reprogrammed No.149-induced iPSCs were determined by immunocytochemistry, karyotyping and teratoma formation. Our results may help to develop more efficient and safe methods to generate functional iPSCs and to better understand the molecular mechanisms underlying reprogramming process.

F-1276

GENERATION OF AN LNCRNA GTL2-GFP REPORTER FOR RAPID ASSESSMENT OF PLURIPOTENCY IN MOUSE INDUCED PLURIPOTENT STEM CELLS

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Epigenetic reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) by overexpression of defined factors holds great promise for disease modelling and regenerative medicine. However, the stochastic reprogramming process often results in variable pluripotency levels of iPSC lines as measured by their in vivo developmental potential, which poses a huge challenge to the application of high quality iPSCs. The activation status of an imprinted

Dlk1-Dio3 region, which encodes three long non-coding RNA (lncRNA) genes *Gtl2, Rian,* and *Mirg,* and a cluster of microRNAs, has been identified as a molecular marker for pluripotency, raising the possibility to generate a reporter for pluripotency through labelling the expression of the *Dlk1-Dio3* region. However, whether it is feasible to generate lncRNA- or microRNA-reporters has not been tested yet. Here we report the generation of cells and mice carrying a *Gtl2-GFP* reporter by inserting a *GFP* gene right after the transcription termination site of *Gtl2*. The synchronous expression of *Gtl2* and *GFP* genes, as well as the normal development of the *Gtl2-GFP* reporter mice, suggested that the lncRNA *Gtl2* was successfully labeled without compromising its developmental function. Moreover, in a proof-of-principle experiment, we have shown that of four iPSC lines expressing key pluripotency marker genes including Oct4, only two *Gtl2-GFP*-positive iPSC lines can produce "all-iPSC" mice. Our results demonstrated that the *Gtl2-GFP* reporter can be used for rapid and noninvasive assessment of iPSC pluripotency and screening of iPSC induction medium that can promote generation of high-quality iPSCs, therefore providing a useful and convenient tool for reprogramming studies, such as screening for good reprogramming components that promote iPSC pluripotency, and choosing the high-quality iPSC clones for further applications.

F-1277

THE NOVEL APPLICATION OF CORDYCEPIN TO MAINTAIN THE STEM CELL PLURIPOTENCY AND INCREASED IPS CELLS GENERATION EFFICIENCY

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To culture the embryonic stem cells (ES) and induced pluripotent stem cells (iPS) needed to maintain the pluripotency for future experiment or therapy. Leukemia inhibitory factor (LIF) was common used to maintain the pluripotency of ES and iPS cells. However, LIF is an expensive reagent. In addition, to improve the iPS cell generation efficiency is also very important to clinical application. The goal of this study was to find out a pure compound that could maintain stem cells pluripotency to replace LIF and improve the iPS cells generation efficiency. From 20 candidates traditional Chinese medicine we found that Cordyceps militaris triggered the up-regulation of stem cells activating genes (Oct4 and Sox2) expression levels in MEF cells. Cordycepin, a major active component of Cordyceps militaris, also could up-regulate Oct4 and Sox2 gene expression. We used ES and iPS cells to treat with different concentrations of Cordycepin (replaced LIF in the culture medium) to test whether it was useful to maintain the pluripotency. The results showed that higher expression levels of several stem cells markers in 10μM Cordycepin-treated ES and iPS cells compared to controls that did not contain LIF, including alkaline phosphatase, SSEA1, and Nanog. Embryonic body formation and differentiation confirmed that 10μM Cordycepin-containing medium was capable to maintain stem cells pluripotency after six times passages. Microarray analysis indicated Extracellular matrix and Jak2/Stat3 signaling pathway as the top two deregulated pathways. In ECM pathway, we determined that the integrin αVβ5 expression levels and phosphorylated Src levels increased after Cordycepin treatment. The phosphorylated Jak2 and phosphorylated Stat3 protein levels were increased after Cordycepin treatment and suppressed with the Jak2 inhibitor, AG490. Subsequence, we used Oct4-GFP MEF cells to determine that 10 μM Cordycepin significantly increased the iPS generation efficiency in day 21. The novel iPS cells also could differentiate into neural stem cells for future application. In conclusion, we demonstrated Cordycepin could maintain the pluripotency of stem cells through both of ECM and Jak2/Stat3 signaling pathway and improved iPS generation efficiency.

F-1278

DISTRIBUTION STRATEGIES FOR PLURIPOTENT STEM CELL LINES: WICELL'S MODEL FOR ENSURING ACCESSIBILITY WHILE MINIMIZING INVESTIGATOR BURDEN

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Based upon conservative estimates, over 300,000 new iPS cell lines will be derived within the next 5 years. To fully realize the potential of these new lines to investigate, understand, and improve human health, they must be secured, characterized, and widely distributed. To achieve the greatest benefit, this must be done in a way that allows inclusion of all lines (including "orphan" cell lines), reduces the financial and technical burden on the generating labs, and eases the process for investigators requesting materials. To meet this need, WiCell (a non-profit institution) has developed a streamlined method for depositing cell lines for global distribution. To eliminate the burden and distraction of distributing from the research laboratory, WiCell offers this service to individual investigators with no cost associated with deposit, banking, characterization, or distribution of cell lines, eliminating any financial concerns associated with deposit while ensuring that cell lines are secure and available globally. Reports indicate that 20-33% of cell lines used are misidentified, resulting in researchers mistakenly studying the wrong models, ultimately slowing progress and wasting research dollars. To assure proper identification and safety, all distributed cell lines are tested for mycoplasma and sterility, and karyotype and identity are confirmed. Funds received from cell line distributions support the rebanking and characterization of in-demand lines, and enable the continued preservation of all materials deposited in the WiCell Stem Cell Bank, allowing so-called "orphan" cell lines to be preserved with the same integrity as more popular materials. Technical support provided helps ensure that receiving laboratories will be successful in establishing working cultures. Through this model, newly derived cell lines will be globally distributed enabling their widespread use in research and maximizing their potential benefit while eliminating the burden on individual laboratories.

F-1279

CULTURE METHOD AFFECTS IN VITRO HAEMATOPOIESIS FROM INDUCED PLURIPOTENT STEM CELLS

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A number of in vitro differentiation methods to promote hematopoiesis of pluripotent stem cells have been reported, each differing in cell preparation, type of cytokines and culture medium used. In this study we used several induced pluripotent stem cells (iPSCs), derived from normal and diseased fibroblasts, to evaluate three methods of differentiation: two utilising iPSCs in embryoid body form (EB), and the other based on adherent

culture (AC) with staged specific addition of differing combinations of cytokines. Hematopoietic differentiation of iPSCs was assessed by 1) flow cytometry detection of hematopoietic surface markers, 2) clonogenic assays and morphology, and 3) changes in gene expression. We found all methods tested produced hematopoietic cells. Flow cytometry time course analysis revealed hematopoiesis transitioned from dual CD34+KDR+ surface expression to CD34+CD33+ and then CD34-CD33+CD45+ expression. The latter population of myeloid lineage cells reached 70% in AC cultures by day 12 in contrast to the 30% produced from the EB methods, comprising mainly of CD34+CD45- cells. The highest yield of CD34+ hematopoietic stem cells (HSCs) was produced using the AC culture method (10-12%) compared to the two EB methods (6 and 4% for staged and non-staged addition of cytokines). However, the clonogenic potential (CFU-GM, CFU-E and CFU-GEMM colonies per 10^3 CD34+ cells) of the HSCs produced by the AC method was lower than the EB method. We observed no difference in the clonogenicity of HSCs derived from diseased and normal iPSCs. Gene expression analysis showed hemoglobin gene synthesis was similar in the erythroid cells (CFU-E colonies) produced from all methods. Epsilon (embryonic) globin gene expression was highest followed by gamma (fetal) globin, but expression of beta (adult) globin was undetectable. In conclusion, in vitro hematopoiesis is dependent the differentiation method used. The EB method provided the most consistent development of functional HSCs and highest yield of mature blood cells.

F-1280

DRUG RE-POSITIONING ENABLES ELIMINATION OF UN-DIFFERENTIATED CELLS IN CLINICAL APPLICATION OF IPS CELLS

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Towards clinical application of human iPS cells (hiPSCs), undifferentiated cells should be removed among iPS-derived cells to reduce risk of tumorigenicity. Our aim is to eliminate undifferentiated cells in vitro through exposure to a drug, for safer clinical application. By adding BET inhibitors to a dish (JQ1 [1 μM], I-BET151 [2 μM], or I-BET762 [5 μM]) (an epigenetic modifier), we observed that human iPSCs (253G1 or 201B7) were totally killed in vitro within 96 hr. On the other hand, we demonstrated that there was no toxicity on differentiated cells, such as hiPS-derived cardiomyocytes (hiPS-CMs) or human fibroblasts. Moreover, we quantitated undifferentiated cells among hiPS-CMs by FACS using TRA-1-60, a marker for pluripotency; the positive fraction for TRA-1-60 was 0.45 ± 0.09% in DMSO-treated hiPS-CMs, whereas that was 0.05 ± 0.01% in JQ1-treated hiPS-CMs, suggesting that BET inhibitor (JQ1) is potent in eliminating undifferentiated cells. Furthermore, we also performed qRT-PCR analysis of Lin28, a pluripotent marker; the relative expression of Lin28 was 0.23 ± 0.05% in DMSO-treated hiPS-CMs, while that was 0.05 ± 0.00% in JQ1-treated hiPS-CMs (compared to untreated hiPSCs). To explore JQ1's effects on tumorigenicity in vivo, hiPS-CM-derived cell sheets with or without JQ1 treatment were subjected to transplantation over the heart surface of NOG mice, which are under investigation. BET inhibitor has been shown to be involved in histone acetylation; furthermore, especially for iPS cells, BET inhibitor has been demonstrated to regulate Super-enhancer-associated pluripotency genes, such as Oct4 or Nanog; in fact, we found that Nanog or Oct4 expression (but not c-Myc expression) in hiPSCs was markedly decreased upon treatment with JQ1. In clinical settings, JQ1 has been tested in phase studies on various cancers. Therefore, we are performing "Drug Re-positioning", where we discover novel application of a drug, thereby enabling faster application of a drug that has been fully validated. Collectively, "Drug Re-positioning" strategy will be expected to accelerate clinical application of iPS cells.

F-1281

UNRAVEL THE MECHANISM OF SOMATIC REPROGRAMMING BY CAPTURING THE INTERMEDIATED STATES OF REPRO-GRAMMING CELLS IN A QUINTUPLE TRANSGENIC MOUSE

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The epigenome of mammalian cells possess flexibility allowing a highly differentiated cell to change its cell fate and even regain the status of pluripotency. As somatic reprogramming with a stochastic onset and gradually toward a deterministic process while approaches pluripotency, it creates several discernable cell statuses during the process of genome remodeling. Even after reaching the ESC-like pluripotency, the oscillatory expression of certain pluripotent factors demonstrates that the unsettled status of cell fate intimately interweaving with the environmental cues is capable to shift beyond pluripotency. Most of those intermediated states represent a unique epigenome in which holds the main routes to warrant pluripotency acquisition and even beyond. Although those transition statuses may not exist in vivo during embryogenesis and therefore represent a kind of cellular neoteny, failing to capture those unique states in vitro, however, accounts for the major barricade to understand the mechanism of somatic reprogramming. To understand the mechanism of somatic reprogramming needs to delineate the routes of remodeling taken by a differentiated cell. The temporally defined markers along the path of remodeling shall facilitate the enrichment of cells synchronized at the similar epigenome status. Previously, we have generated a quadruple transgenic mouse carrying the E-cadhering-YFP to mark the event of MET in addition to the Oct4-gfp pluripotency marker and the rtTA/TetO-mediated MKOS-iPSC inducible system. In the present study, the totipotency marker (2C::tdTomato) will be further introduced to facilitate the identification of somatic reprogramming beyond pluripotency. This quintuple transgenic system will not only allow us to identify new factors participating in various statuses of epigenome establishment, but also enable to link a HTS platform for identifying small molecules potentiating the epigenome remodeling at various stages. To capture the potentiated status of epigenome beyond pluripotency in vitro, manipulating the expression of certain core pluripotency network genes including Nanog, Prdm14, and STAT3 were performed in ESC and iPSC. In this meeting, we will update our progress on the pathfinding of pluripotency acquisition.

F-1282

ESTABLISHMENT OF THERAPEUTIC STEM CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS FOR MALIG-NANT GLIOMA

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Malignant gliomas, such as glioblastoma multiforme, anaplastic astrocytoma and oligo-astrocytoma, are the most aggressive primary brain tumor in human beings. Malignant glioma is consisted of massively proliferative glioma cells and brain tumor stem cells (BTSCs). BTSCs disperse and locate in the brain parenchyma, grow infiltratively and resistant to irradiation and tumoricidal agents, resulting in the incurability by conventional standard therapy including maximal safe tumor resection, adjuvant chemotherapy and irradiation. Although new approaches such as gene therapy have been tried, it showed the poor outcome related to the inability to deliver therapeutic agents to both glioma cells and BTSCs. Recent publications showed that neural stem cells, which haveextensive tropism for tumor cells, would be the possible candidates as the cellular vehicle (CV) for therapeutic agents, however, there are the ethical and practical concerns in the clinical application. To solve those issues, we have been establishing the therapeutic stem cells (TSCs) using induced pluripotent stem cells (iPSCs) as CV to deliver the suicide gene. Lentiviral vector obtained herpes simplex virus-thymidine kinase (HSV-tk) gene has been made with some modification, and transfected to iPSCs, then iPSCs with HSV-tk are stocked as TSCs. We have also established BTSC animal model, which will be intracerebrally injected with NSCs derived from TSCs followed by systemic prodrug ganciclovir administration to assess the bystander therapeutic effect. It is too difficult to destroy glioma cells as well as BTSCs without any damage to the normal brain tissue at moment. Our TSC-based therapies derived from iPSCs can selectively treat glioma cells and BTSCs without harmful effect including tumor formation, and may become a promising strategy for malignant gliomas. We will be presenting our challenges to tackle gliomas.

F-1283

EFFICIENT EMBRYOID BODY FORMATION FROM HUMAN IPS CELLS ON NOVEL MICROFABRIC VESSELS

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Human iPS (induced pluripotent stem) cells have high potential applications in regenerative medicine and drug discovery with their ability of differentiating into a wide variety of cell types. Suspension culture of iPS cell aggregates, named as embryoid bodies (EBs), is one of effective methods for propagation and differentiation of the iPS cells. Furthermore, size and uniformity of the EBs are known to be one of critical factors affecting the differentiation efficiency. However, there are still technical limitations in the generation method of large-number of EBs with uniform size by simple and easy handling. To solve such problems, we attempted to apply novel micro-fabricated culture wares (named EZSPHERE), on which large-number of micro-wells are solely created by laser, followed by low-cell-adhesive coating. The diameter and depth of each micro-well can be altered around 200-1,000 and 100-400 micrometer, respectively. We confirmed that the EZSPHERE is very useful for generating large-number of uniformly-sized EBs, when we inoculate 2.3×10^5 iPS cells into a standard type of the EZSPHERE (35 mm dish with approximately 2,400 micro-wells) in differentiation medium. After cultivation for 4 days, a typical Gaussian distribution was obtained for diametric size (108 ± 33 micrometer) of the generated EBs with the total number of over 2,200 It was found that shape of the micro-wells is suitable for gathering inoculated cells and most of the EBs were formed within 3-6 hours. In addition, it was also confirmed that the obtained EBs could propagate at a good rate and maintain uniformity in growth medium. Differentiation tendency of the EBs was also confirmed by induction into cardiomyocyte or nerve cells. These results indicate that EZSPHERE is a useful tool for the controlled large-scale generation of EBs with uniform size and the differentiation capacity in a reproducible manner. This study was performed as a part of the JST (Japan Science and Technology Agency) project "Research Center Network for Regenerative Medicine"

F-1284

HUMAN PLURIPOTENT STEM CELLS CAN BE CRYOPRESERVED AT HIGH EFFICIENCY BY SLOW COOLING AND SINGLE-CELL DISSOCIATION

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Cryopreservation is a key operation for practical application of human pluripotent stem cells (hPSCs) because it enables permanent preservation and easy transportation. To achieve bulk storage and easy handling, the conventional slow cooling is desirable but the current conventional method leads to poor recovery of hPSCs after thawing. We here demonstrate a high efficient recovery method for hPSC cryopreservation by single-cell dissociation and slow freezing. After confirming hPSC survivability after freeze thawing, we found that hPSCs that were freeze thawed as colonies showed markedly decreased survival, whereas majority of freeze-thawed single hPSCs retained their viability. The higher viability of hPSCs after thawing was confirmed regardless of kinds of the culture medium, freezing medium, and feeder- or feeder-free culture systems. These results indicated that hPSCs should be cryopreserved as single cells under slow freezing method. In addition, freeze-thawed single hPSCs efficiently adhered and survived in the absence of a ROCK inhibitor by optimization of the seeding density. The high recovery rate enabled conventional colony passaging for subculture within 3 days post-thawing. Furthermore, the cell recovery after cryopreservation was highly supported by coating culture surfaces with recombinant human laminins and its fragment. This simple but highly efficient cryopreservation method allows easy handling of cells and bulk storage of high-quality hPSCs.

F-1285

FEEDER-INDEPENDENT AND SERUM-FREE CANINE INDUCED PLURIPOTENT STEM CELLS REPROGRAMMED BY A DRUG-INDUCIBLE SYSTEM

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Canine induced pluripotent stem cells (ciPSCs) are an attractive cell source for veterinary medicine. It is also useful for human medicine in terms of a clinical regenerative disease model. However, for applying ciPSCs from bench to bed, there are many issues to be solved. For example, the contamination of unknown animal components and the difficulty of expanding ciPSC lines are concerned. Although some researchers have reported preparation of ciPSCs, they used unknown animal components like serum and feeder cells for ciPSCs cultures. Moreover, due to a complicated method for the cell passage, it takes much time. In order to resolve these issues, we tried to establish a method to generate serum-free and feeder-independent ciPSCs from canine embryonic fibroblasts and to passage these cells as a single cell. We generated ciPSCs by using DOX-inducible lentiviral vectors carrying four mouse cDNAs (*OCT4, SOX2, KLF4,* and *C-MYC*). Some colonies in serum-free medium exhibited basic FGF dependent proliferation and were maintained over 30 passages. ciPSCs displayed similar characteristics of human embryonic stem cells (hESCs) with a flatted shaped morphology and a critical pluripotency marker, GBX2, which is specific to hESCs. Additionally, ciPSCs had an ability to differentiate into three germ layers via the formation of embryoid bodies. These cells could be dissociated into single cells for passaging and cryopreservation the same as mouse ESCs, and showed more than 80% survival rate after 24 hours of culture with or without ROCK inhibitor. Moreover, we could maintain these cells on matrigel-coated dish without feeder cells. Taken together, we have successfully generated ciPSCs under feeder-independent and serum-free culture conditions using a drug-inducible system. These cells could be simply passaged and cryopreserved as a single cell. These ciPSCs may be suitable for clinical application and would improve difficult situations in using ciPSCs as a clinical cell source in the future.

F-1286

ESTABLISHMENT OF SUITABLE QUALITY ASSURANCE FOR SUPPLY OF QUALITY CONTROLLED IPSC LINES FOR RESEARCH: EXPERIENCE OF THE EBISC BANK FOR EUROPE

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The EBiSC iPSC cell bank is being established under joint Innovative Medicines Initiative (IMI) funding (E24M) from a consortium of European Federation of Pharmaceutical Industries and Associations (EFPIA) members (AstraZeneca AB, H Lundbeck A/S, Janssen Phamaceutica AB, Novonordisk A/S, Pfizer Ltd, UCB Biopharma SPRL) and the European Commission to provide access to 1000 iPSC lines by 2017, from both disease affected and control individuals. An important component in setting up the bank is the implementation of robust procedures for the quality control of cell lines to assure they are fit for purpose of research into disease studies and development of assays for industry. The system for EBiSC has been established with reference to industry standard methodologies and best practice for hPSC cell banking (ISCBI, 2009). Quality control release tests used include cell line viability, growth characteristics, identity (DNA profile), mycoplasma testing, karyology and a virological screen. In addition, cell lines are to be tested for surface markers, self-renewal markers and an assay of pluripotency potential. Details of sample specification, Standard Operating Procedures and criteria for release of cell banks are important to assure users of the bank have a reliable and consistent supply of cells. The poster will describe the development of a suitable quality assurance framework and quality policies to assure delivery of cells suitable for research and industry needs, whilst avoiding gold plating. Professional data management systems are vital to assure security of sensitive data (patient information and certain kinds of genetic data) and retention, stability and traceability of all QC data on cell lines. Considerations in establishing the EBiSC data management system will be described. In the field of stem cell research in particular, QA systems need to be flexible and constant improvement is vital. EBiSC has a programme of development of quality control and characterisation with current effort focused on pluripotency assessment (see poster by O'Shea et al.). Future work will also include establishment of rapid genetic stability testing and detection of viral contaminants.

F-1287

HUMAN INDUCED PLURIPOTENT STEM CELLS DIFFERENTIATE INTO INSULIN PRODUCING CELLS ABLE TO ENGRAFT IN VIVO

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New sources of insulin-secreting cells are strongly required for the cure of diabetes. Recent successes in differentiating embryonic stem cells (ESC), in combination with the discovery that it's possible to derive human induced pluripotent stem cells (iPSC) from somatic cells, have raised the possibility that patient-specific β cells might be derived from patients through cell reprogramming and differentiation. Human iPSC were reprogrammed with Yamanaka's factors by retroviral or sendai virus infection of both fetal and adult fibroblasts. We optimized differentiation protocols already established for ESC. The expression of marker genes of pancreas differentiation was measured through real-time PCR analysis (Taqman) and expressed as fold changes (FC) compared to undifferentiated hiPSC. Proteic expression was confirmed by cytofluorimetric analysis and C-peptide release assessed with ELISA. HiPSC differentiated in vitro were transplanted in NOD/SCID mice and functional and an immunohistochemical analysis of grafts were performed. We obtained down-regulation of the pluripotency genes Oct4 and Nanog and up-regulation of the definitive endoderm Sox17 and Foxa2 (39.25 ± 15.78 and 7.94 ± 4.16 FC) and of the pancreatic endoderm genes Hnf1b, Pdx1, Ngn3 and Nkx2.2 (97.14 ± 77.01, 596.34 ± 368.78 FC, 83.46±80.34 and 1.62 ± 0.90 FC). At the end of the differentiation, the production of insulin mRNA was highly increased (1567.92 ± 785.1 FC) and 5±2.9% cells resulted insulin-positive; terminally differentiated cells also produced C-peptide in vitro (1.7 ± 0.1 ng/mL). In mice transplanted with hiPSC-derived pancreatic progenitor cells, the grafts resulted composed of a mixed population of cells containing mature pancreatic cells (Insulin+, Glucagon+ and Pdx1+), but also pluripotent (Sox2+ and Ki67+) and

some neuronal cells (GFAP+) at 1 and 4 weeks. Mice transplanted with pancreatic progenitor cells responded to glucose secreting human c-peptide. In vitro results showed that hiPSC differentiate in insulin secreting cells. Furthermore, in vivo study suggests that differentiated cells were able to engraft, survive and secrete insulin in the recipient mice, but evidence the necessity of a strategy of selection of target cells before infusion.

F-1288

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM PERIPHERAL BLOOD THROUGH SERIAL SEEDING WITH CENTRIFUGATION

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Pluripotent stem cells are a promising tool for future use in regenerative medicine or drug screening. Previous studies proved that pluripotent stem cells can be induced from adult somatic cells through reprogramming. Various cell types has been tempted and successfully generated induced pluripotent stem cells (iPSCs). One of the attempts was to reprogram blood cells. Blood cells can be easily obtained than skin fibroblasts and takes less time to generate iPSCs. Because of these advantages, several groups used blood origin cells and tried to reprogram with a small amount of blood. However, expansion of cells was needed to accomplish this attempt. In this study, we demonstrated a simple method to improve the original iPSC generation from peripheral blood mononuclear cells (PBMCs). Using this method, we were able to obtain higher amount of iPSC clones in a relatively short period.

F-1289

NEUROSPHERES DERIVED FROM REPROGRAMMED PERINATAL MSCs AUGMENTS FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD INJURY

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Spinal cord injury is a debilitating disorder that results in the progressive loss of motor and sensory functions. The spinal cord injury leads to enervation of normal tissue homeostasis ultimately leading to paralysis. Currently, there is no proper treatment for spinal cord injury. However, recent several preclinical data and preliminary clinical studies indicate cell therapy might serve as an effective tool for the treatment of patients suffering from spinal cord injury. The induced pluripotent stem cells (iPSCs) might serve as an alternate source for cell therapy due to its ability to differentiate efficiently into multiple neuronal subtypes. The roadblocks and ethical issues involving ESCs can be successfully circumvented using iPSCs. In this study, we have utilized human perinatal placental MSCs (PDMSCs) for generation of iPSCs using integration free nucleofection protocol. In-order to make the reprogramming clinically compatible we have utilized autologous feeder for derivation of iPSCs. Treatment of cell undergoing reprogramming with dual kinase inhibitor (2i) and a cytokine leukemia inhibitory factor (LIF) effectively augmented the reprogramming. The derived iPSCs were further subjected to rigorous in vitro and in vivo characterization analysis. We have successfully used Tra1-81 live stain and transient Nanog GFP based transfection method for selecting the fully reprogrammed colonies. For transplantation studies, we have created SCID mice spinal cord injury, paraplegic model with the help of compression injury at T10 level. Our preliminary data with transplantation of iPSCs derived neurospheres (iNSCs) suggest that it effectively augments neural regeneration with motor and sensory recovery. There was no formation of teratoma after transplantation of iPSCs derived neurospheres. Functional recovery was evaluated using trans cranial stimulations. Results also indicated that transplantation of iPSCs derived NSCs led to increased neuronal myelination with reduced inflammation. Physiological data suggest that transplanted mice exhibited moderate bladder control and improved weight gain than the non-transplanted mice. Additionally, in this study, we have successfully employed indocyanine green (ICG) based cell labeling method in order to track the transplanted cells.

F-1290

CYCLIN B1/CDK1 COMPLEX ENHANCES REPROGRAMMING EFFICIENCY POSSIBLY VIA IMPROVING IPSC MATURATION

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Enhancing expression of interphase CDKs and cyclin D1 has been shown to increase somatic reprogramming efficiency, through promoting proliferation or overcoming p53-p21-induced G1 arrest. However, the functional role of cell cycle regulators on reprogramming could be contradictory, and there is no study regarding the role of mitotic CDK/cyclin on reprogramming. In this study, we investigated functional role of cyclin B1 and cyclin B1 associated CDK1 during human induced pluripotent stem cells (hiPSCs) formation in a p53 null background, thereby overcoming p53-p21 mediated cell arrest. Yamanaka factors (OKSM) and LIN28 in episomal vectors were applied to generate hiPSCs. We modulated cyclin B1 and CDK1 levels by overexpression, knockdown, and co-expression in human diploid fibroblast (HDF) primary cell line followed by iPSC reprogramming. Cyclin B1 overexpression significantly improved iPSC formation efficiency in human HDFs. Co-expression of cyclin B1 with low amount but not high amount of CDK1 also displayed a similar effect, whereas expression of cyclin B1 in CDK1 knockdown HDFs blocked iPSC formation. The results suggest that high expression of cyclin B1 is required to bind to intracellular CDK1 for its function on iPSC formation. Cyclin B1 also improved reprogramming efficiency in epithelial cancer cells with the ratio of AP+ iPS colonies was 56% in cyclin B1-expressing versus 41% in GFP-expressing cells. Cyclin B1 only slightly enhanced S and G2/M population at early stage of iPSC formation. Measurement of pluripotency markers from the pool of iPS colonies, we found that Oct4 and SSEA4 were highly expressed in both GFP- and cyclin B1-expressing iPSCs. By measuring TRA-1-60, one of the most specific markers of human pluripotent of stem cells, we noticed that relative proportion of TRA-1-60+ cells was similar in nascent iPSCs, but became 1.6-2.4 fold higher in cyclin B1-expressing iPSCs during subsequent re-plating (fold change was normalized to GFP-expressing cells), suggeting that cyclin B1 might have a role in inhibiting reversion of reprogramming. In summary, cyclin B1 and cyclin B1-CDK1 complex improve reprogramming efficiency. The underlying mechanism of whether and how cyclin B1 promoting

maturation of reprogrammed cells is under investigation.

F-1291

GLYCOSYLTRANSFERASE ST6GAL1 REGULATES PLURIPOTENCY IN HUMAN PLURIPOTENT STEM CELLS

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Many studies have suggested the significance of glycosyltransferase-mediated macromolecule glycosylation in the regulation of pluripotent states in human pluripotent stem cells (hPSCs). We observed that the sialyltransferase ST6GAL1 was preferentially expressed in undifferentiated hPSCs compared to non-pluripotent cells. Lectin which preferentially recognizes α-2,6 sialylated galactosides showed strong binding reactivity with undifferentiated hPSCs and their glycoproteins, but to a much less extent with differentiated cells. In addition, downregulation of ST6GAL1 in undifferentiated hPSCs led to a decrease in POU5F1 protein and significantly altered the expression of many genes that orchestrate cell morphogenesis during differentiation and organ development. The induction of cellular pluripotency in somatic cells was substantially impeded by the shRNA-mediated suppression of ST6GAL1, likely through the perturbation in endogenous POU5F1 and SOX2 expression during cellular reprogramming and the induction of ribosomal stress-mediated inhibition of cell proliferation. Targeting ST6GAL1 activity with a sialyltransferase inhibitor during cell reprogramming resulted in a dose-dependent reduction in the formation of human induced pluripotent stem cells (hiPSCs). Collectively, our work indicates that ST6GAL1 plays an important role in the regulation of pluripotency and differentiation in hPSCs, and that pluripotent state in human cells could be modulated using pharmacological tools to target sialyltransferase activity.

F-1292

AUTOPHAGY AND MTORC1 REGULATE THE STOCHASTIC PHASE OF SOMATIC CELL REPROGRAMMING

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We describe robust induction of autophagy during the reprogramming of mouse fibroblasts to induced pluripotent stem cells (iPSCs) by the 4 Yamanaka factors (Sox2, Oct4, Klf4 and c-Myc). This process occurs independent of p53 activation, and is mediated by the synergistic down-regulation of mechanistic target of rapamycin complex 1 (mTORC1) and the induction of autophagy-related genes. The Yamanaka factors coordinately repress mTORC1, but bifurcate in their regulation of autophagy-related genes, with Klf4 and c-Myc inducing them but Sox2 and Oct4 inhibiting them. On one hand, inhibition of mTORC1 facilitates reprogramming by promoting cell reshaping (mitochondrial remodeling and cell size reduction). On the other hand, mTORC1 paradoxically impairs reprogramming by triggering autophagy. Autophagy does not participate in cell reshaping in reprogramming but instead degrades p62, whose accumulation in autophagy deficient cells facilitates reprogramming. Our results thus reveal a complex signaling network involving mTORC1 inhibition and autophagy induction in the early phase of reprogramming, whose delicate balance ultimately determines reprogramming efficiency.

F-1293

SCREENING OF HUMAN CDNA LIBRARY REVEALS THE PROMOTING EFFECT OF TWO DIFFERENTIAION RELATED GENES ON HUMAN SOMATIC REPROGRAMMING

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Induced pluripotent stem (iPS) cells were initially generated by forced expression of several reprogramming factors, such as Oct3/4, Sox2, Klf4 and c-Myc. However, it is still unclear what happens during the somatic reprogramming. Until now, many researchers have found out the new reprogramming genes that can improve the reprogramming efficiency or replace the first-reported 4 genes. Some of these genes have been reveled to have direct impact on reprogramming process. The analysis of such genes may be useful to clear mechanisms of cellular reprogramming, which is important for not only stem cell biology but also clinical application of iPS cell. To investigate the reprograming process, we tried to find new genes that influence the process. We employed human cDNA library consisted of 2000 transcription factors and 300 kinases as candidate genes. Each of these was transfected into human dermal fibroblast with basic 5 reprogramming factors (OCT3/4, SOX2, KLf4, L-MYC and LIN28) via episomal plasmid method and was evaluated the influence on iPS generation efficiency as the result of its impact on reprogramming process. Some of these factors significantly increased or decreased the iPS generation efficiency. We selected two homeobox transcription factors for further detailed analysis. They are reported as differentiation-related genes that work on visceral endoderm differentiation in mouse development. When used in somatic reprogramming, both factors enhanced the appearance of cells positive for early reprograming marker, TRA1-60. On the other hand, overexpression experiments revealed that they interfered with the maintenance of pluripotent state in human iPS cells. Hence, they showed inverse effects between somatic reprograming and pluripotent maintenance. Our results shed light on the relationship between somatic reprograming and differentiation-related genes. This knowledge may have a chance of deep understanding of reprograming mechanisms and contribution on future regenerative medicine and human developmental biology.

F-1294

OPTIMIZATION OF XENO-FREE CULTURE SYSTEM FOR IN VITRO INDUCTION OF T CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS.

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iPS cell technology has been just delivered to the first in human study in Japan. Since potentially hazardous materials should be avoided from making clinical grade iPS cells and differentiated cells as much as we possible, animal component free (Xeno-Free) culture for pluripotency reprogramming and maintenance are now widely applied. 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 regeneration of antigen specific T cells via iPS cells. The regenerated T cells would be applicable to clinical immunotherapy against various diseases including cancer, but the differentiation process should be optimized for clinical processing. For this purpose, we have developed an animal component free culture protocol to induce clinical grade T cells via hematopoietic stem/progenitor cells derived from iPS cells. We have 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. Concretely, human iPS cells were initially differentiated to hematopoietic cells via embryoid body under xeno-free conditions. Then, the 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 established a xeno-free culture condition to induce regenerated mature T cells from iPS cells through its pluripotency reprogramming to terminal differentiation.

IPS CELLS: DIRECTED DIFFERENTIATION

F-1296

SMALL MOLECULE-BASED DIRECTED DIFFERENTIATION OF BASAL FOREBRAIN CHOLINERGIC NEURONS FROM INDUCED PLURIPOTENT STEM CELLS UNDER ADHERENT CULTURE CONDITIONS

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Basal forebrain cholinergic neurons (BFCN) arise from the medial ganglionic eminence (MGE) and migrate into different regions of the forebrain. Their dysfunction contributes to the pathogenesis of many diseases such as Alzheimer's disease, dyskinesias and dystonias. Differentiating this cell type from induced pluripotent stem cells (iPSC) might therefore help generating in vitro models for a wide variety of diseases. Previous protocols rely on embryoid body formation. We therefore sought for better culture handling and reproducibility to establish a protocol based on adherent conditions. iPSC reprogrammed retrovirally from human adult fibroblasts were plated down on Matrigel coated culture plates and deprived from serum and fibroblast growth factor. Neural induction started by addition of the small molecules SB 431542 and LDN 193189. For rostro-ventral patterning the small molecules purmorphamine and XAV 939 were added in a timed manner for 20 days. RNA was isolated at day 20 for subsequent transcription to cDNA for qPCR analysis. Cells were plated on poly-d-lysine/laminine coated coverslips and differentiated for 30 to 60 days by addition of brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), insuline-like growth factor 1 (IGF-1), dbcAMP, and the notch-inhibitory small molecule DAPT. Thereafter cells either underwent electrophysiological analysis by whole-cell recording or were fixed and stained for immunofluorescence. After 20 days of patterning cells demonstrated a robust up-regulation of markers associated with the MGE and the cholinergic lineage (NKX2.1, LHX8). After differentiation a considerable amount of mature neurons with a large soma size (30 - 40 µm) expressed markers of BFCN as choline acetyltransferase (CHAT), vesicular acetylcholine transporter (VACHT), ISL-1, P75 and NKX2.1. Differentiated cells showed neuronal electrophysiological properties as fast inward sodium and slow outward potassium currents and spontaneous generation of action potentials. Some cells even showed an after-hyperpolarization "sag", typically exhibited by BFCN. It can be concluded that the differentiation protocol possesses the ability of deriving neurons exhibiting features of BFCN on a morphological, expression and electrophysiological level.

F-1297

A NOVEL HEPATIC CORD TISSUE MODEL FOR EVALUATION OF DRUG INDUCED CHOLESTASIS AND HEPATOTOXICITY

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Primary human hepatocytes are utilized in drug development to evaluate human specific drug properties such as drug hepatotoxicity, metabolism, and drug-transporter interactions. However, the demand for primary human hepatocytes far exceeds the available supply and the quality of primary hepatocytes is highly variable. Human pluripotent stem cells could provide a renewable source of human hepatocytes. In this study, we developed a novel hepatic cord cell model from pluripotent stem cells via generation of hepatic progenitors. Pluripotent stem cell-derived hepatic progenitors were able to form colonies and expressed early liver progenitor markers such as DLK, EpCAM, NCAM, AFP, and CK19. Subsequently progenitor cells were induced into hepatocyte-like cells, which are cuboidal in shape and are arranged in irregular rows or columns resembling liver cords. Cells that form the cord structure were observed to express mature hepatocyte and polarity markers and exhibited hepatic functions including CYP enzymatic activity, LDL uptake, albumin secretion, and glycogen and lipid storage. The transporter function of cord cells was examined using carboxy-DCFDA, which is transported specifically by the canalicular transporter MRP2 following cleavage by cytosolic esterases. The resultant fluorescent DCF accumulated in the canaliculi, indicating that cord cells exhibited functional MRP2 transporter activity. Next, cord cells were used as an in vitro model for assessing drug-induced hepatotoxicity using human hepatotoxicants such as troglitazone and acetaminophen. Both drugs caused dose dependent cytotoxicity to cord cells with IC50 values of 24.3 uM and 18.9 mM for troglitazone and acetaminophen, respectively. The exposure of cords cells to cholestatic agents such as cyclosporine A and troglitazone resulted in inhibition of MRP2-mediated transport of DCF in a dose-dependent manner, as shown by a reduction of fluorescent signal in the canaliculi

followed by accumulation of the fluorescent dye in the cytoplasm. Overall, these results suggest that the hepatic cord tissue model can be used as an in vitro model to evaluate drug hepatotoxicity and drug-transporter interactions.

F-1298

GENERATION OF HUMAN NUCLEUS PULPOSUS CELLS FROM STEM CELLS: FIRST STEPS TOWARDS INTERVERTEBRAL DISC REGENERATION

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The nucleus pulposus (NP) is the central part of the intervertebral disc (IVD) and plays a key role in spine kinematic. At birth, the NP is populated by two cell types: the notochord cells (NTCs) known as the NP progenitors and the nucleopulpocytes (NPCytes) which are responsible for the synthesis of NP-specific extracellular matrix. The early decline of the NTCs followed by the progressive disappearance of the NPCytes initiates an irreversible degenerative cascade in the NP ultimately leading to the loss of IVD biomechanical properties. In this context, repopulating a damaged NP with regenerative cells seems to be a promising approach for long-term regeneration of IVD. Our objectives in this study are (i) to generate notochord precursors from human induced pluripotent stem cells (hiPSC) and (ii) NPCyte-like cells from human adipose stromal cells (hASC). Both Nodal and Wnt signaling pathways are required for the formation of the notochord in the mouse embryo. Notochord cells arise from progenitors expressing FoxA2/Brachyury. Human iPSC differentiation was thus induced with activin A and CHIR99021 (inhibitor of GSK-3). Our results showed that 30% of differentiated hiPSC expressed both FoxA2 and Brachyury after 24 hours of treatment by activin A and CHIR99021, whereas activin A alone induced only 10% double positive cells. In parallel, NPCyte-like cells were generated from hASC cultured in the presence of both Transforming Growth Factor-β1 (TGF-β1) and Growth Differentiation Factor 5 (GDF5). Our results showed that differentiation towards NPCyte lineage is robust and highly reproducible (8 patients). In addition, the transplantation of these engineered NPCyte-like cells in nude mice subcutis demonstrated that they survived, maintained their specialized phenotype and secretory activity in vivo. Finally, we have analyzed the role of TGF-β1 and GDF5 canonical pathways during differentiation. Our results indicated that the acquisition of the NPCyte molecular identity was mainly governed by the Smad2/3 pathway while the acquisition of NPCyte morphology was controlled by the Smad1/5/8 pathway. The combination of both engineered cell types may show a great potential for the restoration of the biological equilibrium of the NP and thus provides a long-term regenerative strategy for degenerated IVD.

F-1299

SURVIVAL AND FUNCTIONALITY OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED OLIGODENDROCYTES IN A NON-HUMAN PRIMATE MODEL FOR MULTIPLE SCLEROSIS

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Fast remyelination by endogenous oligodendrocyte precursor cells (OPCs) is essential to prevent axonal and subsequent retrograde neuronal degeneration in demyelinating lesions in Multiple sclerosis (MS). Particularly in chronic lesions of MS, the remyelination capacity of endogenous OPCs has become insufficient. Cell therapy with exogenous remyelinating cells has been forwarded as a potential strategy to replace the failing endogenous OPCs, but the absence of a suitable autologous source for such cells has hampered such an approach so far. The developments in somatic cell reprogramming and the generation of induced pluripotent stem cells (iPSCs) have provided a unique autologous source for remyelinating cells. In the present study, we have differentiated human iPSCs (hiPSCs) into OPCs. After establishing their proper functionality in in-vitro (co-culture with axons) as well as in-vivo (cuprizone-fed mice) myelination models, we stereotactically injected GFP-labelled hiPSCs-derived OPCs in the cortex above the corpus callosum of marmoset monkeys with EAE (experimental autoimmune encephalomyelitis). EAE was induced with recombinant human myelin oligodendrocyte glycoprotein extracellular domain (1-125) (rhMOG) formulated in incomplete Freund's adjuvant; this nonhuman primate EAE model is considered the most adequate animal model for RRMS as it accurately mimics the relapsing-remitting disease course and the occurrence of typical demyelinated, inflammation-invested lesions as found in the brain of MS patients. At 40 days after stereotactical implantation, we could demonstrate that the grafted hiPSCs-derived OPCs do survive and migrate towards the MS-like demyelination lesions in the corpus callosum where they started to remyelinate denuded axons. Our findings provide the first evidence for the therapeutic potential of autologous iPSC-derived OPCs to induce remyelination in MS.

F-1300

FEEDER-FREE PIG IPSCS GENERATED BY A POLYSISTRONIC RETROVIRAL SYSTEM ARE ABLE TO DIFFERENTIATE IN FUNCTIONAL CARDIOMYOCYTES BY WNT PATHWAYS MODULATION

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The beneficial effects of the adult stem cells transplantation post-myocardial infarction are related with cardiac protection but not cardiac regeneration. Induced pluripotent stem-cells (iPSCs) obtained from somatic cells are an unlimited source of embryonic-like cells with a more robust differentiation capacity. Pigs are a suitable large model to test pre-clinic therapeutic approaches using stem cells. We tested the hypothesis that the pig iPSCs generated by polysistronic retroviral approach are able to differentiate in functional cardiomyocytes in vitro by small molecules modulation of Wnt pathways. pASCs from an adult pig, in passage 5, were transduced with a polysistronic lentiviral vector (STEMCCA) in feeder-free conditions and kept until day 15. Colonies were manually picked and cultured on matrigel coated dishes in pig-iPSC-media (KO-DMEM, 20% KOSR, 0.1mM NEAA, 1mM L-Glut, 0.1mM β-mercaptoethanol,

10ng/mL beta-FGF and 10ng/mL LIF) until passage 10. piPSCs were subjected to cardiac differentiation media (RPMI, 10% FBS) plus GSK-3 inhibitor (CHIR) on day 0 and IWP-2/IWP-4 on days 4-5. Beating cells were observed on day 8. Different clones of piPSCs generated by STEMCCA virus, in a feeder-free condition, expressed the pluripotent markers such as: Oct-4, Sox-2, NANOG, TERT, DPPA5, DNMT3-beta and LIFR. These cells showed trilineage differentiation potential by expression of ecto (NeuroD), meso (Enolase) and endodermal (AFP) markers in embryoid body assay. Also, piPSCs showed no chromosomal aberrations up to passage 16. In addition, piPSCs were differentiated in functional beating cardiomyocytes by modulation of Wnt pathways, despite the low efficiency. piPSCs are able to differentiate in functional CM-piPSCs by modulation of Wnt pathways. The efficiency of this protocol must be improved to attain large number of functional cardiomyocytes. These cells can be used for novel cardiac repair therapeutic strategies and as cell model systems for disease modelling.

F-1301

DEFINING THE EARLIEST STEP OF VASCULAR DEVELOPMENT USING CRISPR GENOME EDITING TECHNOLOGY

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We hypothesize that the key vascular genes Mesp1, Isl1, Vegfa and Dlk1 play significant roles in the decision of stem cell fate of iPSCs into vascular progenitor cells. Understanding of vascular development is of great importance in order to facilitate tissue regeneration e.g. in heart failure, by promoting new vessels in the damaged area of the heart. To understand the vascular development it is crucial to investigate how stem cells adopt specific cell fates and subsequently assemble into functional vessels. Novel techniques have emerged that enables genome editing in a variety of cell types, including human induced pluripotent stem cells (iPSCs). These techniques are powerful tools to study how specific genes influence cell fate decisions in the early differentiation stages and throughout development. We study four cardiovascular key-factors (*Mesp1*, *Islet1*, *Vegfa* and *Dlk1*) and their effect on stem cell decision and vascular development. To investigate the impact of each of the chosen key-factors during vascular cell lineage differentiation, we use the CRISPRi technology. This technique facilitates inducible and sequence-specific repression and re-expression of the genes of interest. The modified iPSC lines are characterized during differentiation and analyzed for preferences of cell fate; favoring pluripotentcy, mesodermal or any of the vascular cell types. The analysis is performed using flow cytometry and immunocytochemistry to quantify the number of pluripotent cells (SOX2+ and OCT4+), mesodermal cells (FLK1+), and each of the terminally differentiated vascular cell types; endothelial cells (ECs) (CD31+), including arterial (Cn40+) and venous (EPH4+) endothelial cells and vascular smooth muscle cells (aSMA+). In addition, tube formation assay and LDL uptake assay are used to assess functionality of the vascular cells. We have already generated several highly specific and efficient iPS CRISPRi cell lines. Five guideRNAs targeting each of the promoters of *Mesp1*, *Isl1* and *Dlk1* have been transfected and 5 cell lines per gene has been verified to repress the gene of interest. Results obtained in the present project are expected to add significant knowledge to the regulation of stem cell fate in general, and this may on a long term be used in advancing novel stem cell therapeutics.

F-1302

GENERATION OF HUMAN PLURIPOTENT STEM CELL REPORTER LINES FOR THE ISOLATION OF AND REPORTING ON ASTROCYTES GENERATED FROM REGIONALLY SPECIFIED NEURAL PROGENITORS

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Astrocytes play a critical role during the development and the maintenance of the CNS in health and disease. Yet, their lack of accessibility at embryonic stages, and from diseased patients, has hindered our understanding of their full implication in developmental and pathogenic processes. Human pluripotent stem cells (PSCs) can be used to generate large quantities of astrocytes in vitro, for example for mechanistic studies of development and disease. However, these studies require highly pure populations of astrocytes, which are not always achieved depending on the PSC lines and protocols used. Therefore, there is a need to generate robust cellular models to circumvent this barrier. Here, we describe the generation of human PSC reporter lines expressing TagRFP driven by the *ABC1D* region of the human *GFAP* promoter. *GFAABC1D::TagRFP*-expressing astrocytes generated from regionally defined PSC-derived neural progenitors express NESTIN, S100β, CONNEXIN43 and NF1A markers; they can be purified by fluorescent-activated cell sorting and maintain a bright expression for several additional weeks. These new cellular models, from which highly pure populations of fluorescence-expressing astrocytes can be obtained, provide a new platform for studies where pure or fluorescently labeled astrocyte populations are necessary, for example to assess pro-inflammatory cytokine and chemokine release in response to specific treatment, and uptake and degradation of fluorescently labeled pathogenic proteins, as reported in this study.

F-1303

INDUCED PLURIPOTENT STEM CELLS DERIVED FROM IDIOPATHIC PARKINSON'S DISEASE PATIENTS DIFFERENTIATE INTO MIDBRAIN DOPAMINERGIC NEURONS AND IMPROVE MOTOR FUNCTION OF PARKINSON'S DISEASE MODEL RATS

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Induced pluripotent stem cells (iPSCs) are promising source for cell replacement therapies. However, several problems remain to be solved before they can be used in clinical settings. One of such problems is immune rejection by the hosts, and autologous transplantation of iPSCs derived from patients themselves is a possible solution. In this study, we show that iPSCs derived from patients with idiopathic PD can differentiate into dopaminergic neurons using our feeder-free culture method. First, we generated iPSCs from the peripheral blood of 3 PD patients by reprogramming with episomal vectors. Generated iPSCs have similar morphology with human embryonic stem cells and expressed undifferentiated pluripotent marker genes such as Oct-3/4, Nanog, Tra-1-60 and SSEA-4. Polymerase chain reaction (PCR) confirmed that these iPSCs had no genomic integration of the episomal vectors. Then we differentiated these iPSCs into DA neurons. On the day 0 of neural induction, pluripotent iPSCs were seeded onto laminin E8 fragment-coated well plates in GMEM supplemented with 8% KSR. With the addition of Nodal and BMP inhibitors from day 0 to day 12, most of the cells were positive for PSA-NCAM, an early neuronal marker, at day 12. Purmorphamine and fibroblast growth factor 8 (FGF8) were added from day 1 to day 7 to induce DA neuronal progenitors. At day 12, I performed fluorescence activated cell sorting (FACS) and sorted Corin+ DA neuronal progenitors. From day 12 onwards, the cells were cultured with neurobasal medium supplemented with B-27 supplement, brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), dibutyryl cyclic AMP, and Ascorbic acid for further neuronal maturation. Immunohistochemistry indicated midbrain DA neuronal identity of the generated neurons. We compared the efficiency of DA neuronal differentiation, and found no significant difference between PD patient-derived iPSCs and normal control cells. These neurons improved the motor behavior of PD model rats when transplanted to the brain of the animals. In conclusion, we differentiated iPSCs derived from PD patients into midbrain DA neurons with our feeder-free differentiation method and these cells improved motor behavior of PD model rats.

F-1304

HIGHLY EFFICIENT MEDIA SYSTEM FOR DEFINITIVE ENDODERM INDUCTION FROM HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) provide the potential to differentiate into multiple cell types, thus are useful tools for both basic and therapeutic research. Paramount to reaching their potential is the simple and robust differentiation of stem cells to the desired end cell type. Definitive Endoderm (DE) derived from hPSCs provide a valuable source of cells for hepatic and pancreatic biology basic research as well as applied studies in pharmacological drug discovery, and cellular therapies. Development of a highly pure population of DE is critical to downstream differentiation to multiple lineages including liver, pancreas, and gut. However, optimizing cultures to obtain sufficient differentiated cell yields has been limited by complex steps with multiple component additions, lengthy protocols and high variability between hPSC lines. We have developed a fully defined, ready-to-use, animal origin free media system that allows efficient induction of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) into DE. We demonstrate that our PSC Definitive Endoderm Induction kit generates >90% Cxcr4^{+}/Pdgfrα^{-} cells within 48 hours of induction which is accompanied by loss of pluripotency markers and gain of definitive endoderm markers. Differentiation is highly efficient and reproducible across multiple hESC and iPSC cell lines, yielding cells that express markers of definitive endoderm including CXCR4, SOX17, and FOXA2. In addition, these cells maintain their ability to subsequently differentiate towards anterior, posterior, and mid/hind gut lineages. This simple and highly robust induction of hPSCs to definitive endoderm is an important step to achieve highly functional and pure populations of terminal endodermal lineages which can enable research and therapies.

F-1305

AN EASY TO ACCOMPLISH ENDOTHELIAL CELL DIFFERENTIATION PROTOCOL FROM PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) generated from patients is carrying identical genetic information as in the patients, which may re-implement the disease phenotype in vitro. In the area of vascular disease treatment and drug discovery, endothelial cells (ECs) derived from human induced pluripotent stem cells (hiPSCs) are promising models. Especially for drug screening, in various disease models, hiPSC-derived ECs are highly desirable as they carry the same genetic background as the patients. The high efficiency and functional ECs derived from the patients' specific hiPSC will facilitate the buildup of vascular disease model. Endothelial cells and endothelial progenitors are generated from mesoderm during embryo development. Theoretically, the in vitro differentiation process can recapitulate the in vivo development of ECs. The general endothelial cell differentiation protocol will be generation of the mesoderm cell from pluripotent stem cells (PSC). Following is the derivation of endothelial progenitor, which can be induced into different endothelial cells. As the mesoderm cells, contribute to the derivation of endothelial cells progenitor, the generation of pure mesoderm cells is a necessary step for derivation of high efficiency endothelial cells. Here, by mimicking differentiation of endothelial cells in vertebrates by addition of various combinations of cytokines to hiPSCs in vitro, we show a simple and serum-free way to generate cardiac endothelial progenitor cells. Secondly, we demonstrate how endothelial cells can be generated at high efficiency from these progenitor cells, efficiency can as higher as around 60%. We also provide evidence how specific combinations of cytokines have a diverse effect on subtypes of endothelial cell generation. Furthermore we find that in addition to arterial, venous and lymphatic endothelial cells, a specific subset of endothelial cells can be distinguished from the heterogeneity of endothelial cells. A high efficiency and functional endothelial cells differentiation method is developed in our group. With this method, different further genetic analysis will be performed in the iPSC-EC model, which may provide a deep learning of vascular disease pathogenesis.

F-1306

HUMAN NEURONS DIRECTLY DIFFERENTIATED FROM FXS-IPS CELLS AND ENGINEERED FMR1-NULL ES REVEAL DEFECTS IN HOMEOSTATIC PLASTICITY ASSOCIATED WITH FRAGILE X SYNDROME

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The pathogenesis of Fragile X Syndrome (FXS) remains elusive because studies of the human brain are largely restricted to imaging or post-mortem analyses. Animal models only partially recapitulate the defects observed in FXS and the success rate for translating drugs effective in rodents is disproportionately low. Numerous defects associated with synapses morphology and function have been described in the fragile X mental retardation protein (FMRP) knock-out mice but the extent of these findings to human remains to be confirmed. Recently, it has been show that FMRP is essential for the increase in synaptic strength induced by retinoic acid (RA) in the mouse hippocampus. This is a particular form of homeostatic plasticity that is essential to stabilize the overall activity of neural networks. Suppression of synaptic activity increases synaptic strength by inducing synthesis of retinoic acid (RA), which activates postsynaptic synthesis of AMPA-type glutamate receptors (AMPARs) in dendrites and promotes synaptic insertion of newly synthesized AMPARs. The in vitro differentiation of induced pluripotent stem (iPS) cells provides unprecedented opportunities. However, the unique genetic background of each individual causes a line-to-line variability, which greatly hampers the study of subtle phenotypes such as the synaptic related ones. Here we present an isogenic model of human embryonic stem (ES) cells carrying a floxed allele for the Fmr1 gene that can be used both as control and Fmr1 knockout (KO) upon Cre-mediated recombination in a time-controlled manner. This approach utilizes a gene targeting system based on adeno associated virus (AAV). The obtained Fmr1 conditional KO lines were subsequently used for transcription factor - based direct differentiation to induce ES into synaptically competent excitatory neurons (ES-iN). We analyzed the homeostatic synaptic plasticity of ES-iN by comparing iN infected with Cre and delta-Cre (a catalytically inactive mutant) and iN derived from FXS patient and control iPS cells. Consistently with the findings in mouse we observed that loss of FMRP in human neurons completely abolishes synaptic scaling. Our results raise the possibility that at least some of the symptoms of fragile X syndrome reflect impaired homeostatic plasticity and impaired RA signaling.

F-1307

OPTIMIZATION OF CULTURE CONDITIONS FOR DIRECTING OSTEOGENESIS DIFFERENTIATION OF MOUSE INDUCED PLURIPOTENT STEM CELLS

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Application of iPS cells derived osteoprogenitor cells for skeletal regenerative medicine is expected to be a promising stem cell source for future therapies of bone regeneration in response to large defects, bone tumor resection and traumatic bone loss. However, the problems of production efficiency and application safety for generating osteoprogenitor cells from iPS cells remain unsolved. It is extremely difficult to commit osteoblast lineage cells and efficiently induce osteoblast differentiation from iPS cells under serum-free culture condition. Therefore, it will be necessary to carefully consider optimizations of not only culture media along with osteogenesis, but also other environmetal factors such as oxygen tension, surface-treated dishes and ECM proteins. In addition to optimized cytokine and compound cocktails, a combination of low oxygen tension (hypoxia) and surface-treated dish, along with ECM proteins was utilized to establish a novel efficient serum-free culture system for osteoblast differentiation of mouse iPS cells. All the appropriate concentrations of selected cytokines and compounds such as Activin, Wnt, BMP and ATRA were determined in different developmental stage from mesendoderm into mesoderm derivatives using flow cytometry and gene expression analysis. Consequently, a combination of various surface-treated dishes and ECM proteins in hypoxic culture condition was performed in an optimized culture medium which promotes osteogenesis. Effects of stepwise hypoxic conditions (for 3 days and 14 days), surface-treated dishes of these two types (commercial products A and B) were investigated. Serum-free culture conditions for osteoblast differentiation of mouse iPS cells were optimized in combination with six cytokines and two compounds. Furthermore, remarkable effects of osteogenesis could be shown by the combined use of stepwise hypoxic conditions, surface-treated dishes and ECM proteins. Hypoxia has an important role for proliferation and differentiation of mesendodermal and mesodermal cells. Surface treatments of culture dishes (i.e. plasma, thermal CVD, etc.) may contribute to promote differentiation of osteoblast lineage cells and inhibit proliferation of undifferentiated cells.

F-1308

NADPH OXIDASE 2 CONTROLS NEURAL PRECURSOR CELLS IN MOUSE AND HUMAN

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The majority of research on reactive oxygen species (ROS) has been dedicated to its deleterious effect and cellular toxicity in various pathological conditions of the central nervous system (CNS). However, recent studies have shown that a non-toxic endogenous level of ROS plays an important role during physiological signaling such as cellular growth and survival. One of the main sources of ROS in many types of cells is the NADPH oxidase (NOX) family of enzymes. Thus, we investigated the role of NOX enzymes during an in vitro neural differentiation of human induced pluripotent stem cells (iPSC) derived from normal and patients affected with chronic granulomatous disease (CGD: NOX2-/-). During neural differentiation, we observed that NOX2 expression was only detected once neural induction was triggered. Moreover NOX2 protein and ROS generation was clearly enriched in

rosette structures, known as developmental signature of neural progenitor cells in vitro. To verify our observations, we assessed the neurogenic region of wild type and NOX2-deficient adult mouse by microdissection of dentate gyrus (hippocampus) and subventricular zone. Interestingly, NOX2-deficient neurogenic regions had lower signs of redox activity and in both human and mice, NOX2 deficiency results in significantly lower expression of neural stem/progenitor genes including nestin and neurogenesis regulatory neurotrophic factor (BDNF). This study has identified a redox-mediated mechanism of neural stem/precursor cells regulated by NOX2 during early stages of neural differentiation, which may have significant implication for neurogenesis during physiological condition such as memory and learning as well as CNS pathologies and cellular repair.

F-1309

ANALYSIS OF DIFFERENCES IN GENE EXPRESSION PATTERNS OF HUMAN INDUCED PLURIPOTENT STEM CELLS FOR SELECTION OF CELL LINES SUITABLE FOR DIFFERENTIATION

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Differentiation directivities of induced pluripotent stem cell (iPSC) lines have to be well analyzed before clinical application because iPSCs need to be differentiated efficiently into intended cells. Embryoid body (EB) formation is one of the most important methods for iPSC differentiation. We previously found that the capabilities of forming EBs differed between iPSC lines, even with the same generation methods, same passage numbers, and same cell types of origin. Therefore, the objective of this study is to identify gene expression patterns that give an indication of human iPSC lines suitable for differentiation. Human peripheral mononuclear cells, which were activated with CD3 antibody and IL-2 for T-cell proliferation, were infected with Sendai virus vectors containing Oct3/4, Sox2, Klf4 and c-Myc. 12 colonies were passaged and established as iPSC lines. Efficiencies of forming EBs were compared at 10 passage intervals. Expression of pluripotent markers and differentiation markers were analyzed with RT-PCR. Global gene expression patterns were analyzed using DNA chips and ingenuity pathway analysis (IPA) on these iPSC lines. RT-PCR analyses showed that expression of pluripotent markers in these cell lines did not correlate with efficiencies of forming EBs. However, expression of differentiation markers correlated with efficiencies of forming EBs. IPA revealed that expression of BMP signal pathway-associated genes increased in iPSC lines that efficiently formed EBs. Upstream analysis also showed that genes associated with the activation of the BMP signal pathway acted as upstream regulators of gene expression in iPSC lines efficiently forming EBs. iPSC lines that efficiently formed EBs showed relatively high expression of genes associated with the differentiation signal pathway even in culture condition which maintained pluripotent states. Applying these gene expression patterns is useful for selection of cell lines suitable for clinical application.

F-1310

SMALL MOLECULE DRIVEN HEPATOCYTE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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The differentiation of pluripotent stem cells to hepatocytes is well established, yet current methods suffer from drawbacks including a lack of definition and reproducibility, which in part stems from continued reliance on recombinant growth factors. This has remained a stumbling block for the translation of the technology into industry and the clinic for reasons associated with cost and quality. We have devised a growth factor-free protocol that relies on small molecules to differentiate human pluripotent stem cells towards a hepatic phenotype. The procedure can efficiently direct both human embryonic stem cells and induced pluripotent stem cells to hepatocyte like cells. The final population of cells demonstrates marker expression at the transcriptional and protein levels, as well as key hepatic functions such as serum protein production, glycogen storage and cytochrome P450 activity.

F-1311

EFFICIENT DERIVATION OF MESENCHYMAL STEM CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS IN DEFINED CULTURE CONDITIONS

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Mesenchymal stem/progenitor cells (MSCs) are typically isolated from adult bone marrow mononuclear cells. However, their low frequency and heterogeneity, and the invasive nature of isolation from adult tissues have prompted researchers to use human induced pluripotent stem cells (hiPSCs) as an alternative source. Current approaches to derive MSCs from hiPSCs involve culturing cells in serum-containing medium on feeder layers of animal cells or using the embryoid body method but this requires extensive time in culture. We have developed a method to efficiently induce direct differentiation of hiPSCs into cells with MSC-like properties under completely defined culture conditions. Briefly, cells from a hiPSC line previously cultured in mTeSR1™medium on Matrigel® were dissociated into single cells using Gentle Cell Dissociation Reagent (GCDR) and seeded at 1×10^5 cells/cm^2 in a monolayer culture. The cells reach confluency in 5-7 days and are then dissociated using animal component-free (ACF) dissociation reagents (STEMCELL Technologies), counted and seeded at $1.5 – 7.5 \times 10^4$ cells/cm^2 on a defined substrate. Three different ACF media formulations (MesenCult™-ACF, and two variants thereof; ACF2 and ACF3) were tested. For subsequent passages, cells were seeded at $3 – 6 \times 10^3$ cells/cm^2. The proliferative potential of hiPSC-derived MSCs in each medium was measured by counting cells at each passage. Cell phenotype was analyzed by flow cytometry at different stages during the differentiation process. After 1 week in culture, hiPSCs started adopting MSCs-like morphology and could be expanded for up to 10 passages in all three ACF media. Preliminary data (n=1) indicate that the average expansion at each subculture from P1 to P10 was

lower in MesenCult™-ACF (2.5-fold) than in ACF2 (4.7-fold) and ACF3 (5.3-fold) media. More than 90% of MSC-like cells expressed markers typical of MSCs, including CD73, CD105, CD90 and CD146 at 21 days. The hiPSC-derived MSCs were able to differentiate into adipocytes, osteogenic cells and chondrocytes in vitro. These data indicate that MSC-like cells can be derived efficiently from hiPSCs using completely defined culture conditions. Studies are underway to characterize other hiPSC lines and to further improve the culture conditions.

F-1312

DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO MATURE FUNCTIONAL PURKINJE NEURONS

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It remains a challenge to differentiate human induced pluripotent stem cells (iPSCs) or human embryonic stem (ES) cells to Purkinje cells. In this study, we derived iPSCs from human fibroblasts and directed the specification of iPSCs first to Purkinje progenitors, by adding Fgf2 and insulin to the embryoid bodies (EBs) in a time sensitive manner, which activates the endogenous production of Wnt1 and Fgf8 from EBs that further patterned the cells towards a midbrain-hindbrain-boundary tissue identity. Neph3-positive human Purkinje progenitors were sorted out by using flow cytometry and cultured either alone or with granule cell precursors, in a 2-dimensional or 3-dimensional environment. However, Purkinje progenitors failed to mature further under the above conditions. By co-culturing human Purkinje progenitors with rat cerebellar slices, we observed mature Purkinje-like cells with right morphology and marker expression patterns, which yet showed no appropriate membrane properties. Co-culture with human fetal cerebellar slices drove the progenitors to not only morphologically correct but also electrophysiologically functional Purkinje neurons. Neph3-posotive human cells could also survive transplantation into the cerebellum of newborn immunodeficient mice and differentiate to L7- and Calbindin-positive neurons. Obtaining mature human Purkinje cells in vitro has significant implications in studying the mechanisms of spinocerebellar ataxias and other cerebellar diseases.

F-1313

RAPID INDUCTION AND FUNCTIONAL MATURATION OF CORTICAL INTERNEURONS FROM HUMAN PLURIPOTENT STEM CELLS

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GABAergic interneurons are the major cellular elements that control hyperexcitability in the brain. Alternations in GABAergic circuit function have been associated with multiple neurodevelopmental and neurodegenerative disorders. The ability to derive human interneurons from patients could provide a foundation for studying the pathogenesis and identifying the potential therapeutic targets for these diseases. Available methods for differentiating human pluripotent stem cells (hPSCs) into interneurons require the deviation of medial ganglionic eminence (MGE)-like progenitor cells and stepwise functional maturation of human interneurons, which take a prolonged timeline of up to 7 months. The protracted procedures present technical challenges of reproducibility and consistency of neurons derived, and renders large-scale studies difficult. In the current study, we set out to seek for transcription factor (TF) combinations that allow rapid and reproducible production of inhibitory human induced neuronal (iN) cells from hPSCs. To this end, we identified a combination of two TFs that could induce iN cells with features of interneurons with mature physiological properties. Our approach requires eight weeks to generate human GABAergic interneurons that can form functional synapses and express cortical interneuron markers, including Somatostatin, Calretinin and Calbindin demonstrating the feasibility to induce human cortical interneuron differentiation from hPSCs in vitro despite their protracted development in vivo. Given the implication of inhibitory neurons in in a broad range of disorders, our method may become useful to better model such diseases with cultured human neurons.

F-1314

USING SYNTHETIC RNA TO PRODUCE SKELETAL MUSCLE FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) have enormous potential for use in regenerative medicine. We have developed human induced pluripotent stem cells (hiPSCs) from patients with a devastating muscle disease, Duchenne Muscular Dystrophy (DMD). Skeletal muscle myofibers contain muscle stem cells called satellite cells, which regenerate muscle after acute injury. In degenerative muscle diseases including DMD, the satellite cells can become exhausted leading to failed regenerative ability. Skeletal muscle progenitor cells (SMPCs), or satellite cells in the adult muscle, lie outside the muscle fiber and refurbish the endogenous muscle stem cells upon damage or exhaustion. However, once these muscle stem cells are depleted, damaged muscles are replaced by excessive fat and extracellular matrix deposition leading to muscle deterioration and fibrosis. One goal of this work is to identify the mechanism/s through which SMPCs can be developed from hPSCs for use in muscle replacement for DMD. To develop these SMPC populations we will test the hypothesis that over-expressing the key SMPC transcription factors MYOD or PAX7 using a synthetic RNA construct will produce a SMPC-like cell (with PAX7) or myoblast (with MYOD) from multiple hPSC lines. This will be compared to the myogenic potential of the gold standard lentiviral mediated overexpression of myogenic transcription factors. Once expression is confirmed via RNA and protein analysis using multiple SMPC markers, the SMPCs will be characterized for the ability to fuse in vitro and engraft in vivo. We also plan to compare the hPSC derived SMPCs to fetal SMPCs isolated from early human fetal tissue in order to better understand the timing and specification of human SMPCs during human development.

F-1315

MATURATION OF IPSC-DERIVED GRANULOSA CELLS ARE PROMOTED BY MIRNA INHIBITION

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Human induced pluripotent stem cells (iPSCs) have the ability to self-renew and differentiate into different cell types of three germ layers, which have the great potential to be used for cell therapy. However, it remains to be a big challenge to get the functional somatic cells from iPSCs. In this study, we used multistep approaches including different combinations of many growth factors for the differentiation of human iPSCs to ovarian granulosa cells. The iPSC-derived granulosa cells showed high expression of FOXL2, AMH, AMHR2 and FSHR, but low expression of CYP19A1 by RT-PCR, immunofluorescence and Western blot, suggesting that these cells are immature. Because CYP19A1 expression is regulated by AMHR2 and FSHR, the expression of CYP19A1 in the iPSC-derived granulosa cells may be inhibited post-transcriptionally. Through miRNA microarray analysis, we identified that miR-151b is highly expressed in the iPSC-derived granulosa cells and CYP19A1 is the predicted target gene of miR-151b. Through luciferase reporter assay and Western blot, we demonstrated that miR-151b could down-regulate the expression of CYP19A1 through binding with the 3' UTR of CYP19A1 gene. Therefore, we inhibited the expression of miR-151b in the iPSC-derived granulosa cells by transfection of miR-151b inhibitors and resulted in the increased expression of CYP19A1. Subsequently, the production of estradiol by the iPSC-derived granulosa cells was significantly increased. Our results suggested that miRNA inhibition may be used to promote the generation of functional cell types differentiated from iPSCs.

IPS CELLS: DISEASE MODELING

F-1317

TARGETING HEMOGLOBIN EXPRESSION IN ERYTHROCYTES FROM SICKLE-CELL DISEASE PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

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We have recently described the directed differentiation of human cord blood CD34+ hematopoietic stem cell - derived induced pluripotent stem cells into hematopoietic progenitor and mature red blood cells (Haematologica, 2015). In line with other reports, erythroid differentiation of our induced pluripotent stem (iPS) cell lines resulted in terminal maturation into normoblasts and enucleated reticulocytes presenting predominantly fetal hemoglobin ($\alpha 2\gamma 2$). We have now generated and characterized human iPS cells from two Sickle-cell disease (SCD) patients. We can demonstrate a similar differentiation pattern towards erythroid cells resulting in more than 95% GPA+ cells with characteristics of normoblasts (89%) and enucleated reticulocytes (11%). In line with our previous report, cells expressed mainly fetal hemoglobin. The application of the CRISPR/CAS nuclease system utilizing different targeting vectors allowed us to rescue the Val-Glu6 transition in these SCD iPS cells. To investigate erythroid differentiation of rescued iPS cells in its entirety, it is necessary to induce the switch from fetal to adult hemoglobin ($\alpha 2\beta 2$). KLF1 and BCL11a are potential target genes to induce up-regulation of adult hemoglobin. We demonstrate successful transduction of iPS derived hematopoietic progenitor cells with KLF1 in the course of our erythroid differentiation protocol and the associated modulation of hemoglobin expression. The combination of SCD patient-derived iPS cells, genome editing of the disease causing mutation and ectopic expression of factors regulating hemoglobin expression allows us a more detailed description of human globin switching under normal and pathophysiological conditions and brings us closer to the in vitro production of red blood cells for clinical transfusion.

F-1318

DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FROM PATIENTS WITH RAG1 MUTATIONS INTO T-LINEAGE CELLS AS A MODEL FOR PRIMARY IMMUNODEFICIENCY DISEASE

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Primary immunodeficiency diseases (PIDs) arise from mutations within genes that are critical for immune cell development and function, which prevent the body from mounting a proper immune response and can also lead to autoimmune reactions. Due to differences between mouse and human lymphocyte development, and the rarity of access to human tissue, it is difficult to study how specific gene mutations result in PIDs. Here we report using human induced pluripotent stem cells generated from dermal fibroblasts of patients with Rag1 mutations that resulted in severe combined immunodeficiency (SCID), Omenn syndrome, or leaky SCID, and differentiated these in vitro in order to assess the alterations in T cell development and V(D)J recombination. While the RAG1-null SCID cells are blocked at the CD7+ CD5+ stage of T cell development, the hypomorphic RAG1 Omenn syndrome cells show an earlier block at the CD7+ CD5- stage. Furthermore, V(D)J rearrangement analyses of genomic DNA isolated from CD4+ CD8- and CD4+

CD8+ immature T cells indicate a restricted V and D gene usage in Omenn syndrome versus wild-type control cells. Finally, deep sequencing analyses showed an increase in CDR3 length, which together with an altered T cell receptor repertoire may explain the autoimmune phenotype seen in Omenn syndrome patients. Our findings point to a primary early defect in T cell development and repertoire selection associated with different RAG1 mutations, and illustrates the efficacy of using mutant iPSCs as a model for PIDs.

F-1319

PATIENT IPSC-DERIVED NPCS AS A MODEL FOR NEURODEGENERATIVE MTDNA DISORDERS

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Mitochondria fulfill a central role in the cellular energy metabolism. Since the editing the mitochondrial DNA (mtDNA) by traditional gene targeting approaches is inefficient there is a considerable lack of suitable mitochondrial disease models analysing mtDNA mutations in the respective nuclear genome background. However, patient cells containing the mutated mtDNA as well as the nuclear genome can be reprogrammed to induced pluripotent stem cells (iPSCs) and further differentiated to neuronal cells debilitated by mitochondrial dysfunction. We generated iPSC clones from control and patient individuals. Furthermore, we adapted a protocol to derive neural progenitor cells (NPCs) that is solely based on neural induction in defined media supplemented with small molecules. Therefore, the neural induction is very stable, reproducible, and operator-independent. We extensively characterized our NPCs with electron microscopy and at the bioenergetic as well as transcriptomic level. Analysing also numerous other published NPC populations derived with various protocols, we established a comprehensive comparison that could be relevant for the whole field of stem cell-derived and ex vivo NPCs/NSCs. All our three patients carry an mtDNA mutation in MT-ATP6. Mutations in this gene -encoding a mitochondrial ATP synthase subunit- are associated with severe infantile maternally inherited Leigh syndrome (MILS). When a certain genotype is predominant throughout the mtDNA copies per cell it can reach a grade of homoplasmy with disease penetrance. Importantly, all our iPSC lines still displayed the same homoplasmy in mtDNA as their parental fibroblasts, even upon prolonged cultivation and differentiation confirmed by mtDNA deep-sequencing. We established high-throughput-scalable screening methods to assess the mitochondrial disease phenotype. Overall, our NPCs as well as their functional neuronal derivatives could be utilized for high-content screening (HCS) as well as bioenergetic profiling. Both approaches could help to unravel molecular pathological mechanisms of disorders in the basal ganglia and to identify novel drug candidates for therapeutic intervention.

F-1320

GENERATION AND CHARACTERIZATION OF AN IPSCS-BASED MODEL FOR FAMILIAL MENIERE DISEASE FROM REPROGRAMMED MONONUCLEAR CELL

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Meniere's disease (MD) is an inner ear disorder characterized by sensorineural hearing loss, episodic vertigo and tinnitus. Using whole exome sequencing we have identified for the first time two mutations in the FAM136 and DTNA genes which can be candidate disease-causing genes. However, the functional role of these genes in MD is unknown. Since inner ear biopsies are not possible, the generation of a cellular model of MD using human induced pluripotent stem cells (iPSCs) will help to determine the pathways involved in the disease. In this study, we have generated iPSCs from a MD patient and a healthy relative from peripheral blood mononuclear cells (PBMCs) using non-integrative Sendai viruses containing Sox2, Oct3/4, c-Myc and Klf4 (CytoTune®-iPS 2.0 Sendai Reprogramming Kit; MOI = 3, Life Technologies). We used 9x105 PBMCs from two controls and one patient. The first colonies started to emerge as early as 9 days after transduction. Mature iPSC colonies could be selected on day 19, with an efficiency of iPSC generation of 0.01% and 0.03% for patient and control, respectively. Two clones, MD-iPSCs (W7) and control-iPSCs (A2), were established and characterized. First, we determined the expression of the exogenous reprogramming factors by quantitative PCR. A2 and W7 clones were maintained in culture > 25 passages on irradidated human foreskin fibroblasts feeder cells. Control and patient-derived-iPSCs have been also adapted to feeder-free cultures on matrigel. Both cell lines express the pluripotency markers hOCT4, hSOX2, hKLF4, hMYC and hNANOG at the mRNA levels, and they also express OCT4, SSEA4 and TRA-1-60 proteins as assessed by immunocytochemistry. Of notice, both cell lines showed different proliferation rates, as MD-iPSCs W7 have a doubling time of 48-53 hr, while WT A2 cells divide every 36-39 hr. Furthermore, both cell lines demonstrated pluripotency by differentiating into three germlayers by in vitro and in vivo differentiation tests. In summary, we describe the generation and characterization of patient-specific MD-iPSCs compared with control-iPSCs from a healthy relative with a common genetic background. Future studies with this cellular model, including in vitro differentiation towards otic precursors, will improve the understanding of the molecular pathways involved in MD.

F-1321

A NOVEL DISEASE MODEL TO STUDY LOW-DENSITY LIPOPROTEINS RECEPTOR FUNCTIONS

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To treat metabolic liver disorders, autologous transplantation of genetically corrected hepatocytes represents an alternative to allogenic transplantation as it circumvents the problems of

immune rejection and immunosuppression. In this context, induced pluripotent stem cells (iPSCs) represent a potential source of hepatocytes for generating patient- and disease-specific cells not only for drug screening but also for clinical applications after in vitro correction. Familial Hypercholesterolemia type IIa (FH) is a life threatening genetic disorder causing high cholesterolemia in serum and early cardiovascular diseases. It is due to mutations in the Low Density Lipoprotein Receptor (LDLR), which uptakes circulating cholesterol conjugated to Low Density Lipoprotein particles. Liver transplantation is the only curative treatment because hepatocytes are the only cells able to eliminate cholesterol via bile and are therefore the target for cell/gene therapy techniques. Using a non-integrative approach, we have generated FH-iPSCs from one patient homozygous for the Q12X mutation in the LDLR, resulting in a truncated non-functional receptor. Cells were characterized for pluripotency. We used a multi-step protocol recapitulating liver embryogenesis to differentiate them into bipotent hepatic progenitors expressing FOXA2, α-fetoprotein and cytokeratin 19, then into more mature hepatocyte-like cells expressing transcription factors such as HNF4α and specific markers such as albumin and α-1 antitrypsin. These iPSCs were also genetically corrected by targeted insertion of the therapeutic transgene cassette (LDLR cDNA controlled by ApoA-II promoter) in the AAVS1 safe harbor genomic site using CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated endonuclease 9) technology. Corrected FH-iPSCs were selected by puromycin resistance and most of the clones showed accurate targeted integration at molecular level. Phenotypic correction was demonstrated in corrected differentiated cells by internalization of a fluorescent receptor ligand (DiI-LDL). This disease model will be useful to study cholesterol pathway regulation and to further understand the entry of some viruses (Hepatitis C and VSV-G) as LDLR is one of their co-receptors.

F-1322

ALPHA-SYNUCLEIN EXPRESSION IDENTIFIED DURING OLIGODENDROCYTE DIFFERENTIATION USING RODENT PRIMARY CULTURES AND NEW HUMAN PLURIPOTENT STEM CELL MODELS OF SYNUCLEINOPATHIES

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Multiple system atrophy (MSA) is an adult-onset, rapidly progressing, fatal neurodegenerative disease of unknown etiology. The disease involves a combination of atypical Parkinsonism, cerebellar ataxia, and pyramidal dysfunction, and at later stage autonomic failure. MSA is classified as a synucleinopathy. The hallmark of the disease is the presence of alpha-synuclein (α-syn)-rich glial cytoplasmic inclusions (GCIs) in the oligodendrocytes. α-syn is an intracellular protein encoded by the SNCA gene, synthesized in neurons. In the healthy brain, aSYN protein is absent in mature oligodendrocytes. Thus, it is hypothesized that the presence of α-syn in the GCIs in MSA may have an extracellular origin, presumably neuronal. However, it is possible that under disease condition, oligodendrocytes express SNCA. This could be answered by analyzing SNCA expression in oligodendrocytes generated from MSA patient induced pluripotent stem cells (iPSCs). Here, we generated induced pluripotent stem cell from MSA, Parkinsonian and healthy patients, which we differentiated into oligodendrocytes. We showed the presence of aSYN in immature oligodendrocytes and neurons, and absence in astrocytes. Interestingly, we found that SNCA expression is not restricted to MSA iPSC-derived oligodendrocytes, implying SNCA is expressed during oligodendrocyte development. Importantly, confirm abundance of SNCA transcripts following purification of immature oligodendrocytes by fluorescence activated cell sorting. Our results therefore demonstrate that SNCA is expressed by immature oligodendrocytes. This novel finding will be of high interest to those studying the origin of α-syn in GCIs, and open new research avenues using patient iPSCs for assessing the consequences and functional role of α-syn in oligodendrocytes under physiological and pathological conditions.

F-1323

EFFICIENT AND SPECIFIC MODIFICATION OF PATIENT-SPECIFIC IPSC USING CAS9 NICKASE

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We have used Cas9_D10A nickase and specific guide RNAs (gRNA) for the targeted modification of the human SERPINA1 locus in iPSC from patients with severe α1-Antitrypsin (A1AT) deficiency. To this end, we have used an allele-specific gRNA, which can only target the mutant allele and is inactive on the wild type allele. Moreover, we have evaluated the homology directed repair (HDR) based gene targeting potency of different gRNAs together with Cas9_D10A nickase in an in vitro reporter assay. Using the two most potent opposite strand gRNAs for introduction of a double nick with 5' overhang and a high donor : Cas9+gRNA ratio we were able to reach more than 1% gene targeting efficiency. Finally, we compared the gene targeting accuracy of Cas9 nuclease and Cas9 nickase in patient-specific A1AT deficiency iPSC by co-transfection of a piggyBac-flanked puroΔtk-selectable donor. Using a multiplex PCR based analysis we found that most of the Cas9 nuclease targeted clones had off-target integrations, while all of the nickase targeted clones showed correct monoallelic integration in the A1AT locus. Moreover, when we applied the double nick-technique we found biallelic donor integration in 40% of all clones. We demonstrate that we can use the silencing-resistant CAG promoter for expression of the puroΔtk-cassette and use it for puromycin selection and FIAU counter-selection, after piggyBac excision. Using a silencing-resistant promoter, we should be able to increase the efficiency of the FIAU counter-selection, which relies on functional expression of the thymidine kinase (Δtk). Taken together, we have successfully used CRISPR/Cas9 for high efficiency and high accuracy precision genome engineering in human cells. Using an allele-specific double-nick strategy and a selectable donor with a silencing-resistant promoter, we have established a system which will allow for high-throughput genome engineering in the future.

F-1324

UNCOVERING THE ROLE OF HYPERMETHYLATION BY CTG EXPANSION IN MYOTONIC DYSTROPHY TYPE 1 USING MUTANT HUMAN EMBRYONIC STEM CELLS

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Myotonic dystrophy type 1 (DM1) results from CTG repeat expansions in the 3`UTR of the myotonic dystrophy protein kinase (*DMPK*) gene. The CTG expansion commonly results in hypermethylation and reduced expression of the downstream neighbor gene, *SIX5*. The contribution of hypermethylation to the disease pathogenesis is poorly understood, nor are the mechanisms by which SIX5 expression is reduced. Here we characterized DNA methylation upstream of the CTG repeats in mutant human embryonic stem cells (HESCs), and explored its regulatory role in local gene transcription. Using a wide range of DM1-affected HESCs (14 cell lines), as well as DM1 affected iPS cells, we identified a disease-associated differentially methylated region (DMR) upstream to the CTG repeats that abnormally gains methylation in a way that strongly correlates with expansion size specifically in undifferentiated cells, and coincides with the reduction in SIX5 expression. Using in vitro and zebrafish functional assays, including in vitro differentiated transgenic cardiomyocytes, we provide evidence for the regulatory activity of the DMR that is lost by the gain of abnormal methylation. Taken together, our study is the first to describe a disease-associated DMR that functions as an exon coding sequence in addition to a regulatory element, whose activity is epigenetically hampered by a heritable mutation. In addition and more generally, this study emphasizes the power of mutant HESCs in deciphering mechanistic relations that are exclusive in undifferentiated cells such as the relation between methylation spreading and expansion size (associations would not have been revealed if not for the availability of a large and heterogeneous cohort of mutant HESC lines). Lastly, this study emphasizes, the strength of mutant HESCs in disease modeling by affording the exploration of human based disease relevant tissues, impaired cardiomyocytes, that are otherwise inaccessible for research.

F-1326

GENERATION OF DISEASE-SPECIFIC INDUCED PLURIPOTENT STEM CELLS FOR CELL-BASED DISEASE MODELING AND DRUG SCREENING OF HEREDITARY SPASTIC PARAPLEGIA TYPE 5

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Hereditary spastic paraplegia (HSP) refers to a group of rare monogentic disorders which are characterized by a progressive axonal degeneration of corticospinal motor neurons, leading to spasticity and weakness of the lower limb. 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. In cultures of motor neuron like cells (NSC-34) and 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. Research into molecular pathogenesis of HSP is limited by the restricted access to primary neurons and hepatocytes 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. We reprogrammed primary fibroblasts of five SPG5-patients and two age-matched controls using non-integrative episomal plasmids and characterized genetic integrity and pluripotency of the generated iPS cells. Differentiation into iPSC-derived neurons and hepatocyte-like cells could be established, leading to an in vitro human cell model genetically identical with our patients. We aim to use this cell model to study further molecular mechanisms of SPG5 and to screen compounds for potential disease-specific biochemical or functional effects. These studies will improve our insight in pathogenesis of SPG5 and may help to develop new therapeutic approaches for the treatment of HSP.

F-1327

INVESTIGATING THE UNDERLYING MECHANISMS IN HIGH RISK CAD BY STUDYING PATIENT SPECIFIC IPSCS DERIVED HEPATOCYTE MODELS IN VITRO

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Coronary artery disease (CAD) remains asymptomatic for decades and conventional laboratory tests such as LDL-C are known as poor predictors of chronic diseases. At least 10% of the chronic events occur in apparently healthy individuals in the absence of traditional risk factors. In addition, while some of the atherosclerotic plaques remain stable during the lifetime, others can be highly vulnerable and lead to heart attack and sudden death. In this study we investigate if molecular lipid species such as distinct phospholipids and sphingolipids could be better predictors of clinical outcome of CAD. Here, we aim to develop an in vitro model of CAD patient's hepatocytes to study key lipids involved in formation of vulnerable atherosclerotic plaques. To achieve our aim, induced pluripotent stem cells (iPSCs) have been developed from the skin biopsies of three patient groups: acute, stable CAD and control. Then iPSCs have been differentiated to functional hepatocyte-like cells. Three hepatic differentiation methods were tested to find the best protocol for our purpose and results have been compared. The gene expression of SOX17, FOXA2, AFP, ALB, and LDL-R was evaluated by qPCR and confirmed by Immunocytochemistry. We show that hepatocyte-like cells were able to uptake LDL, store lipids, and secrete LDL, TG, albumin, as well as urea. However, we did observe that various methods produced hepatocytes with different morphology and functionality. Lipidomic data by mass spectrometry revealed that lipid profile varies between hepatocytes produced by different methods. In conclusion, we have successfully set up an in vitro patient specific hepatocyte model which is functional and is capable of secreting lipids. This model can be an invaluable platform to investigate the underlying mechanisms in CAD which can lead us to discover novel lipid biomarkers for high risk CAD. We already have nominated some candidate lipids as reliable predictors of risky CAD.

F-1328

HUMAN STEM CELL DERIVED MOTOR NEURONS TO MODEL MULTIFOCAL MOTOR NEUROPATHY

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The lack of understanding of the complex underlying immune pathological disease mechanisms in multifocal motor neuropathy (MMN) and other inflammatory neuropathies represents a major challenge in the discovery of novel therapeutic targets. Functional screening for compounds that prevent neuronal damage is a hurdle due to a lack of available in vitro disease models. MMN is an immune-mediated pure motor neuropathy, associated with IgM antibodies against GM1 gangliosides in the serum of approximately 50% of patients. There is indirect evidence that anti-GM1 IgM antibodies cause damage to neurons, but their pathogenicity remains controversial. Here we examined antibody binding and pathogenicity of IgM anti-GM1 antibodies in MMN using human iPSC-derived motor neurons (MNs) as a novel disease model for inflammatory neuropathies. We show for the first time that IgM anti-GM1 antibodies present in the serum of MMN patients bind to GM1 gangliosides of iPSC-derived motor neurons, and did this in a titre specific manner. Furthermore IgM anti-GM1 antibodies show complement activation, and in vitro incubation of patient serum leads to axonal damage. Our findings show for the first time binding of IgM anti-GM1 antibodies to human motor neurons in MMN, and pathogenic effects of these antibodies using iPSC-derived motor neurons. We describe a novel platform that can be used for (i) examining the pathogenic effects of autoantibodies and (ii) as a potential screening method for therapeutics for MMN and other inflammatory neuropathies.

F-1329

HGF RELEASING HYDROGEL PROMOTED ANTIOXIDANT ABILITY OF DENTAL PULP DERIVED IPSC DIFFERENTIATED HEPATOCYTES

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Acute hepatic failure (AHF) is a severe liver injury leading to sustained damage and complications. Induced pluripotent stem cells (iPSCs) may be an alternative option for the treatment of AHF. Because of its accessibility and differentiation potential, dental pulp has gradually drawn attention in regenerative medicine. However, it is unknown whether the resultant dental pulp-reprogrammed iPSCs could be further differentiated into iPSC-derived hepatocyte-like cells (iPSC-Heps) with potential hepatoprotective activity. In this study, we reprogrammed human dental pulp-derived fibroblasts into iPSCs (DP-iPSCs), which exhibited pluripotency and the capacity to differentiate into tridermal lineages, including hepatocyte-like cells (iPSC-Heps). These iPSC-Heps resembled human ESC-derived hepatocyte-like cells in gene signature and hepatic markers/functions. Hepatocyte growth factor (HGF) is a multifunctional polypeptide implicated in embryogenesis, angiogenesis, organ regeneration, and wound healing. To improve iPSC-Heps engraftment, we next developed an injectable carboxymethyl-hexanoyl chitosan hydrogel (CHC) with sustained hepatocyte growth factor (HGF) release (HGF-CHC) and investigated the hepatoprotective activity of HGF-CHC-delivered iPSC-Heps in vitro. Notably, we demonstrated that the CHC nanoscale hydrogel with sustained release of HGF (HGF-CHC), and validated the growth capacity and hepatic-like functions of iPSC-Heps cultivated in HGF-CHC. In conclusion, our findings demonstrated that HGF mediated the enhancement of iPSC-Hep antioxidant capacities, suggesting that HGF-CHC is as an excellent platform for iPSC-Hep cytoprotection.

F-1330

SOMATIC MITOCHONDRIAL GENOME MUTATIONS IN INDUCED PLURIPOTENT STEM CELLS DERIVED FROM OLDER MICE

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iPSCs offer an unlimited source of cells and tissues through autologous replacement therapies for many age-onset degenerative diseases. However, accumulations of somatic mtDNA mutations have been implicated in age-related diseases and aging itself. We investigated mtDNA integrity in skin fibroblasts and their iPSCs derivatives isolated from young and old mice. Whole mitochondrial genome sequencing using Miseq platform did not reveal any mtDNA mutations in fibroblasts or iPSCs derived from fetal or adult mice (up to the 8 month old). However, several homoplasmic and high level heteroplasmic mtDNA mutations were discovered in older animals (> 10 month old). Parental fibroblasts and ten examined individual iPSC lines from the 34-month-old mouse carried non-synonymous 13029 G>A point mutation in ND5 gene (86-100% heteroplasmy). In addition, heteroplasmic coding mutations in 16S-rRNA (2008 G>A) and ND1 (3625 Del A) genes were found in some but not other iPSC lines. We induced reprogramming of the same 34-month-old mouse fibroblasts by SCNT into enucleated oocytes and, as expected, the resulting NT-ESC line carried donor oocyte mtDNA with no detectable mutations. In summary, these results suggest that pathogenic mtDNA point mutations accumulate with age in mouse skin fibroblasts and are transmitted to iPSC derivatives. Further studies are warranted to explore functional effect of these somatic mtDNA mutations on iPSC potential in regenerative medicine.

F-1331

DIFFERENTIAL ISOFORM EXPRESSION IN AN IN VITRO MODEL OF CARDIAC HYPERTROPHY USING HIPSC-DERIVED CARDIOMYOCYTES

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Left ventricular hypertrophy (LVH), defined as a pathological increase of LV mass, is an independent risk factor for cardiovascular diseases. LVH is accompanied by significant differential gene expression in cardiomyocytes (CMs). However, little is known about alternative splicing and the role of differentially expressed isoforms (DEIs). We performed Next-Generation RNA sequencing in an in vitro model of cardiac hypertrophy using hiPSC derived CMs (hiPSC-CMs) to determine the prevalence, pattern and inter-individual variability of DEIs. Hypertrophy was induced by endothelin-1 stimulation in hiPSC-CM lines from two LVH patients, who vary by ejection fraction and the severity of LVH. Transcripts were analyzed for relative abundance using Cufflinks2. We found that 433 and 770 genes showed significant expression changes in the two hiPSC-CM lines respectively. Interestingly, more than half of these genes have two or more annotated isoforms. A significant portion (~5%) of identified DEIs was unique to an individual CM line. Subsequent Gene Ontology analysis of these genes showed enrichment in cardiac contraction and myofibril assembly pathways. Further, several well-known genes associated with hypertrophic cardiomyopathy showed patient-specific DEIs. For example, out of the seven isoforms of Troponin T type 2 (TNNT2), two DEIs were found in one CM line while four DEIs were found in the other line. In order to explore potential functional impacts of DEIs, we compared the exons that were unique to DEIs to those that were common to all isoforms. These unique exons contained several well-characterized functional domains encoded by genes implicated in cardiac hypertrophy and cardiomyopathy, including hypoxia-inducible-factor 1, POPDC3 and FHL1. Intriguingly, over 40% of the DEIs exhibited exon length variation at the 3'end, suggesting a potential role of alternative 3' splice sites in isoform regulation. Taken together, we illustrated that changes in isoform expression were prevalent in an hiPSC-CM model of cardiac hypertrophy with a subset of DEIs unique to each patient. Our study indicated that DEIs contributed to the overall disease phenotype and demonstrated the power of hiPSC-based disease modeling to dissect genetic, molecular, and environmental factors in complex diseases.

F-1332

THE TRANSCRIPTOME OF THE HUMAN BLOOD-BRAIN BARRIER USING NORMAL AND HUNTINGTON'S DISEASE IPSCS

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With the advent of iPSC technologies we have been given the opportunity to model new cellular systems and human diseases in a novel and powerful context with limited perturbations to the normal genetic background. Utilizing this technology with next generation sequencing not only allows us to investigate how well in vitro models recapitulate the in vivo system but also permits us to determine what attributes of a model can be accurately assessed for changes during disease. Here we use iPSCs to recapitulate the human Blood-Brain Barrier (BBB) transcriptome through the differentiation of human iPSCs into brain endothelial cells that express the proper markers and functionally maintain barrier properties equivalent to the in vivo system. Whole transcriptome analysis (RNA sequencing) was used to determine global expression profiles of these cells and how they compare to publicly available datasets of the human BBB transcriptome. We highlight the similarities and differences observed to establish the parameters of the iPSC model for use in studies of the BBB. Potential disease related phenotypes were investigated using HD patient cells. HD is a devastating, neurodegenerative disease that typically strikes in mid-life and is caused by a CAG repeat expansion within the coding region of the HD gene. Due to the genetic basis of HD, unique opportunities exist to model the disease and extend scientific findings to other neurodegenerative diseases. Recent evidence of vascular abnormalities and changes in genes controlling the BBB in the CNS of HD patients and animal models of HD, suggests that the NVU and BBB may contribute to HD pathology. We therefore hypothesized that abnormal signaling exists in and between BECs, astrocytes and neurons which causes abnormal expression of BBB proteins. Our data shows transcriptional changes in these cells that correspond to observed CAG repeat-associated functional deficits that could directly contribute to HD pathogenesis. Future studies will focus on whether these changes are due to cell autonomous effects or altered signaling between HD astrocytes or neurons.

F-1333

IPSC-DERIVED NEURONS WITH LRRK2 MUTATIONS ASSOCIATED TO PARKINSON DISEASE SHOW DEFECTIVE NFKB RESPONSES TO PRO-INFLAMMATORY STIMULI

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Mutations in leucine-rich repeat kinase 2 (LRRK2) contribute to both familial and idiopathic forms of Parkinson disease (PD), but the normal function of the protein and its role in the disease is not clear. Neuroinflammation is a key factor in neurodegeneration and aging. Previously, we found that the inflammatory response was attenuated in dermal fibroblasts from patients with two mutations in LRRK2 (G2019S and R1441G) and with idiopathic PD, which showed weak induction of pro-inflammatory cytokines and reduced NFκB activation. To study this pathway in a disease-relevant cellular context, we generated iPS cell lines harboring LRRK2 mutations and differentiated them into dopamine neurons. At day 50, neurons with LRRK2 mutations showed reduced NFκB stimulation with IL1β in luciferase reporter assays. This defect was particularly prominent in LRRK2^{R1441G} neurons (82±7% decrease *v.* control neurons). NFκB activation with TNFα was also impaired while baseline NFκB activity appeared higher in PD neurons. Following an inflammatory stimulus (IL1β), protein levels of the NFκB inhibitor IκBα showed a rapid degradation in all neurons, but the recovery curve was protracted in neurons with LRRK2 mutations. Further confirming LRRK2 involvement in the NFκB pathway in neurons, lentiviral silencing of LRRK2 expression (66±12% decrease) markedly reduced the IL1β-

induced NFκB response (63±15% v. stimulated mock control) in control neurons. The effect of LRRK2 silencing on NFκB signaling was less clear in neurons with LRRK2 mutations, suggesting that pathway activity may benefit from knocking down the defective protein. Several lines of evidence indicate that the pathogenic mechanisms of LRRK2 and α-synuclein converge. Indeed, LRRK2 silencing in control neurons decreased α-synuclein protein levels (29±14% reduction). Furthermore, we observed that IL1β treatment reduced α-synuclein levels concomitantly to the degradation of IκBα. This suggests that impaired NFκB signaling in PD neurons may underlie the dysregulation of convergent pathogenic pathways, such as α-synuclein dynamics. iPS cell-derived DA neurons offer a powerful tool to discover novel targets with disease-modifying potential.

F-1334

CUREMOTORNEURON - A NOVEL IPSC APPROACH TO ADVANCE ALS RESEARCH

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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. *'CureMotorNeuron'*, 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.

F-1335

ESTABLISHMENT OF NEW DRUG SCREENING SYSTEM USING FIBRODYSPLASIA OSSIFICANS PROGRESSIVA IPS CELLS

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Fibrodysplasia Ossificans Progressiva (FOP) is a rare genetic disease characterized by progressive ectopic ossifications, which are often initiated after physiological stimuli. The responsible gene for FOP is the ACVR1 gene, which is one of type I receptors for BMP. The replacement of one amino acid in ACVR1 seems to activate downstream genes regulating bone formation, although the precise mechanisms are still to be investigated. Application of patient-derived iPS cells will be useful for the research of this disease, because harvesting target tissues from patients is strictly prohibited because tissue damage accelerates the ectopic ossification. Recently we have reported that iPSCs derived from FOP patients showed enhanced osteogenic and chondrogenic differentiation properties. Because experiments were done under the culture condition without exogenous BMP signals, these data may support the hypothesis that the mutant ACVR1 can be activated via BMP-independent mechanisms. However, the precise molecular pathway responsible for the enhanced differentiation properties are unknown, which requires to investigate how and when the ACVR1 is activated during the differentiation process. Here we addressed this issue by a new approach using the differentiation system through the neural-crest cell lineage. Multi-potent neural crest cells (NCCs) were efficiently induced from iPSCs, from which multipotential mesenchymal stromal cell (MSC)-like cell (iMSCs) population can be obtained. Therefore we are able to analyse the signal pathway at each of iPSC, NCC, iMSC, and terminally differentiated stage. In addition, to minimize the effect of genetic background other than ACVR1 mutation, we established genetically rescued cell lines from parental FOP-iPSCs by the homologous recombination. FOP-iMSCs possessing enhanced chondrogenic ability were transcriptionally distinguishable from resFOP-iMSCs and activated the SMAD1/5/8 and SMAD2/3 pathways at steady state. Using this method, we identified responsible genes for accelerating the chondrogenesis of FOP-iMSCs. This indicates that iMSCs through NCC lineage are useful for investigating the molecular mechanism of FOP and corresponding drug discovery.

F-1336

GENERATION OF CYSTIC FIBROSIS-PATIENT DERIVED IPS CELLS AND SELECTION-FREE CFTR GENE CORRECTION USING TALENS AND SINGLE STRANDED OLIGONUCLEOTIDES

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Cystic Fibrosis (CF) is a monogenetic disease caused by mutations in the CFTR gene, which affects multiple organs. Effective drugs are only available for the treatment of a minority of CF patients with very specific mutations, and lung and liver pathologies are still untreatable with current interventions other than an organ transplant. Hence, novel compounds have to be identified and combinations with known CFTR correctors, potentiators and other small molecules have to be tailored to specific CFTR mutations or even to the individual patient. Obviously, the complexity of the mutant CFTR maturation requires the use of advanced cellular models that closely recapitulate the specific properties of the most clinically affected organs. With patient-specific induced pluripotent stem cells (iPSCs) and their CFTR gene-corrected controls, a valuable new platform is now available for a better understanding of different CF disease phenotypes, and for the identification of novel drugs. IPSC lines were generated from the peripheral blood of CF patients with the most common (F508del) trafficking mutation. The resulting CF-iPSCs showed a normal karyotype, expressed pluripotency markers and could be differentiated in vitro into derivatives of all three germ layers. Expression analysis of the mutated locus of our CF-iPSCs revealed CFTR mRNA expression on a similar level to a wild-type iPSC line and the disease-related presence of immature CFTR protein was confirmed. We applied our recently developed TALEN-based targeting protocol for efficient footprint less correction of the

F508del mutation using single stranded oligonucleotides (ssODNs). Although CFTR is not expressed in undifferentiated iPSCs, and adaptation of the screening procedure to relatively low targeting efficiencies was required, direct PCR screening revealed correctly targeted single iPSC clones without any pre-selection. Together with the targeted insertion of a fluorescence reporter in the endogenous CFTR locus and insertion of a halide-sensitive reporter system, CF-iPSC lines and their gene-corrected counterparts after differentiation into CFTR expressing epithelia provide a valuable new platform for CF disease modelling, and for the identification of drugs that functionally correct the organ-specific phenotype of different CFTR mutations.

F-1337

GENERATION OF INDUCED PLURIPOTENT STEM CELLS DERIVED FROM A NEUROFERRITINOPATHY PATIENT

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Neuroferritinopathy is an autosomal dominant neurodegenerative disease caused by FTL (ferritin light chain) gene mutation. Mutant and normal ferritin aggregate in the cytoplasm and nucleus of neurons, oligodendrocytes and astrocytes, thus the patient suffers from various clinical symptoms such as involuntary movements, cognitive impairment and cerebellar ataxia. The role of ferritin is to storage irons in cytoplasm mainly in the central nervous system. In the early stage of neuroferritinopathy, it is conceivable that iron atoms are emitted from ferritin polymer during axonal transport, and injure axons. However there are neither effective pathological primate models nor clinical treatments. In order to determine the cellular consequences of the disease, we used somatic cell reprogramming technology to generate the patient-specific induced pluripotent stem cells (iPSCs) by transfecting the episomal plasmids into T lymphocytes. The derived patient is a 44-year-old, Japanese female, presented initially with chronic headaches at the age of 42, later developing progressive orolingual and arm dystonia, dysarthria, cerebellar ataxia, pyramidal tract signs, and psychiatric symptoms. The findings of head magnetic resonance imaging were typical for neuroferritinopathy. Biochemical studies on the patient showed normal serum ferritin levels, but remarkably low cerebrospinal fluid (CSF) ferritin levels. She had a mutation in exon 4 of one allele of the FTL gene, c.468_483dupTGGCCCGGAGGCTGGG. We confirmed that the established cells showed a typical morphology of human pluripotent stem cells, no episomal vectors remained, normal karyotypes, and the expression of pluripotency markers. We successfully differentiated these iPSCs into neurospheres, and furthermore induced into neurons and glial cells, and detected the aggregation of ferritin light chain in the neurons and astrocytes. Additionally we are now investigating disease-specific phenotypes in the disease-specific neuronal cells.

F-1338

TRANSCRIPTOME OF THE DEVELOPING ATAXIA-TELANGIECTASIA CEREBELLUM: RNA SEQUENCING OF HUMAN IPSC-DERIVED CEREBELLAR PROGENITORS

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We report the use of patient-derived induced pluripotent stem cells (iPSCs) to generate neuronal-like cells similar to those affected in the neurological disorder Ataxia-Telangiectasia (A-T), namely Purkinje and granule cells of the cerebellum. Generation of cerebellar-like cells from murine ESCs and human embryonic stem cells has previously been reported, however isolation and manipulation of discrete sub-populations of cerebellar neuronal cells has proven challenging. Building on these studies, we successfully induce wide scale expression of mid-hindbrain markers EN1 and GBX2, and the transcription factors MATH1 and PTF1α, which demarcate rhombic lip (granule cell) and ventricular zone (Purkinje, Golgi, and Stellate cell) progenitors, respectively. We then expand these progenitors to produce cells that are morphologically similar to neuronal cells of the cerebellum. To gain insight into the early events that occur during the formation of the cerebellum and how these may be affected in the absence of ATM, RNA sequencing of neuronal progenitors was performed. After 34 days of differentiation down-regulation of pluripotency genes and up-regulation of neural commitment and anterior/posterior patterning gene programs was observed, including EN1, ISL1, MEIS1, SHH, REELIN, LHX9, WNTLESS, NFIX and members of the HOX gene family. Comparative pathway analysis between control and A-T neurons revealed gene expression patterns indicative of neurological disease, in particular progressive motor neuropathy and cerebellar ataxia, major hallmarks of A-T. This exemplifies the concept that iPS cells can be used for disease modelling purposes and presents a unique view on a window of human brain development, which has not been previously investigated. We were able to detect gene expression evidence that is congruous with a number of prominent theories based on other cellular systems and animal models regarding the nature of the neurodegeneration in A-T including dysregulation of genes involved with reactive oxygen species, DNA repair/cell cycle regulation, and neuronal long term potentiation. These data are a valuable resource for researchers to formulate and test hypotheses regarding the early developmental events that occur in the absence of ATM.

F-1339

EXPLORING THE PATHOGENESIS OF TRANSIENT MYELOPROLIFERATIVE DISORDER USING IPSCS

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Transient myeloproliferative disorder (TMD) is one of hematopoietic complication with Down syndrome (DS), characterized by transient abnormal proliferation of blastic cells. TMD blast cells have trisomy of chromosome 21 and mutations in GATA1 gene. Although TMD usually resolves spontaneously within 3 months after birth, twenty to thirty percent of TMD patients develop acute megakaryoblastic

leukemia within several years afterward. This leukemogenic transition is considered as a good model for multi-step tumorigenesis. However, it is still unclear that how GATA1 mutation promotes TMD development and that why GATA1-mutated progenitors prevail during embryonic hematopoiesis only in trisomy 21 patients. In order to address these issues, a strictly controlled isogenic cell panels that can reproduce human emboryonic hematopoietic development is needed. Human induced pluripotent stem cells (iPSCs) derived from DS patients are a promising platform for this, but so far there is no report regarding GATA1-mutated TMD-associated iPSCs. Therefore, we set out to establish an iPSCs panel that covers each genomic status of chromosome 21 and GATA1 gene. First, we established isogenic iPSCs derived from 2 mosaic DS patients. We reprogrammed these cells by introducing 5 episomal vectors, pCE-hOCT3/4, pCE-hSK, pCE-hUL, pCE-mp53DD and pCXB-EBNA1, under feeder free condition. There is no morphological difference between disomy and trisomy iPSC clones in both patients. We next introduced disease-associated GATA1 mutation into established isogenic trisomy and disomy iPS clones using transcription activator-like effector nuclease (TALEN) technology. Next, we established TMD-blast-derived iPSCs and non-blast-derived iPSCs from peripheral blood mononuclear cells of TMD patient. And we corrected GATA1 gene in TMD-blast-iPSCs by TALEN technology. In conclusion, we successfully established a comprehensive panel of iPSC clones for evaluating the hematopoietic consequence associated with the GATA1 genotype and the ploidy of chromosome 21. We believe that comprehensive understanding of TMD and AMKL pathogenesis provides a fruitful insight into our understanding of human leukemogenesis.

F-1340

RETINAL CILIOPATHY DISEASE MODELLING USING DISEASE-SPECIFIC CRISPR/CAS9-GENERATED AND PATIENT-DERIVED HUMAN INDUCED PLURIPOTENT STEM CELLS

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Vision impairment is a global health issue estimated to affect 285 million people worldwide. A major cause of visual disorders is the dysfunction or loss of photoreceptors, a condition known as retinal degeneration. Due to the burden of eye disease and its surgical accessibility, the retina is a prime target for novel cell therapies. While gene therapy approaches can slow down the loss of photoreceptors they cannot replenish the lost cells. The isolation of human embryonic stem cells (hESCs) and generation of patient-specific human induced pluripotent stem cells (iPSCs) offer us the possibility to potentially use these cells as a source for cell therapy. However, two key challenges in the field are the demonstration of disease-related phenotypes in hESC-derived and iPSC-derived retinal cells and the ability to rescue them for transplantation purposes. Our aim is to use hESCs/hiPSCs and CRISPR/Cas9 genome editing technology to successfully model certain retinopathies that affect cilia formation in photoreceptors. Cilia are complex organelles that protrude from the cell membrane and have signalling, sensory and motility functions that are vital for normal tissue development and homeostasis. In photoreceptors, cilia are essential for transforming light into vision. Photoreceptor cilia degeneration results in a wide range of syndromes affecting a large portion of the visually impaired population, these include macular degeneration, retinitis pigmentosa, Leber congenital amaurosis, cone-dystrophy, cone-rod dystrophy, as well as retinal degenerations associated with Usher syndrome, Joubert syndrome, primary ciliary dyskinesia and Bardet-Biedl syndrome among others. We have generated disease-specific clonal hESCs and hiPSCs using CRISPSR/Cas9 technology and patient-derived disease-specific hiPSCs. We are differentiating these cells towards photoreceptor lineage to model certain ciliopathies and compare any differences between CRISPR/Cas9-generated and patient-derived disease models in order to establish the suitability of these novel cell lines for disease characterisation studies and future disease treatment approaches.

F-1341

ANTISENSE U7 SNRNA-MEDIATED CORRECTION ABERRANT SPLICING OF BETA-THALASSEMIC ERYTHROID CELLS WITH IVS-2 654/BETA E DERIVED FROM PATIENT-SPECIFIC IPS CELLS

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Beta-thalassemia is an inherited blood disorder prevalent in Southeast Asia. A large number of mutations affecting splicing consequently cause the decrease or ab¬sence in production of β-globin chain, resulting in low level of hemoglobin. Here, we report a successful generation of β-thalassemic iPS cells harboring IVS2-654 (C>T)/βE (G>A) mutations, leading to the inclusion of intronic sequences and deletion of exonic sequences, respectively. In this study, we focus on the point mutation at position IVS2-654 in β-globin gene which creates the aberrant 5 ´ splice site and in turn activates a cryptic 3 ´ splice site, generating the inclusion of intronic sequences in spliced β-globin mRNA. In addition, we aim to correct this mutation in β-thalassemic iPS cells by using modified double-target U7 snRNA to antisense against both the cryptic 3 ´ splice site (ss) and a potential branch point upstream of the cryptic 3 ´ ss and to skip aberrant splicing. The β-thalassemia iPS cells were transduced with lentiviral vectors encoding the U7 snRNA and later differentiated into erythroblasts. We successfully demonstrate the restoration of correct spliced β-globin pre-mRNA in erythroblasts derived from iPS cells stably expressing the U7 snRNA. This study potentially shows that abnormal splicing β-globin pre-transcripts could be corrected in thalassemic iPS cells and differentiated to hematopoietic stem cells before autologous transplantation to the patients using this antisense-based therapy alternative to gene replacement approach.

F-1342

INVESTIGATION OF HNF1A AND HNF4A MODY MUTATIONS IN INDUCED PLURIPOTENT STEM CELLS USING CRISPR TECHNOLOGY

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Maturity onset diabetes of the young (MODY) is a rare inherited form of diabetes caused by a mutation in a single diabetes-associated

gene. To date, eleven MODY mutations have been described and two of these, HNF4A (MODY1) and HNF1A (MODY3), are known to cause changes in beta cell mass and to impair beta cell function, leading to early-onset diabetes. Recently, induced pluripotent stem cells (iPSCs) from healthy individuals have been shown to differentiate to functional insulin-secreting beta cells in vitro. Hence, iPSCs containing MODY mutations could be useful for elucidating diabetes related mechanisms and for screening of new therapeutic drugs. In the present study, artificial HNF4A or HNF1A mutations are introduced in iPSCs derived from a healthy individual using CRISPR technology. Eight different CRISPRs as well as two single stranded oligodeoxynucleotides (ssODNs) containing either an HNF1A Pro291fsinsC or an HNF4A pR309C mutation were generated. The ability of the CRISPRs to cut the DNA was verified in HEK293T cells using a plasmid containing the target gene surrounded by EGFP and analysed by flow cytometry. Transfection of iPSCs with CRISPRs and ssODNs is performed with the Amaxa 4D nucleofector and single, gene edited clones are isolated using neomycin selection or by flow cytometry with GFP. To compare their differentiation potential, the MODY and healthy iPSC lines are subjected to differentiation toward pancreatic endoderm (PE) and analyzed by qPCR, ICC and FACS with the PE markers PDX1, NKX6.1, NGN3 and MAFA. In addition, phenotypic in vitro assays will be performed including gene expression and DNA methylation analysis of the disease-causing genes, measurements of glucose stimulated insulin secretion (GSIS) in response to different concentrations of glucose, as well as Ca2+ imaging. The present proof of concept study could provide valuable cues on the effect of HNF1A and HNF4A MODY mutations in diabetes, and serve as an important in vitro platform for manipulating insulin secretion in pancreatic beta cells.

F-1343

TRANSMISSION OF MITOCHONDRIAL DYSFUNCTION FROM DISEASED CELLS TO HEALTHY CELLS IN AN ISOGENIC STEM CELL MODEL OF PARKINSON'S DISEASE

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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. Recently, a pathophysiological connection between mitochondrial dysfunction and proteostsis in PD has been postulated that centers on redox signaling. We thus asked whether seeding of α-syn oligomers can transmit mitochondrial deficits to healthy neurons by triggering redox stress. We utilized a patient-derived hiPSC model of PD that allows for comparison of A53T-SNCA mutant cells against isogenic mutation-corrected controls, in addition to a hESC model with the A53T-SNCA mutation introduced. Following a floor plate-derived differentiation paradigm to DA neurons (hNs), we determined that A53T hNs display fragmented mitochondria, oligomeric α-syn and increased oxidative stress relative to controls. To test whether seeding of α-syn oligomers can transmit mitochondrial deficits, we first drove cells to a neural progenitor cell (NPC) and then labeled control NPCs with GFP plus mitoDSRed and A53T NPCs with mitoDSRed alone, allowing us to discriminate between mitochondria of both cell types. We next combined and co-cultured A53T-hNs with their genetically corrected controls to assess whether mitochondrial impairments could be transmitted from A53T to controls hNs. To assess the contribution of seeded α-syn, co-cultured NPCs were differentiated in either the presence of mAB-α-syn (to capture secreted α-syn) or IgG until terminal differentiation was complete. We found that at 35 days of differentiation the mitochondria of control hNs had taken on the fragmented morphology of their diseased counterparts. This was blocked by mAB-α-syn, suggesting that mitochondrial impairment is transmitted by seeded oligomeric α-syn. In addition to informing on the mechanism of α-syn mediated mitochondrial damage, our data offer insight into the mode of protection of mAB-α-syn based therapeutics.

F-1344

METABOLOMIC PROFILE REVEALS OXIDATIVE STRESS IN POMPE DISEASE IPSC DERIVED CARDIOMYOCYTE

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We have previously shown that induced pluripotent stem cell derived from late-onset Pompe disease patient is useful in disease modeling and drug screening after cardiac differentiation. The molecular mechanism of cardiac complication of Pompe disease is almost unknown but it is shown that even in late-onset Pompe disease, which has mild phenotype, hypertrophic caridomyopathy is often seen and it is one of the important complications. To investigate cellular mechanism of cardiac complication of late-onset Pompe disease, we have conducted metabolomic profiling by CE-TOF-MS. Compared to healthy control iPSC derived cardiomyocyte, glutathione redox ratio is significantly reduced in Pompe disease iPSC derived cardiomyocyte. Glutathione redox ratio is the parameter of oxidative stress in the cell. It is suggested that oxidative stress might be associated with the mechanism of cardiac complication of late-onset Pompe disease. Mitochondria impairment triggers cellular oxidative stress and is considered to be the disease mechanism of a lot of degenerative disease. Mitochondiral dysfunction is shown to be associated with some lysosomal storage diseases including Pompe disease. Our results are compatible with the theory that mitochondrial dysfunction is associated the disease progression of Pompe disease through oxidative stress.

F-1345

MODEL OF IPS CELLS DERIVED FROM NIEMANN-PICK DISEASE TYPE C REVEALS NEWLY EFFECTIVE DRUG CANDIDATE HPGCD

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Niemann-Pick disease type C (NPC) is a lysosomal storage disease associated with mutations in NPC1 or NPC2. These mutations result in the abnormal accumulation of free cholesterol and glycolipids in lysosomes and late endosomes. Liver enlargement and neural dysfunction are major symptoms of NPC patients. The cellular model of NPC is essential tool for studying the molecular mechanism of diseases and developing the new therapies. Recently, the induced pluripotent stem (iPS) cells are established from the patient cells and are expected to be applied for studying both pathologic events of disease and drug development. Our purpose is to establish a cellular model of NPC and is to find drug candidates for NPC using NPC-derived iPS cells. We newly generated iPS cell lines from NPC using Sendai virus vectors which express reprograming factors, OCT3/4, SOX2, KLF4, and c-MYC, and directed the differentiation into hepatocyte-like cells and neural progenitors. Using this system, we attempted to find new drug candidates. NPC-derived hepatocyte-like cells and neural progenitors markedly accumulated free cholesterol. The analysis of hepatocyte-like cells and neural progenitors revealed the obstacles of ATP production and autophagy function were impaired in the NPC-derived cells. These findings indicate that iPS cell-derived cells can phenocopy human NPC. Using this system, we have screened drug candidates and finally found a new candidate "2-hydroxypropyl-γ-cyclodextrin (HPGCD)" that could reduce the cholesterol accumulation. HPGCD treatment could restore the functional and molecular abnormalities in the NPC-derived cells, and do so more effectively than 2-hydroxypropyl-β-cyclodextrin treatment, which is known as effective for reducing of cholesterol accumulation in NPC1-defective cells. The treatment with HPGCD was effective on the liver injury of NPC model mice and prolonged the survival of these mice. These results clearly show that our iPS cell lines were useful as cellular models of the disease. The new chemical "HPGCD" found by us is an attractive candidate for NPC therapy.

F-1346

MODELING FRONTOTEMPORAL DEMENTIA USING NEURONS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Individuals carrying a microtubule-associated protein tau (MAPT) R406W mutation develop a form of Frontotemporal dementia, which clinically resembles Alzheimer's disease. Neurons derived from iPSCs have potential to improve in vitro models for neurodegenerative diseases. For this application, a clear understanding of their functional matureness is crucial. In this study, neurons were derived from five iPSC lines from patients with a MAPT R406W mutation and five control individuals. The ten neuronal cultures were characterised for marker expression and functional properties. Furthermore, we investigated the potential of these cells to model disease mechanisms. The neurons exhibited clear neuronal morphology with neurite outgrowth. Real-time PCR showed up-regulation of neuronal marker genes and immunocytochemistry demonstrated the appearance of βIII-tubulin, microtubule-associated protein 2, and tau positive cells. Western blot showed the presence of the embryonic tau isoform (3RN0) exhibiting an embryonic phosphorylation pattern. Depolarisation of the membrane potential by increasing the extracellular potassium levels and stimulation with neurotransmitters (GABA or glutamate/glycine) resulted in a considerable increase in cytosolic calcium as detected by fluorescent probes. One control line was further analysed by image analysis demonstrating the presence of spontaneous calcium spikes, and by patch clamp recordings showing the presence of voltage-gated Na^+ and K^+ currents in a subpopulation of the cells. Overall, the ten neuronal cultures did indeed exhibit neuronal marker expression and functional characteristics but encompassed a relatively immature neuron population. We are investigating if we can identify a disease phenotypic difference in the five MAPT versus five control lines. Currently, we have not found differences in tau subcellular localisation or phosphorylation levels in standard growth conditions, but we have indication of stronger stress responses in neurons derived from patients. Moreover, we have shown that iPSC neurons release endogenous tau into the cell culture media and that they can take up extracellular recombinant tau implying their applicability in modelling the cell-to-cell spread of tau that has been implicated in the pathogenesis of tauopathies.

F-1347

ELECTROPHYSIOLOGICAL ANALYSIS OF A 2D CARDIOMYOCYTE SHEET DERIVED FROM LONG QT TYPE1 PATIENT INDUCED PLURIPOTENT STEM CELLS USING A MEMBRANE POTENTIAL DYE

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Human induced pluripotent stem cell (hiPSC) technology can be used for recapitulation of the disease phenotype of inherited long QT syndrome (LQTS). So far, several reports have successfully reproduced LQTS phenotype from hiPSC-derived cardiomyocyte (iPS-CM) models using a single cell patch clamp method, multi-electrode array (MEA) system, [Ca(2+)]i transients, or a voltage sensitive dye, di-4-ANEPPS. Recent improvement of voltage-sensitive dye enabled us to detect changes in membrane potential with higher magnitude and sub-millisecond resolution. We generated patient specific iPSCs from LQTS type 1 (LQT1) and type 2 (LQT2) patients and cardiomyocytes were derived from these iPS cells. While the action potential duration (APD) prolongation of the LQT2 patient iPS-CMs was observed by a patch clamp method at single cell level, those from LQT1 patients displayed no APD prolongation (now without β-stimulation). We applied a new voltage sensitive probe FluoVolt to the analysis of LQT1 patient iPS-CMs sheet. LQT1-iPS-CMs at day30 were dispersed to form 2D cardiac sheet after sorting by a cardiac surface marker, SIRPa. Additional

15-20 days culture showed APD prolongation on LQT1-iPS-CMs sheet (average APD90c ; LQT1 691±97 ms vs control 295 ms±92 ms). Furthermore, LQT1-iPS-CMs were more susceptible to an IKr blocker, E4031 compared to control-iPS-CMs, resulting in marked APD prolongation. These data suggest that culture on 2D cardiac sheet led to electrophysiological maturation of iPS-CMs, supporting the recapitulation of disease phenotype of LQT1. In addition, APD analysis on 2D sheet provided more information, such as AP waveforms, which are not measured using MEA or [Ca(2+)]i transients assay. These results demonstrated that the analysis of a 2D CM sheet using a membrane potential dye can be a useful tool for studying the disease phenotype of LQTS.

F-1348

GENERATION AND CHARACTERIZATION OF RAT-INDUCED PLURIPOTENT STEM CELLS USING MESENCHYMAL STROMAL CELLS FROM A NEW MODEL OF METABOLIC SYNDROME

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We characterized DahlS.Z-$Lepr^{fa}$/$Lepr^{fa}$ (DS/obese) rats, derived from a cross between Dahl salt-sensitive rats and Zucker rats, as a new animal model of metabolic syndrome (MetS). DS/obese rats develop obesity as well as hypertension, dyslipidemia, insulin resistance, and type 2 diabetes mellitus. Although the phenotype of DS/obese rats is similar to that of humans with MetS, the pathophysiological and metabolic characteristics in each cell type remain to be clarified. Hence, the establishment of induced pluripotent stem cells (iPSCs) derived from MetS rats is essential for investigations of MetS in vitro. Reports of rat iPSCs (riPSCs), however, are few because of the difficulty. Recently, the advantage of using mesenchymal stromal cells (MSCs) as a cell source for generating iPSCs was described. Then we established a novel method to generate riPSCs from MSCs, and presented at 11th ISSCR (2013 at Boston). MSCs were collected from adult rat subcutaneous adipose tissues of DS/obese and their lean littermates, DahlS.Z-$Lepr^{+}$/$Lepr^{+}$ (DS/lean) rats. MSCs from rats were treated with three mouse reprogramming factors *(Oct3/4, Sox2,* and *Klf4*) through lentiviral vectors. These cells were then termed obese riPSCs (o-riPSCs) for those derived from DS/obese rats and lean riPSCs (l-riPSCs) for those derived from DS/lean rats. Morphological data clearly showed that both riPSCs became adipocytes after induction of adipogenesis by insulin, T3, and dexamethasone. Moreover, quantitative RT-PCR analysis also revealed that both riPSCs and the adipose tissue from DS/obese and DS/lean rats possess similar expression patterns of adipocyte differentiation-related genes. Adipose tissues in teratomas from o-riPSCs and l-riPSCs showed similar morphology, and adipogenesis using both riPSCs in vitro resulted in no differences between the two groups. These data suggest that physiological and/or metabolic differences in surrounding adipose tissues between DS/obese and DS/lean rats in vivo had a greater effect on the adipocytes than on expression of their own genes. Further studies are required to test this hypothesis. We succeeded in generating riPSCs effectively from MSCs of both DS/obese and DS/lean rats. These riPSCs may well serve as highly effective tools for the investigation of MetS pathophysiology in vitro.

F-1349

USING INDUCED PLURIPOTENT STEM CELLS TO MODEL LIVER DISEASE IN ALPHA-1 ANTITRYPSIN DEFICIENCY

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A major obstacle to the development of new therapies is the poor understanding of how genetic modifiers alter the onset and outcome of various diseases. A classic example is alpha-1 antitrypsin (AAT) deficiency, a metabolic liver disease in which the mutant gene and its product are known, but where clinical progression and outcome are extremely variable and thought to be influenced by genetic modifiers. We have generated hIPSC lines from existing AAT patients (ZZ) with variable degrees of liver disease, including those without evidence of liver damage and those who have suffered a more aggressive course leading to end stage liver disease. We are using control and AAT hIPSC-derived hepatocyte like cells (HLCs) to probe the hypothesis that the significant heterogeneity seen in disease progression due to AAT ZZ mutations is related to genetically determined variability of fundamental biological hepatocyte processes involved in cellular disposal, stress response, and cell survival pathways, including proteasomal degradation, ER stress, autophagy, and apoptosis. Prior data obtained in mouse and cell line models has shown that autophagy may act as a primary route of intracellular degradation of mutant AAT protein thus providing an important hepatoprotective mechanism. Our preliminary results show that HLCs derived from AAT mutant patients with no evidence of liver disease (AAT NLD) have increased activation of autophagy at baseline compared to AAT mutants with severe liver disease (AAT LD). Furthermore, AAT hepatocytes from patients with severe liver disease show a weaker autophagy induction response upon serum deprivation. Our data supports a role for autophagy as a potential modifier in the pathobiology of AAT related liver disease, and opens the way for mechanistic studies involving this and other basic biological pathways that may modulate hepatic injury in AAT. Our studies can impact the way we approach AAT deficiency: 1) by developing predictive diagnostics through discovery of biomarkers that identify patients at risk for severe liver disease, and 2) by promoting therapeutic candidate discovery through validation of new or existing therapeutic targets in live human hepatocytes.

F-1350

THE INDUCTION OF KERATINOCYTE FROM THE IPS CELLS DERIVED FROM THE PATIENT OF KINDLER SYNDROME

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Kindler syndrome is an autosomal recessive disorder caused by the mutations in the kindlin-1 gene, which is known to bind to integrins and regulate integrin activation at cell adhesions. Kindler syndrome is characterized by skin blistering, erosion and photosensitivity. Radical treatments, such as regenerative therapies using stem cells are strongly desired because of its difficulties of complete cure. Therefore, we decided to establish induced pluripotent stem cells (iPSCs) from human adipose tissue-derived multilineage progenitor

cells (hADMPCs) derived from patient of Kindler syndrome, and differentiate them into keratinocytes in order to discover the pathogenic mechanism. In this study, we have established disease-specific iPSCs by introducing four transcription factors, OCT3/4, SOX2, KLF4, and c-MYC and cultured under the of 5% O2 or 20% O2 condition. Consistent with previous report, the rate of iPS-like colony formation significantly increased under the 5% O2 condition. The expression of pluripotent stem cell markers including OCT4, Nanog, Tra-1-60, Tra-1-81, E-cadherin, and SSEA3 were observed by immunofluorescent staining. Also, flow cytometry analysis revealed that Tra-1-81, E-cadherin, SSEA4 were positive in these cells. Furthermore, we investigated spontaneous tripotent differentiation capacity of the patient specific-iPSCs via embryoid bodies formation. After 21 days of induction, expressions of βIII-tubulin, α-smooth muscle actin, α-fetoprotein were observed by immunofluorescent staining. These results indicate that iPSCs from patient's hADMPCs were successfully established. When the patient specific-iPSCs were differentiated into keratinocytes by adding BMP4 and retinoic acids, the expression of keratinocyte markers were found to be markedly increased in these cells. Also, the expressions of keratinocyte markers K14, Involucrin, and Loricrin were observed by immunofluorescent staining. Moreover, expressions of CD49f (α6 integrin) and CD29 (β1 integrin) were also observed by flow cytometry analysis, suggesting that iPSCs established from patient have the ability to differentiate into keratinocytes. This study is expected to be a first step in the investigation of the underlying mechanism and a novel therapy development of Kindler syndrome.

F-1351

MODELING OF VALPROIC ACID- INDUCED HEPATOTOXICITY IN MITOCHONDRIAL RECESSIVE ATAXIA SYNDROME WITH PATIENT HIPSC DERIVED HEPATOCYTES

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Hepatocyte like cells (HLCs) derived from human pluripotent stem cells provide powerful tools for toxicology assays. In addition, hiPSCs derived from patients with hepatic diseases enable studies of disease mechanisms. We aimed to develop a hiPSC based model to study Valproic acid (VPA)- induced toxicity in Mitochondrial Recessive Ataxia Syndrome (MIRAS) patients. MIRAS is the most common hereditary ataxia in Finland, caused by mutation in the mitochondrial DNA polymerase (POLG). Patients with age of onset at or before 20 years, often present with seizures. Interestingly, these early onset MIRAS patients develop acute severe liver failure if VPA is administrated for treatment of epilepsy. The mechanisms for VPA toxicity and whether VPA is toxic for the late onset MIRAS patients, who generally do not need anticonvulsants, remain unknown. We generated two early- and two late onset MIRAS-hiPSC lines. Hepatic differentiation was induced by a stepwise protocol through definitive endoderm into hepatic progenitors by 5 days with BMP4 and FGF2 and then further 5 days with HGF. Final maturation was induced by HGF and Oncostatin M. Cells were analyzed before and after VPA treatment for cell death by immunostaining, gene expression by QPCR and metabolomics by mass spectrometry. Differentiated MIRAS-HLCs expressed genes characteristic for fetal hepatocytes, such as Albumin and HNf4α and secreted albumin similarly as healthy control HLCs. During hepatic differentiation, MIRAS-HLCs accumulated excessively Oil-red-O positive lipids. VPA induced morphological changes and cell death in patient cells at low concentrations that were non-toxic in control cells. Metabolomics revealed clear differences between the patient and control cells both in untreated conditions as well as in response to low VPA doses. MIRAS-HLCs provide a platform for studies on mechanisms leading to VPA induced hepatotoxicity.

F-1352

HUMAN IPSC GLIAL MOUSE CHIMERAS REVEAL GLIAL CONTRIBUTIONS TO SCHIZOPHRENIA

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Recent genetic and neuroradiological studies have suggested a role for glial pathology in the genesis of schizophrenia. To assess this possibility, we established human glial chimeric mice using glial progenitor cells (GPCs) produced from human induced pluripotential cells (hiPSCs) derived from patients with juvenile-onset schizophrenia, or from gender-matched controls. We did so by neonatally implanting donor hiPSC GPCs into either of two murine recipients: 1) into normally-myelinated immunodeficient rag2-/- hosts, in which implanted GPCs remain as such or become astrocytes; and 2) into hypomyelinated shiverer (MBP $^{shi/shi}$) x rag2-/- mice, in which the donor GPCs also develop as myelinating oligodendrocytes. When implanted into shiverer mice, by 4 months of age the schizophrenia-derived iPSC GPCs exhibited less white matter engraftment than control iPSC GPCs, instead migrating prematurely into the cortex. The lower density of GPCs in the white matter of schizophrenic hiPSC GPC-transplanted shiverer hosts resulted in their significant hypomyelination, relative to control hiPSC GPC-engrafted shiverers. When established in myelin wild-type hosts, the schizophrenia hiPSC glial chimeras exhibited an aberrant behavioral phenotype, characterized by diminished prepulse inhibition, higher anxiety, and social avoidance compared to control chimeras. RNAseq revealed significant differences in glial differentiation- and migration-associated gene expression by schizophrenia-derived hiPSC GPCs, relative to those derived from control patients. These data suggest a significant contribution of cell-autonomous glial pathology to the development of juvenile-onset schizophrenia. *Supported by NIH grants R33MH087877, R01MH104701, R01MH099578 and R01NS75345, the Leila Y. and Harold G. Mathers Charitable Foundation, and the Novo Nordisk Foundation.*

F-1353

DERIVE INDUCED PLURIPOTENT STEM CELLS AND NEURAL STEM CELLS SIMULTANEOUSLY FROM HUMAN HEMATOPOIETIC PROGENITOR CELLS FOR AN IN VITRO AUTOLOGOUS MODEL OF NEUROINFLAMMATION

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Recent advances in the field of cell transformation provide unique opportunities for in vitro human disorder modeling, especially for neurological disorders. However, it is still challenging to model neuroinflammatory disorders using autologous cells due to limited sample size. To develop a convenient method to generate autologous neuroinflammatory cultures, we optimized conditions of PBMC separation and CD34 cells purification, and further generated induced neural stem cells (iNSC) and induced pluripotent stem cells (iPSC) simultaneously from CD34 cells transfected with Sendai virus vectors containing Yamanaka factors followed with selective media. The neural stem cells could be derived within two weeks after transfection and iPSC appeared in three weeks, as confirmed by immunostaining for cell specific markers. Meanwhile, we could still collect PBMCs from the same blood sample for co-culturing with neural cells, thus creating an autologous model of neuroinflammation from as low as 10 ml of human blood. This method is relatively easy to master and has been used to generate iNSC/iPSC for a variety of neurological disorders. We expect this model could be adopted by researchers in non specialized labs and help them generate autologous models for neuroinflammatory disorders.

F-1354

MODELING AE-BART'S DISEASE USING PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

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Thalassemia syndromes are common monogenic disorders resulting from genetic defects in α- or β-globin gene(s) leading to decreased production or structural alteration of their respective α- or β-globin chain. In Thailand and Southeast Asia several different combinations of such abnormal genes can give rise to over 60 thalassemia syndromes. In the present study, we generated iPSCs from a patient with thalassemia intermedia (AE-Bart's disease), a form of thalassemia resulting from complex gene interactions between α-thalassemia (α-thalassemia 1/α-thalassemia 2) and heterozygous Hb E. The AE-Bart's disease-specific iPSCs (AEB-iPSCs) express pluripotent markers and can be spontaneously differentiated in vitro (embryoid body formation) and in vivo (teratoma formation) into cells derived from each of the three embryonic germ layers. The AEB-iPSCs exhibit a normal karyotype and carry the patient's genetic mutations, i.e. two types of deletion, -α3.7 and --SEA, in each of their respective α-globin alleles (Hb H disease), and a heterozygous point mutation at codon 26 in one of β-globin alleles (Hb E). Results from a controlled study of hematopoietic differentiation in a feeder-free culture system will be discussed.

F-1355

A NOVEL MODEL FOR PREECLAMPSIA IN DISH:FROM DISEASE SPECIFIC PLACENTAL CELLS TO IPS DERIVED TROPHOBLASTS

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As a common placenta-related disease affecting 5-8% human pregnancies, preeclampsia continues to be one of the leading causes of maternal death, fetal and neonatal adverse outcome, and even higher long-term cardiovascular risk. Studies have been underwent to uncover the pathogenesis at the first trimester for optimizing earlier prediction and therapy, however, little was found due to possible adverse effects in sampling tissues with a gestational age less than 12 weeks. Since 2006, induced pluripotent stem cells (iPSCs) have been successfully introduced to model kinds of disease. Hence we speculate that reprograming the preeclampsia-specific placental cells including amniotic epithelial cells (AECs), amniotic mesenchymal cells (AMCs) and trophoblasts into iPSCs and then differentiating them to be trophoblasts may mimic the early abnormal development of preeclampsia trophoblasts, which would give a clue to the detailed molecular mechanism in dish. Firstly, we isolated placental cells (AECs, AMCs) from placenta of preeclampsia soon after birth. Following culturing and expansion, target cells were reprogrammed into iPSCs under feeder-free condition with episome system, where miR-302-367 cluster and OCT4, SOX2, SV40T, KLF4 were simultaneously transfected through electroporation. And then resembled ES colonies were picked up and purification for identification, which showed a normal karyotype of 46, XY and positive expression of SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81. Meanwhile, higher expressions of endogenous gene (OCT4, SOX2, and NANOG) were detected. Also, embryoid bodies and teratomas in immune-deficient mice representing of three germ layer lineages both in vivo and vitro were demonstrated. Secondly, placental cells-derived iPSCs were treated with BMP4. At Day 7, the harvest induced cells showed positively staining of CDX2, CK-7 and EOMES and expression of trophoblast lineage specific genes (CDX2, GATA3, and HAND1). Also, syncytial cells were observed and secretion of hCG and progesterone were detected. Totally, we successfully generate iPS cells from preeclampsia-specific placental cells and differentiate them into trophoblasts in vitro, where a novel model to understand the early changes of preeclampsia involving gene mutation and abnormal trophoblast invasion would be established.

F-1356

USING PLURIPOTENT STEM CELLS TO MODEL OPTIC NEUROPATHIES

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Optic neuropathies are blinding diseases characterised by damaged retinal ganglion cells (RGCs), specialised neurons which relay visual information obtained by the retina for processing in the brain. An example of an optic neuropathy is glaucoma, the leading cause of irreversible blindness worldwide that affect one in ten Australians

over 80. A major barrier to studying optic neuropathies is the lack of in vitro models, due to the extreme difficulty of obtaining ocular tissue from living patients. To address this, we use induced pluripotent stem cell (iPSC) technology to model optic neuropathies. We focus on generating patient-specific iPSCs and subsequent differentiation into RGCs. Using episomal reprogramming method; we have generated 6 integration-free iPSC cell lines from patients and control. Characterisation of these iPSCs demonstrated that the cells are positive for pluripotent markers TRA160 and OCT4. These iPSCs are differentiated in vitro by embryoid bodies formation and we showed the presence of cells representative of ectoderm (NESTIN positive cells), mesoderm (SMA positive cells) and endoderm (AFP positive cells). Also, the generated iPSCs are differentiated by teratoma assay and we confirmed that their potential to differentiate in vivo. To ensure the quality of the iPSCs generated, we performed genome-wide copy number variation analysis using SNP arrays. Our results demonstrated that all 6 iPSC cell lines possess normal karyotype. Generation of disease-specific iPSCs will allow us to study the precise mechanism underlying the pathology of optic neuropathies, providing a platform for screening drugs to improve treatment options and development of gene therapy and cell replacement therapy.

F-1357

MODELING MICROCEPHALY DISEASE BY ORGANOID CULTURE FROM IPSCS

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Modeling neuronal progenitor proliferation and organization that produces mature subcortical neuron subtypes during early stages of development is essential for studying human brain developmental diseases; however, current organoid culture systems are not elaborative enough to study circuitry formation and maturation in vitro. Here, we demonstrate a new method to culture cerebral organoids by induced pluripotent stem cells (iPSCs), recapitulating the inside-out organization of 6-layer neocortex and exhibiting characteristics of neuronal network formation. The observation of spontaneous excitatory postsynaptic currents (sEPSCs) suggests that chemical synapses are formed among cells in the organoids. More importantly, a transition from NDMA-receptor- to AMPA-receptor-mediated excitatory neurons indicates mature synapse formation in these neurons and integration into the existing circuitry. In addition, using patient-specific iPSCs with dysfunction of the Aspm gene from a patient with primary microcephaly, we demonstrate layer formation defects in patient organoids, suggesting a new strategy to study human developmental diseases.

F-1358

VASCULAR SMOOTH MUSCLE PHENOTYPES OF MARFAN SYNDROME PATIENT-DERIVED PLURIPOTENT STEM CELLS REVERSED FOLLOWING CORRECTION OF FIBRILLIN-1 MUTATION

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Marfan syndrome (MFS) is a common connective tissue disorder caused by mutations of a gene encoding fibrillin-1 (FBN1). This disorder is associated with skeletal, ocular, skin and cardiovascular complications, and even death due to ruptured aortic aneurysm. Here we generated a cellular model of MFS in which we derived induced pluripotent stem cells (iPSCs) from MFS patient fibroblasts, corrected the FBN1 mutation in one MFS-iPSC line, and differentiated the cells into vascular smooth muscle cells (SMCs) to recapitulate phenotypes of MFS patients in the context of the developmental program and functions of SMCs. Compared to the mutation-corrected SMCs, MFS-SMCs had a reduced contractility and calcium signal in response to carbachol, elevated expression of pro-inflammatory factors, and reduced expression of extracellular matrix components. Interestingly, MFS-SMCs also showed reduced expression of contractile phenotypic markers, and increased expression of synthetic phenotypic markers, indicating a synthetic phenotype of MFS-SMCs. Together, our findings suggest that SMCs derived from MFS-iPSCs can manifest phenotypes specifically attributable to FBN1 mutation, which may serve as readout for screening of novel therapies of the disease.

F-1359

MODELING INFANTILE POMPE DISEASE WITH PATIENT DERIVED IPS CELLS

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Pompe disease, also known as glycogen storage disease II, is a rare autosomal recessive disease. It is caused by the defect of the lysosomal enzyme, acid alpha-glucosidase (GAA), which converts glycogen into glucose. The lack of GAA causes abnormal glycogen accumulation in the lysosomes of various organs, including skeletal muscle, liver and heart. A severe "infantile" form of Pompe disease shows almost no residual GAA activity and early death within 2 years. The enzyme replacement therapy with recombinant human GAA has dramatically improved the patients' survival, but its effect to skeletal muscle symptom is limited. For the purpose of resolving this remaining clinical problem, we have been investigating the mechanism of this ineffectiveness of the enzyme replacement therapy for skeletal muscle symptom by using patient-derived induced pluripotent stem cells (iPSCs). We generated myocytes and hepatocytes from three infantile Pompe patients' iPSCs for the establishment of disease modeling system. First, we introduced doxycycline-inducible expression system of MYOD1, master regulator of myogenic differentiation, into iPSCs with piggyBac transposon vector. After myogenic differentiation in culture media with doxycycline, we analyzed differences between myocytes derived from patients' iPSCs (Pt-iPSCs) and those from controls. We also compared hepatocytes derived from Pt-iPSCs and controls. As a result, substantial myogenic and hepatogenic differentiation was achieved in both Pt-iPSCs and controls. Pt-iPSC-derived myocytes and hepatocytes showed glycogen accumulation in the lysosomes. Quantitative analysis revealed higher lysosomal glycogen levels in Pt-iPSC-derived myocytes and hepatocytes. The accumulation of lysosomal glycogen was improved by adding recombinant human GAA in the culture media in a dose-dependent manner. In conclusion, we succeeded in in vitro recapitulation of both skeletal muscle and liver phenotypes of infantile Pompe disease using patient-derived iPSCs. We expect our modeling system will help elucidate more details of disease mechanism or establish an efficient drug-screening platform.

F-1360

MITOCHONDRIAL ALTERATIONS BY PARKIN IN DOPAMINERGIC NEURONS USING PARK2 PATIENT-SPECIFIC AND PARK2 KNOCK-OUT ISOGENIC IPSC LINES

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In this study, we used patient-specific and isogenic PARK2 induced pluripotent stem cell (iPSC) lines to show that alterations in PARK2 function alter neuronal cell proliferation and percentage of TH-positive neurons was decreased in PD patients-derived neurons carrying various mutations in PARK2 gene when compared to an age-matched control subject, and this reduction was accompanied by alterations in mitochondrial: cell volume fraction (mitochondrial volume fraction). The same phenotype was confirmed in the PARK2 null line we generated using Zinc Finger Nuclease technology. 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. Whole genome expression profiling at the fibroblast, iPSC, neural stem cell, neuron and dopaminergic neuron stages confirmed the mitochondrial phenotype and identified pathways altered by PARK2 dysfunction that include PD related genes. Our results are consistent with the current model of PARK2 function where damaged mitochondria are targeted for degradation via a PARK2/PINK1 mediated mechanism. Currently, we are extending this study with Pink1 KO and PARK2/PINK1 double KO isogenic iPSC lines.

F-1361

CARDIAC DISEASE PHENOTYPES AND PHARMACOLOGICAL RESCUE IN HUMAN IPS CELL MODELS OF JERVELL AND LANGE-NIELSEN AND LQT3 SYNDROME

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Long QT syndrome (LQTS) is a rare inherited or acquired heart condition in which delayed repolarization of the heart causes high risk of ventricular arrhythmia, which may lead to sudden cardiac arrest. LQTS can be inherited in an autosomal dominant or autosomal recessive fashion. Here, we generated human induced pluripotent stem cell (hiPSC) models of the autosomal recessive LQTS, the Jervell and Lange-Nielsen syndrome (JLNS), as well as of a dominant LQTS variant, LQT3, by reprogramming of patient fibroblasts. In cardiomyocytes of both human induced pluripotent stem cell (hiPSC) models, typical LQTS disease features were recapitulated at the celular level. This included significant prolongation of the QT-like interval on multielectrode arrays (MEAs), as compared to wild-type controls, as well as increased propensity for developing stress-induced arryhthmia and/or early afterdepolarisations (EADs) in the ground state. Interestingly, these LQTS phenotypes were caused by distinct mechanisms at the molecular level. While the JLNS hiPSCs were deficient in the potassium channel-encoding KCNQ1 gene, LQT3 hiPSCs harboured a missense mutation in the sodium channel-encoding SCN5A locus. Specifically, JLNS hiPSC-derived cardiomyocytes (hiPSC-CMs) displayed severely increased field potential durations while showing arrhythmic spontaneous beating at low supplied dosages of the proarrhythmic compound cisapride. In contrast, LQT3 hiPSC-CMs showed EADs, known triggers of arrhythmia, already in the ground state at high probability. Based on the distinct underlying disease mechanisms, drug testing was performed to correct these specific phenotypes pharmacologically. JLNS hiPSC-CMs could be rescued using an activator of the hERG potassium channel in that induced arrhythmias were fully suppressed. In LQT3 hiPSC-CMs, in comparison, EADs could be dose-dependently repressed by means of a pharmacological SCN5A inhibitor, balancing the gain-of-function phenotype in this model. In sum, we demonstrate successful pharmacological disease phenotype correction in two distinct models of LQTS. Our data hence highlight the utility of patient-specific hiPSCs for personalized drug evaluation in case of inherited cardiac channelopathies.

IPS CELLS: EPIGENETICS

F-1363

DIFFERENTIATION OF HUMAN PARTHENOGENETIC PLURIPOTENT STEM CELLS REVEALS MULTIPLE TISSUE AND ISOFORM SPECIFIC IMPRINTED TRANSCRIPTS

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Parental imprinting results in a monoallelic parent-of-origin dependent gene expression. However, many imprinted genes previously identified by differential methylation do not exhibit complete monoallelic expression. This may be partly explained by previous findings demonstrating a complex tissue-dependent expression patterns for some imprinted genes. Still, the complete magnitude of tissue specific imprinting remains largely unknown. In this study we differentiated human normal pluripotent stem cells and parthenogenetic pluripotent stem cells (which harbor only maternal alleles) into different cell types. This enabled the identification of two novel paternally expressed genes which are not expressed in undifferentiated cells but are expressed in a monoallelic fashion in the differentiated cells. Interestingly, we also found that nearly half of all known paternally imprinted genes are expressed in the parthenogenetic cells which lack the paternal allele. Our analysis identified for these genes both biallelic and monoallelic isoforms that are controlled by tissue-specific alternative promoters. The overlap of biallelic and monoallelic isoforms poses difficulties in identifying monoallelic expression by using the commonly available genome wide expression methods. Therefore in order to search for putative tissue- and isoform-dependent imprinted genes in a genome wide manner, we performed a novel 5' RNA high throughput sequencing, and combined the results with whole genome DNA methylation data. Collectively, our analysis proposes multiple putative imprinted differentially methylated regions (iDMRs) located in genes with alternative promoters. We demonstrate that the vast majority of the newly identified iDMRs are also present in chimpanzee, supporting

their evolution conservation. Taken together, our study implies that the control of alternative promoters is an important mechanism for tissue-dependent expression of imprinted genes. This study provides the first global analysis of tissue-specific imprinting in humans, and implies that alternative promoters are central in the regulation of imprinted genes.

F-1364

OPPOSING ROLES OF PRMT2 AND PRMT8 IN MOUSE SOMATIC CELL REPROGRAMMING

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Methylation of arginine residues on histones regulates gene function by modulating their interactions with other regulatory proteins. The methylation at arginine residues is catalyzed by the activity of a class of enzymes known as protein arginine methyl transferases (PRMTs). The regulatory PRMTs such as CARM1 (PRMT4), PRMT5 and PRMT6 have been previously implicated in maintenance of pluripotency and reprogramming. Further, combined inhibition of PRMTs and TGFβ signaling with small molecules enabled *Oct4* induced reprogramming of MEFs. To understand the role of different classes of PRMTs in regulating mouse somatic cell reprogramming, we measured the mRNA expression levels of *Prmt* 1 to 8 in MEFs, SSEA1$^+$ reprogramming intermediates, pre-iPSCs, iPSCs and R1ESC. *Prmt2* was expressed at significantly high levels in MEFs and, following reprogramming factor expression its levels sharply declined and was barely detectable in SSEA1$^+$ reprogramming intermediates, pre-iPSCs, iPSCs and R1ESCs. In contrast *Prmt8* expression could be detected only in R1ESCs and one of the established iPSC clones but not in others. When the pre-iPSC clones were cultured in presence of KOSR, the expression of *Nanog* and *Prmt8* was induced at levels similar to that of R1ESCs. None of the small molecule inhibitors of HDACs and DNMTs and ascorbic acid could induce *Prmt8* expression. To further elucidate the roles of *Prmt* 2 and 8 in reprogramming, knockdown and overexpression constructs were prepared. The constitutive overexpression of *Prmt8* yielded two fold higher alkaline phosphatase colonies, while *Prmt2* overexpression reduced the number of alkaline phosphatase colonies by two folds compared to empty vector controls. Consistently, the constitutive knockdown of *Prmt2* generated two fold higher numbers of alkaline p

F-1365

EPIGENETIC BIOMARKER TO SUPPORT CLASSIFICATION INTO PLURIPOTENT AND NON-PLURIPOTENT CELLS

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Several methods can be used as surrogate assay to determine pluripotency of induced pluripotent stem cells (iPSCs) - e.g. analysis of iPSC-colony morphology, surface marker expression, analysis of individual genes or gene expression profiles, test for multilineage differentiation potential in vitro, or teratoma formation. There is a clear trade-off between cost and labor-intensive methods on the one hand and reliability on the other. Differentiation potential of iPSCs is also reflected by a unique epigenetic makeup in DNA methylation (DNAm) profiles. Here we describe a simple method to estimate pluripotency which is based on the DNAm level at only three CpG sites. Two of these CpG sites were selected based on their discriminatory power in 258 DNAm profiles (63 pluripotent, 195 non-pluripotent; 450k Illumina BeadChips, www.ncbi.nlm.nih.gov/geo/). They become either methylated or demethylated in iPSCs and their combination is referred to as "Epi-Pluri-Score". In addition, a third CpG located in the pluripotency-associated gene POU5F1 (OCT4) was considered. This epigenetic signature was validated on independent DNAm datasets (264 pluripotent and 1951 non-pluripotent samples; 27k Illumina BeadChips) with 99.9% specificity and 98.9% sensitivity. Notably, the method could also discriminate partially or improperly reprogrammed cells. Subsequently, we established pyrosequencing assays to specifically analyze DNAm at the CpGs of the Epi-Pluri-Score. The results allowed reliable classification of 18 pluripotent cell lines and 31 non-pluripotent cell lines. DNAm changes at these three CpGs were subsequently analyzed in the course of differentiation of iPSCs towards mesenchymal stromal cells demonstrating that particularly the CpG site in POU5F1 demarcates early differentiation events. Taken together, DNAm level of three specific CpG sites provides a simple and robust biomarker for analysis of pluripotency with high sensitivity and specificity.

F-1366

EPIGENETIC VARIATIONS OF IMPRINTED GENES IN MOUSE PLURIPOTENT STEM CELLS

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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. iPSCs have therapeutic potential as the source of personalized stem cells and offer a unique tool to dissect the principles of cell fate determination during normal development and its dysregulation in disease. Dynamic epigenetic alterations such as DNA methylation and histone modifications 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. Indeed, a previous study reported variable DNA methylation at some imprinted genes such as Meg3 and Rian, and that such altered expression levels are associated with the quality of iPSCs. Here, we examined DNA methylation status at H19 DMR (differentially methylated region) and Peg10 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 Peg10 DMR 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

F-1368 Note: this work will not be presented as a poster but as an oral presentation in Concurrent IVB: Friday 26 June at 13:50

THE HISTONE DEACETYLASE SIRT6 CONTROLS EMBRYONIC STEM CELL FATE VIA TET-DEPENDENT PRODUCTION OF 5-HYDROXYMETHYLCYTOSINE

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How embryonic stem cells (ESCs) commit to specific cell lineages and ultimately yield all cell types of a fully formed organism remains a major question. ESC differentiation is accompanied by large-scale histone and DNA modifications, but the relations between these two categories of epigenetic changes are not understood. Here, we demonstrate the interplay between the histone deacetylase Sirtuin 6 (SIRT6) and TET (Ten-eleven translocation) enzymes. SIRT6 targets deacetylation of histone H3 at lysine's 9 (H3K9ac) and 56 (H3K56ac), while TET enzymes convert 5-methylcytosine (5mC) into 5-hyfroxymethylcytosine (5hmC). We found that ESCs derived from SIRT6 knockout (S6KO) mice are skewed towards neuroectoderm development. This phenotype is associated with an increased expression of the core pluripotent genes Oct4, Sox2 and Nanog, which are directly regulated by SIRT6-dependent deacetylation of H3K9ac and H3K56ac. We show increased recruitment of OCT4 and SOX2 to regulatory regions of Tet genes in S6KO versus WT ESCs. Genome-wide analysis revealed an upregulated expression of neuroectoderm genes, which are marked with higher levels of 5hmC in S6KO versus WT ESCs, thereby implicating TET enzymes as responsible for the neuroectoderm-skewed differentiation of S6KO ESCs. Strikingly, knockdown of TET1 and TET2 fully rescued the differentiation defect in S6KO ESCs. Additionally, we found the role of SIRT6 in ESC differentiation to be conserved in human ESCs (hESCs). Overall, we demonstrate a new role for SIRT6 as a chromatin regulator safeguarding the balance between ESC pluripotency and differentiation through TET-mediated production of 5hmC, which functions as an epigenetic determinant to control gene expression.

F-1369

THE CHROMATIN REMODELER CHD7 IS ESSENTIAL FOR NEUROGENESIS AND TUMORIGENESIS

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Neurogenesis is the process by which neurons are generated from neural stem cells. Aberrant neurogenesis occurs frequently in human brain disorders. Many chromatin factors are mutated in various brain disorders, however, the contribution of these factors in diseases are not well understood. Mutation of the chromatin remodeler CHD7 (chromodomain-helicase-DNA-binding protein 7) is the major cause of human CHARGE syndrome, a severe developmental disease affecting multiple organs including brain. Our data show that CHD7 is selectively expressed in active neural stem cells and neuronal precursors in mouse brain. Genetic inactivation of CHD7 in forebrain NSC and cerebellar granular cell precursors blocks neuronal differentiation, induces cell death and leads to hypoplasia of affected brain regions. Mechanistically, CHD7 binds to regulatory elements of essential neuronal genes and controls the expression of these genes via modeling local chromatin structure. Moreover, deletion of CHD7 in cerebellar granular cell precursors alters SHH and WNT signaling pathways that are essential for both normal cerebellar development and the development of medulloblastoma, childhood tumors of cerebellum. Our results from genetic mouse models suggest that CHD7 is as a tumor suppressor for granular cell precursers-originated medulloblastoma. In sum, we provides in vivo evidences showing that CHD7 is essential for the activation of neuronal differentiation program during neurogenesis, and blocked such differentiation could lead to tumor formation. Thus our study provides new insights into epigenetic regulation of stem cell differentiation and molecular mechanism of human CHARGE syndrome and medulloblastoma.

F-1370

CHROMATIN ARCHITECTURE DICTATES REPLICATION-TIMING PROGRAMME IN EMBRYONIC STEM CELLS

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DNA replication is temporally and spatially organized in all eukaryotes. The DNA replication timing programme has been shown as a highly stable cell type-specific epigenetic feature. At molecular level, the replication timing correlates with gene density, transcriptional activity, chromatin structure and nuclear position. Mouse Embryonic stem cells (ESc) are characterized by a distinct higher-order global chromatin structure and a unique replication-timing programme which is shared with somatic cells reprogrammed to pluripotency. Moreover, the replication-timing profile undergoes profound changes during differentiation suggesting that the ESc-specific temporal and spatial organization of DNA replication is a signature of the pluripotent state. Therefore an understanding of

how the replication-timing programme in embryonic stem cells is controlled is crucial to get a more comprehensive understanding of pluripotency. We recently identified Rif1 as the master regulator of replication-timing programme, highly expressed in pluripotent ESc. To dissect Rif1 molecular mechanisms governing the replication timing we decided to establish mouse ESc carrying a conditional Rif1 allele. By Chromosome conformation capture, here we demonstrate that Rif1 controls the three-dimensional chromatin organization. Indeed, loss of Rif1 modifies chromatin architecture, in turn impacting on the replication-timing programme. In the long term these changes lead to the alteration of gene expression and pluripotency. Our data indicate chromatin architecture as the common denominator of replication timing and pluripotency, proving for the first time a hierarchical and integrated organization of nuclear function.

F-1371

A SYSTEMATIC APPROACH TO INVESTIGATE THE FUNCTION OF SUPER-ENHANCERS IN EMBRYONIC STEM CELLS

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The β-globin locus control region (LCR) is responsible for tissue-specific robust transcription of the β-globin genes. Recent studies have identified LCR-like elements throughout the genome in many cellular contexts. These clusters of enhancer-like regions have been referred to as super-enhancers or stretch-enhancers, however, limited functional investigation has been conducted to evaluate their role in gene transcription. We conducted CRISPR/Cas9-mediated deletions in F1 ES cells (*Mus musculus*[129] x *Mus castaneus)* to study transcriptional regulation by super-enhancers. The effect of super-enhancer deletion was first investigated in heterozygous enhancer deleted clones by allele specific RNA-Seq to identify regulated genes. These regulatory interactions were next confirmed in additional heterozygous and homozygous clones. Our results revealed that most super-enhancers regulate a single gene, which is often the closest gene, and have only a modest effect on transcriptional activity. Specifically we found that in most cases >40% of the transcript remained when a super-enhancer was deleted. In only a few cases, *Sox2* and *Klf4,* we found that the super-enhancer was required to maintain the majority of transcriptional activity. Many designated super-enhancers overlap genes and as a result are more difficult to study using a deletion approach. We hypothesized that these regions contain independent regulatory regions which have been algorithmically clustered with the gene promoter. To investigate this we studied regulation of *Dppa5a,* which is contained within a super-enhancer. Our results revealed that deletion of either the downstream or upstream region affected expression of *Dppa5a* although neither abolished expression of *Dppa5a.* Interestingly deletion of the upstream enhancer also affected expression of the nearby *Ooep* gene revealing the only example in our study of an enhancer regulating more than one gene. Our data indicate the need for caution in interpreting designated regulatory regions without conducting functional studies. In addition we suggest that robust, distal, clustered regulatory elements which are responsible for the majority of transcription for a particular gene are relatively rare and that in general more dispersed elements regulate gene transcription in an additive manner.

F-1372

GENOMIC DISTRIBUTION AND CHROMATIN LANDSCAPE OF HISTONE H2B GLCNACYLATION DURING ADIPOSE TISSUE STROMAL CELL DIFFERENTIATION

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Stem cells display metabolic plasticity to support differentiation towards various lineages. The major switch in energy metabolism in stem cells is between glycolysis and oxidative phosphorylation. Intermediates in glycolysis are required for synthesis of N-acetyl glucosamine, the substrate used by *O*-linked β-N-acetylglucosamine transferase (OGT) for post-translational modifications (*O*-GlcNAcylation) of proteins, including histones. *O*-GlcNAcylation is required for adipogenesis, and has been suggested to be implicated in the regulation of gene expression. Thus *O*-GlcNAcylation may constitute a link between cellular metabolic state and chromatin organization, but this has not been shown. We report the genome-wide distribution of H2B GlcNAcylated on Ser112 (H2BGlcNAc) by ChIP-sequencing during five stages of differentiation of human adipose tissue stromal cells (ASCs) into adipocytes. We show that H2BGlcNAc occupies large chromatin domains which we refer to as H2BGlcNAcylated domains, or GADs. GADs range from ~10 kilobases to ~10 megabases. GADs are gene-poor and genes within these domains are repressed; accordingly, H2BGlcNAc enrichment negatively correlates with gene expression level determined by RNA-seq. GADs are stable during adipogenic differentiation, with only the smaller domains showing variations in gene-rich areas. We find that adipogenic and osteogenic genes are located outside GADs in undifferentiated ASCs, consistent with a potential for activation on differentiation induction. Lastly, we show that the formation of *de novo* nuclear lamin A-interacting domains (LiDs) on adipogenic induction spatially coincides with domains pre-enriched in H2BGlcNAc, suggesting that GADs constitute a permissive chromatin environment for differentiation-induced anchoring of loci to nuclear lamin A, and thereby spatial organization of the genome.

F-1373

APP INTRACELLULAR DOMAIN SUPPRESSES NEURONAL DIFFERENTIATION THROUGH TRANSCRIPTIONAL REGULATION OF MIR-663

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Amyloid precursor protein (APP) is best known for its involvement in the pathogenesis of Alzheimer's disease. We have previously demonstrated that APP intracellular domain (AICD) regulates neurogenesis; however, the mechanisms underlying AICD-mediated regulation of neuronal differentiation are not yet fully characterized. Using genome-wide chromatin immunoprecipitation approaches, we found that AICD is specifically recruited to the regulatory regions of several microRNA genes, and acts as a transcriptional regulator for miR-663, miR-3648 and miR-3687 in human neural stem cells. Functional assays show that AICD negatively modulates neuronal differentiation through miR-663, a primate-specific microRNA. Microarray data further demonstrate that miR-663 suppresses the expression of multiple genes implicated in neurogenesis, including FBXL18 and CDK6. Our results indicate that AICD plays a novel

role in suppression of neuronal differentiation via transcriptional regulation of miR-663 in human neural stem cells.

GERMLINE CELLS

F-1374

THE ROLE OF UCHL1 IN MOUSE SPERMATOGONIA

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Lifelong male fertility relies on the spermatogonial stem cell (SSC) population of the testis achieving a balance between self-renewal and differentiation. The molecular and physical environment that supports this fate decision balance is not completely understood. The deubiquitinating enzyme Ubiquitin C-terminal Hydrolase L1 (uch-l1) is highly expressed in spermatogonia, but not in further differentiating germ cells of the testis. Previous studies have shown that UCH-L1 is important for normal spermatogenesis; however, its specific role has not been determined. We hypothesize that UCH-L1 is important for spermatogonia maintenance. uch-l1-/- mice were compared to aged matched heterozygous (+/-) and wild type (+/+) controls at key time points during spermatogenesis (7, 17, 30, 60, 90 and 120 days old). Testes were analyzed by immunohistochemistry for differences in undifferentiated spermatogonia numbers (PLZF), somatic Sertoli cell numbers (SOX9), proliferating cells (PCNA) and apoptotic cells (TUNEL) (n=6/genotype/time point). Samples were also analyzed by qPCR for differences in gene expression. Number of Sertoli and apoptotic cells were not significantly different at any time point. The number of PLZF positive cells was also not statistically different at any time point likely due to high variability; however, at 120 days uch-l1-/- testes had a trend to decreasing numbers of PLZF positive cells. Furthermore, qPCR analysis revealed lower expression of spermatogonia associated gene Gpr125 ($p<0.05$) in 120 day old uch-l1-/- testes compare to uch-l1+/+ testes. At 120 days there was a significantly higher number of proliferating cells in uch-l1-/- testis compared to +/+ controls ($p<0.05$). These results indicate that UCH-L1 may be important for maintenance of the spermatogonia pool. The decrease in Gpr125 gene expression coupled with increased cell proliferation in older uch-l1-/- testes suggest that fate decision is skewed towards differentiation. A stronger phenotype is expected at later time points; however, this cannot be studied in vivo due to a strong neurodegenerative phenotype in the uch-l1-/- animals which limits their humane lifespan to 120 days.

F-1375

GERMLINE AND PLURIPOTENT STEM CELLS IN ADULT MOUSE OVARIES AND THEIR IN VITRO DIFFERENTIATION POTENTIAL

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During the last ten years, the presence of germline and somatic stem cells in the adult mammalian ovary and their differentiation into oocyte and somatic cells has been led to controversy. The aim of this study is investigation of in vitro differentiation potential of putative germline stem cells into oocytes in adult mouse (8 week-old) ovaries and analysis of these cells in mRNA and protein levels. As a result of our study, immunohistochemical staining of ovarian frozen sections revealed the presence germline stem cells (DDX4 (VASA) and DAZL) and pluripotent stem cells (Oct-4, Nanog, Sox2 and SSEA1) proteins only in the oocytes of adult mouse ovaries. However, formation of embryoid-bodies (EBs), oocytes, follicles and parthenogenetic embryo-like structures (PELS) form putative pluripotent and germline stem cells were observed in adult mouse ovaries cell culture. Germline and pluripotency potential of ovarian stem cells and EBs were demonstrated in cell culture using immunofluorescence staining of pluripotency-specific (Oct-4, Nanog, Sox2 and SSEA1) and germline-specific (DDX4 and DAZL) proteins. Analysis of mRNA and proteins of EBs, differentiated oocytes, and PELS by qRT-PCR and western blot methods are in progress. Results of this study may support the presence of germline and pluripotent stem cells in adult mouse ovaries cell culture and their differentiation potential into oocyte, follicle and PELS under in vitro circumstances.

F-1376

IN VITRO SPERMATOGENESIS USING TESTIS TISSUE CULTURE TECHNIQUE IN RODENTS

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Spermatogenesis is a complex developmental process of continuous cell division involving a phase of germ cell expansion, meiotic division and cytodifferentiation that ultimately generates mature gametes. The objective of this study was the development of an optimal testis tissue culture system and its establishment in order to induce spermatogenesis in vitro. We collected immature testicular tissues from different developmental-stage of mice and rats which were subsequently cultured on agarose gel with serum-free culture media. After appropriate culture period, we counted multiple layered seminiferous tubules in the cultured testis tissue to find out whether any differentiation had occurred following spermatogenesis. Between our experimental mouse strains ICR and C57, as a result, we observed significantly increased spermatogenic tubules in case of C57 mouse strain in neonatal stage. In our optimized culture condition, we detected the lectin PNA positive spermatid resulted from spermatogenesis in cultured testis tissue after 6 weeks of incubation. We also injected the lentiviral transduced rat spermatogonial stem cells (SSCs) into recipient testis to generate the transgenic gametes and the testicular fragments were then cultured based on our optimized culture condition. After culturing 8 weeks, we observed expression of GFP in differentiated germ cells including post meiotic germ cells, which suggested the induction of donor-derived spermatogenesis. Also, in order to optimize the in vitro culture system for rat testis, we have tested the additional effect of hormones and growth factors, but could not observe any significant enhancive effects among the treated groups than the control. In conclusion, our in vitro tissue culture methods could be applicable for production of in vitro transgenic gametes in various kinds of mammalian species including domestic animal. *This study was supported by the Next-Generation BioGreen 21 Program (No. PJ011347), the Korea Healthcare technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI12C0055).*

F-1377

MITOCHONDRIA-LOCALIZED GASZ LINKS MITOFUSION TO DIFFERENTIATION OF SPERMATOGONIAL STEM CELLS

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Nuage is an electron-dense cytoplasmic structure containing ribonucleoproteins and participates in piRNA biosynthesis and transposon repression. Despite the observation that clustered mitochondria are associated with a specific nuage called intermitochondrial cement in germ cells, the importance of multifaceted mitochondrial function during spermatogenesis is yet to be determined. We show that a germ cell specific protein GASZ contains a mitochondrial targeting signal, and is specifically localized at mitochondria both endogenously in germ cells and in somatic cells when ectopically expressed. In addition, GASZ forms dimers at the outer membrane of mitochondria and promotes MFN1-dependent mitofusion. Either deletion of mitochondrial targeting signal from GASZ or MFN1 deficiency leads to defects of nuage formation and male infertility in mice. Interestingly, either GASZ or MFN1 mutation only affects differentiation of spermatogonial stem cells, but not their self-renewal. Our data thus highlight a crucial role of GASZ/MFN1-dependent mitochondrial function in nuage formation, and demonstrate unequivocally the essential requirement of GASZ/MFN1-mediated mitofusion in germ cell differentiation.

TOTIPOTENT/EARLY EMBRYO CELLS

F-1378

RNA PROCESSING SHAPES CELL FATE AND CELLULAR HOMEOSTASIS

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All organisms maintain homeostasis by dynamically regulating gene expression. RNA processing encompasses key regulatory processes that affect almost all mammalian genes both during and after transcription. In particular, alternative splicing increases the mRNA and protein repertoire and provides a source for qualitative differences between cells because the vast majority of alternatively spliced transcripts are expressed only in specific cells, or at specific times during development. SR proteins comprise a family of essential RNA binding proteins which play a critical role in the regulation of pre-mRNA splicing and also execute functions in other steps of gene expression. How SR proteins coordinate gene expression programs in cells is poorly understood and comprehensive knowledge of their RNA targets is lacking. We use mouse and cell line models together with genome-wide approaches and bioinformatics to decipher SR protein functions in vivo and to identify SR protein regulated gene networks. Functional studies have shown unexpected regulatory roles of SR proteins in the control of gene expression output of cells which is a central feature in the determination of cell fate and maintenance of cellular homeostasis.

F-1379

MICRORNA PROFILE OF THE EARLY PERIOD MOUSE EMBRYOS AND MOUSE EMBRYONIC STEM CELLS AFTER RAPAMYCIN ADMINISTRATION

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Rapamycin is a macrolide antibiotics which inhibits mTOR and has immunosuppressant effects, antitumor effects and antiangiogenic effects. mTOR (Mammalian target of rapamycin) is a serine-threonine protein kinase which regulates protein synthesis, cell growth and proliferation. Each step in mammalian embryonic development is important, especially molecular basis of the division and genetic-epigenetic factors must be revealed. MicroRNA (miRNA)', small and noncoding RNA's, which are important in the regulation of posttranscriptional gene expression have an important role in embrionic development and differentiation. In this study it is aimed to investigate the effect of Rapamycin (mTOR inhibition) administration on the microRNA profile of early stage embryos and mouse embryonic stem cells. Mouse embryos and embryonic stem cells have been investigated in two groups; group I: control group, group II: Rapamycin group. Mouse embryos were further divided into two sub-groups; a) 8-16 blastomere, b) blastocyst group. Microarray analysis was performed to determine the miRNA profile of all groups. The differentially expressed miRNAs were determined by bioinformatics analysis (hierarchical cluster analysis). In addition, common miRNAs between mouse and human was detected from the differentially expressed miRNAs. miRNA heat-maps of all groups was detected, and the most specific changes was found between Rapamycin applied 8-cell embryo group and untreated group. From the perspective of the common miRNAs between mouse and human, 8 different miRNA was expressed in embryos after rapamycin application, 7 different miRNA was expressed in mouse embryonic stem cells after rapamycin application. Although the studies about the role of mTOR in embryogenesis still carry on, it has not been fully elucidated. It is expected that, stem cell and targeted microRNA therapies which are very topical in recent years will take place in embryonic development and the anomaly, in the future.

F-1380

CONSTITUTIVE EXPRESSION OF SERPIN-CI PROTECTS EMBRYONIC STEM CELLS AGAINST LYSIS BY NK CELLS

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Undifferentiated human and murine embryonic stem (ES) cells express very low to undetectable levels of MHC class I molecules. Thereby fulfilling the 'missing self' criterion for recognition and lysis by natural killer (NK) cells, ES cells should be readily lysed by these cytotoxic effector cells. However, ES cells were previously reported to be, if at all, poorly lysed by NK cells. In this study we characterize

the interaction of NK cells with ES cells. Resting or activated NK cells were used as effector cells against ES cells in cytotoxicity assays in vitro. We confirmed and extended previous reports by showing that murine ES cells were poorly lysed by NK cells, regardless whether resting NK cells or activated NK cells were used as effector cells. Importantly, each of these NK cell populations fully recognized ES cells as effectively as a cell line established as standard target cell for murine NK cells, i.e. YAC cells. This was shown by secretion of IFN-gamma in response to target cell contact. We revealed that murine ES cells express the serine protease inhibitor involved in cytotoxicity inhibition (SPI-CI), the specific inhibitor of granzyme M. In NK cells, granzyme M is an important cytotoxic effector molecule stored in cytotoxic granules. Stable knock down of SPI-CI by lentiviral expression of specific shRNA rendered ES cells susceptible to lysis not only by resting NK cells, but also by activated NK cells. The constitutively expressed specific inhibitor of granzyme M, SPI-CI, protects ES cells against NK cell-mediated cytotoxicity in vitro. The biomedical implications of these findings, for example for ES cell-based regenerative medicine or embryonic development, will be discussed.

EMBRYONIC STEM CELL DIFFERENTIATION

F-1381

TCR ENGINEERING OF NK CELLS FOR MELANOMA TREATMENT

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Melanoma is the most dangerous form of skin cancer, and it is resistant to many common cancer treatments, such as chemotherapy and radiation. Although the novel therapies based on the blockage of the PD-1/PDL-1/2 signaling pathway have resulted in tumor regression in some individuals, not all the patients benefited from this treatment. Therefore, developing an effective melanoma treatment is still an urgent mission. The goal of this proof of principle study is to evaluate whether we can augment the ability of natural killer (NK) cells to recognize and kill melanoma cells by engineering them to express a T cell receptor (TCR) specific for melanoma antigen Mart-1. TCRs are not normally expressed on NK cells, although the protein components necessary for TCR signaling exist in these cells. So, by using this approach, we hope to consolidate the NK and T cell anti melanoma properties within one cell type. Furthermore, as melanoma cells generally use different strategies to suppress T and NK cells, we also postulate that TCR expressing NK cells can compensate for the lack of T cell activity when these cells are absent or immunosuppressed. In this study, we opted to use human embryonic stem cells (hESC) as the source of genetically modified NKs, as they are relatively easy to genetically manipulate, expand to clinically relevant quantities and to differentiate into NKs that are phenotypically and functionally indistinguishable from their blood derived counterparts. TCRs require the CD3 complex of proteins for transport to the cell surface and proper signaling. So, to provide all the elements needed to support normal TCR function, we introduced the coding sequences for the CD3 delta, epsilon, gamma and zeta chains, along with the Mart-1 TCR genes, into H1 hESC. We selected individual transgenic hESC lines and then established that these hESC express both Mart-1 TCR and CD3 on their surface. Now we are in the process of differentiating TCR/CD3 hESC into NKs, which will then be used in in vitro and in vivo experiments to establish whether the presence of the Mart-1 TCR endows these transgenic NKs with a higher anti-melanoma cytotoxic potential. Importantly, if our approach is successful, this technology could be adapted and expanded for treatment of other tumors and malignancies.

F-1382

PHF8 REGULATES CARDIAC LINEAGE DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS VIA CONTROLLING PROGRAMMED CELL DEATH

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Spontaneous apoptosis is concomitant with the differentiation of embryonic stem cells (ESCs) and plays a critical role in specific lineage commitment. However, the role of histone demethylases (KDMs) during this process is poorly understood. In this study, we screened the expression profile of 18 KDMs during mouse ESC (mESC) cardiac differentiation and found that plant homeo domain finger protein 8 (PHF8) was increased during early differentiation. Then we knocked out the X-chromosome-encoded phf8 gene in one allele of male SCR012 ESCs (phf8-/Y). Deletion of phf8 did not significantly affect the self-renewal of ESCs, but the cell viability was greatly increased during ESC differentiation. Further analysis showed that phf8-/Y significantly reduced apoptosis in the early differentiating ESCs, while no difference in the proliferation of differentiating cells between the wild type ESCs (phf8+/Y) and phf8-/Y. The mRNA micro-array analysis showed an increase of pro-apoptotic gene expression in early differentiating phf8+/Y ESCs, but opposite in early differentiating phf8-/Y ESCs. ChIP-seq analysis demonstrated that PHF8 regulated the apoptosis by removing the repressive mark H3K9me2 and activated the transcription of pro-apoptotic gene. Moreover, deletion of phf8 promoted the differentiation of mesoderm and cardiac lineages, and the downregulation of the pro-apoptotic gene enhanced the mesodermal and subsequent cardiac lineage commitment in phf8+/Y cells. In conclusion, we reported here the previously unrecognized effect of the epigenetic regulator on germ layer commitment and differentiation by modulating key mediators of apoptosis. These findings extend the knowledge in understanding of the epigenetic modification in apoptosis during ESC differentiation and in the link between apoptosis and cell lineage decision as well as cardiogenesis.

F-1383

EFFECTS OF BDNF ON SPECIFICATION OF GLUTAMATERGIC PROGENITORS IN FRAGILE X SYNDROME

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Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability and a well characterized form of autism spectrum disorder. A triplet repeat expansion in the *FMR1* gene leads to transcriptional silencing and the absence of FMR1 protein (FMRP) in FXS. FMRP is a RNA binding protein essential for maturation and function of synapses and neuronal networks. Neural progenitor differentiation is altered in the absence of FMRP. Studies of *Fmr1* knockout (KO) mice, a mouse model of FXS, have revealed that glutamate and brain-derived neurotropic factor (BDNF)/TrkB signaling are implicated in the neurobiology of FXS. We have studied specification of FXS neural progenitors to glutamatergic neurons and examined the contribution of BDNF to the alterations in the differentiation of neuronal cells in human and mouse FXS neurosphere cultures. We reprogrammed somatic cells of individuals with FXS to induced pluripotent stem (iPS) cells and examined functional responses to activation of glutamate receptors by intracellular calcium recordings with Fura-2 at different stages of neuronal differentiation. We observed alterations in the responses to activation of both metabotropic glutamate receptors by (S)-3,5-dihydroxyphenylglycine (DHPG) and ionotropic glutamate receptors by kainate and N-methyl-D-aspartate (NMDA) in FXS progenitors when compared to that of controls. Treatment with a specific antagonist of metabotropic glutamate receptor 5 prevented some of these changes. Furthermore, we observed that findings in human FXS neurospheres correlated with findings in mouse FXS neurospheres and that reduced BDNF modified neuronal specification of progenitors derived from mice deficient of both FMRP and BDNF. Altogether our results show that glutamate and BDNF contribute to altered neural progenitor specification in FXS and that the patient-specific neural progenitors provide a promising new tool to study involvement of glutamate signalling in FXS.

F-1384

EARLY FATE SPECIFICATION OF PLURIPOTENT STEM CELLS IMPROVES DOPAMINERGIC DIFFERENTIATION AND ISOLATION OF PROGENITORS FOR TRANSPLANTATION INTO A RODENT MODEL OF PARKINSONISM

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While pluripotent stem cells (PSCs) represent a potentially unlimited cell source for the purpose of transplantation, poorly specified and undifferentiated cells result in suboptimal functionally integrated grafts and risks of tumors. To overcome these limitations we have developed an improved differentiation protocol for mouse PSC and made use of a reporter line (Lmx1a-GFP), enabling isolation of high proportions of dopaminergic progenitors for the purpose of transplantation into an animal model of Parkinson's disease. By comparison to pre-existing differentiation protocols our new method, involving early fate specification without the need for stromal-derived feeder layer, results in significantly greater proportions of correctly specified DA progenitors (FoxA2+/Lmx1a+/Nurr1+) and mature DA neurons (Nurr1+/TH+/Pitx3+), as revealed by FACS analysis and immunocytochemistry. Using the LMX1a-GFP mouse reporter line and aforementioned differentiation protocol, we were able to demonstrate the benefit of transplanting FACS isolated LMX1a+ dopamine progenitors compared to Lmx1a- or unsorted cells. Lmx1a+ grafts showed enhanced numbers of DA neurons, graft innervation and significant improvements in motor function. This improved protocol enables the enrichment of dopamine progenitors for the purpose of transplantation, and additionally provides high proportions of mature dopamine neurons that may be of benefit for disease modelling and drug discovery.

F-1385

ROLE OF MEDIATOR COMPLEX IN HEPATOCYTE DIFFERENTIATION

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Several protocols have been developed recently to differentiate human embryonic stem cells (hESCs) into definitive endoderm, hepatic progenitors and mature hepatocytes progressively. This provides an excellent model to study hepatocyte differentiation which still remains poorly understood in human. Previous studies using animal models have identified several signaling pathways and transcription factors such as Foxa1-2, GATA 4/6, Hhex and HNF4α in hepatocyte differentiation; however the molecular mechanisms of human hepatocyte differentiation still remain obscure. Mediator is a transcriptional coactivator complex known to regulate gene expression by bridging gene specific transcription factors to RNA Pol II. It's a large multi-subunit complex comprising 30 protein subunits in mammals. Mediator subunits are known to interact with a large number of transcription factors including liver specific transcription factors such HNF4α to regulate gene expression. Mutations in various subunits of Mediator complex are known to cause different developmental disorders indicating its role in development. Here we have identified a specific subunit of Mediator complex to regulate the expression of various effectors of insulin signaling pathway including insulin receptor, PI3K and AKT in liver. Insulin is known to enhance hepatocyte differentiation from hESC-derived DE through the activation of PI3K/AKT pathway. We are currently focused on understanding the role of this Mediator subunit in the hepatocyte differentiation with the hypothesis that it acts as a master regulator in insulin signaling pathway and regulates early stages of liver development using an in-vitro model of embryonic hepatogenesis by differentiating hESCs into hepatocytes. Understanding the transcriptional regulatory mechanisms of hepatogenesis could lead to better exploit hES or hiPS cell derived hepatocytes in the treatment of liver diseases.

F-1386

EXPOSURE TO IONIZING RADIATION IMPAIRS MOUSE EMBRYONIC STEM CELL DIFFERENTIATION

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The use of therapeutic and diagnostic procedures based on ionizing radiation has increased in the last decades, which at certain circumstances may represent a thread to the embryo of early-stage pregnancies. The embryo is particularly sensitive to ionizing radiation at early developmental stages. Taken this information together with the scarce data collected mainly from survivors of

nuclear catastrophes like Hiroshima or Chernobyl, further studies to assess the radiation-caused damage are required. Hence, pluripotent embryonic stem (ES) cells are ideal models to study the embryotoxicity of ionizing radiation, due to their ability to differentiate into all cells of the three germ layers, resembling an in vivo embryonic development. The European Center for the Validation of Alternative Methods has validated the Embryonic Stem cell Test (EST) as a model system to assess the toxicity of chemical compounds via differentiation of mouse ES cells into beating cardiomyocytes as one of the endpoints of the EST. We studied the effects of sparsely ionizing X-rays and densely ionizing carbon ions (C-ions), two different radiation qualities, on the differentiation capacity of the mouse ES-D3 cell line. Pluripotent cells were differentiated via Embryoid Body (EB) formation directly after exposure. In control cultures spontaneously beating areas containing cardiomyocytes could be observed from day 10 onwards. The percentage of beating EB at day 10 was lower in exposed samples compared to controls, and it increased with time. Gene expression analysis by quantitative RT-PCR revealed a deregulated expression of pluripotent and cardiac markers in the exposed samples. To further investigate the differentiation process, germ layer-specific markers were analyzed and an alteration of the expression pattern was observed in the samples exposed to ionizing radiation. Moreover, an increase in liver markers was observable at late differentiation time points (30 days) in samples exposed to C-ions. In conclusion, our results suggest that exposure to ionizing radiation at pre-implantation stages could affect organogenesis.

F-1387

EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO NEURAL CREST AND NEURAL CREST DERIVATIVES UNDER FEEDER-FREE, CHEMICALLY DEFINED CONDITIONS

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The neural crest (NC) is a multipotent progenitor population capable of differentiating to a wide variety of different cell types including smooth muscle cells (SMC), neurons, mesenchymal stem cells (MSC), adipocytes, chondrocytes, osteocytes, and melanocytes. Whilst numerous methodologies have been reported to generate NC from human pluripotent stem cells (hPSC), further work is required to facilitate the production of this cell type in a fully chemically defined manner, and to reduce the complexity of the differentiation conditions required. Here we report a simple, robust, and efficient protocol to differentiate hPSC to bona fide NC under feeder-free, chemically defined conditions. The NC population generated expresses the markers B3GAT1, NGFR, TFAP2A, ZIC1, PAX3, FOXD3, SOX9, SOX10, and S100B after 12 days in culture. This population is 90-95% NGFR+/TFAP2A+, and is obtained without the use of cell sorting. The generated NC cells are migratory, and respond to the well-established NC chemoattractants Wnt3a and FGF8. We demonstrate that the NC population can be matured in vitro, and that the efficiency of differentiation to NC-derivatives is increased by maturing the population. SMCs derived from the NC are contractile and demonstrate high expression of the markers CNN1, MYH11 and ACTA2. NC-derived SMCs demonstrate a specific requirement for MRTF-B activation during development in vitro, as previously demonstrated in vivo. Additionally, NC-derived SMCs maintain expression of specific NC markers, which are not present in SMCs derived from a mesodermal origin, which may act as a marker of embryological origin. As this protocol generates a proliferating population of NC which is expandable and cryopreservable, we believe that this can be used to generate the high numbers of cells required for drug screening and human disease modelling studies. This could be of great use in studies of NC disorders such as Treacher-Collins syndrome and DiGeorge syndrome.

F-1388

MODELLING THE FATE DECISIONS OF HUMAN PLURIPOTENT STEM CELLS

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The use of human pluripotent stem cells (hPSC) in replacement therapies is greatly affected by the inherent heterogeneity found within the cultures and a large proportion of cells being lost between subsequent passages leading to culture adaptation.
An improvement in the ability to expand cultures and generate differentiated cells may be facilitated by developing computational models that characterise the shift between the three choices hPSC have to make: self-renewal, differentiation and death. We investigated the spatio-temporal characteristics of hPSC fate decisions at the onset of differentiation in cells maintained in a controlled culture environment and observed using a time-lapse microscope. Cell cultures were exposed to precisely controlled time-series of factors triggering differentiation delivered through a microfluidic platform. Information extracted from time-lapse was used to generate models capturing the shift in fate of hPSC and investigate the effect of colony topology on efficiency of conversion to differentiated cell types.
Our study combines time-lapse analysis, computational techniques and immunocytochemistry to provide insight into how decisions at the single cell level are responsible for complex heterogeneous behaviour visible in hPSC cultures. In particular, the modelling helps explain the observation that cell position and colony size affect the fate that hPSC ultimately adopt. The models we have developed will form the basis for the identification of innovative strategies to control stem cell behaviour.

F-1389

TBX3 FUNCTION IN HEPATIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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End stage liver disease represents a growing health challenge while organ transplantation remains the only therapy available. However, shortage in organ donors and side effect of immunosuppressive treatment are major limiting factors. Consequently, production of hepatocytes like cells from Human pluripotent stem cells (hPSCs)

represents a major objective for the regenerative medicine field. However, generation of fully functional hepatocytes from hPSCs continue to be problematic. Here, we studied regulators of hepatic specification to understand human liver development and to generate hIPSCs derived hepatocyte like cells with improved functional characteristics. One of these regulators is TBX3, a T-box transcription factor involved in liver organogenesis and hepatoblast differentiation toward hepatocytes vs cholangiocytes. We showed that TBX3 has a distinct pattern of expression in relation to other reported transcription factors during differentiation of hPSC toward the hepatocyte lineage. Microarray analysis confirmed the specific pattern of TBX3 expression and also uncovered that TBX3 is co-expressed with genes mainly involved in alternative splicing and mRNA regulation. Using this knowledge, we developed knockout strategies with the CRISPR-Cas9 system and also conditional overexpression approaches using Oestrogen-Receptor fusion proteins in hESCs. These gain and loss of function experiments enable us to validate targets genes identified by gene expression analyses and also to investigate the molecular role of TBX3 in liver lineage differentiation. Together, these results demonstrate that hPSCs can be used to model liver development in vitro and to study the function of key factor directing hepatocytes production in vivo.

F-1390

MULTPOTENT CAUDAL NEURAL PROGENITORS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS THAT GIVE RISE TO LINEAGES OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM

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The caudal neural plate is a distinct region of the embryo that gives rise to major progenitor lineages of the developing central and peripheral nervous system, including neural crest and floor plate cells. We show that dual inhibition of the glycogen synthase kinase 3β (GSK3β) and activin/nodal pathways by small molecules differentiate human pluripotent stem cells (hPSCs) directly into an early OCT4-/SOX2+/PAX6- caudal neural progenitor cell (CNP), which are equivalent to progenitors of the embryonic caudal neural plate. CNPs can efficiently generate neural crest, floor plate, and caudally-specified neuroepithelial cells. Neural crest derived from CNPs differentiated to neural crest derivatives and demonstrated extensive migratory properties in vivo. Our studies define a novel progenitor derived from human pluripotent stem cells, which is the precursor to major caudal lineages of the embryonic neural tube.

F-1391

HIGHLY EFFICIENT NEURAL CONVERSION OF HUMAN PLURIPOTENT STEM CELLS IN ADHERENT AND ANIMAL-FREE CONDITIONS

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Neural differentiation of human embryonic stem cells (hESCs) and induced pluripotent stem cells (ihPSCs) can produce a valuable and robust source of human neural cell subtypes, holding great promise for the study of neurogenesis and development, and for treating neurological diseases. However, current hESCs and ihPSCs neural differentiation protocols require either animal factors or embryoid body formation, which decreases efficiency and yield, and strongly limits medical applications. Here we develop a simple, animal-free protocol for neural conversion of both hESCs and ihPSCs in adherent culture conditions. A simple medium formula including insulin induces the direct conversion of >98% of hESCs and ihPSCs into expandable, transplantable, and functional neural progenitors with neural rosette characteristics. Further differentiation of neural progenitors into dopaminergic and spinal motoneurons as well as astrocytes and oligodendrocytes indicates that these neural progenitors retain responsiveness to instructive cues revealing the robust applicability of the protocol in the treatment of different neurodegenerative diseases. The fact that this protocol includes animal-free medium and human extracellular matrix components avoiding EBs makes this protocol ready for the use in clinic.

F-1392

INSULIN SIGNALING AND RESISTANCE IN HUMAN PLURIPOTENT STEM CELL-DERIVED MODELS OF METABOLIC DISEASE

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We are in the midst of a worldwide epidemic of type 2 diabetes (T2D) and closely related problems of obesity and metabolic syndrome. These disorders represent a complex interaction between genes and environment. In the case of T2D there is a well-defined progressive pathogenesis of disease, beginning with insulin resistance in peripheral tissues such as muscle, fat and liver. This is initially compensated for by increased insulin secretion, but eventually beta cells exhaust and there is a gradual decline in insulin level leading to clinical onset of hyperglycemia. While some alterations in insulin action leading to insulin resistance have been defined and genome wide association studies have identified many genes associated with risk of T2D, the primary defect(s) in peripheral tissues leading to the insulin resistance remains unclear. To elucidate the molecular basis of insulin resistance, we generated several genetic models in human pluripotent stem cells. These models replicate both impaired and constitutively activated insulin signaling through the knockout or modification of genes involved in the insulin signaling pathway. To mimic impaired signaling, we knocked out the insulin receptor INSR, as well as AKT2, the main intracellular insulin signaling node, and FOXO1, a major insulin-regulated metabolic transcription factor. In addition, we have knocked out PTEN and knocked-in a mutation in AKT2 (E17K) to model activated insulin signaling. We

are currently characterizing these genetic models in a number of relevant metabolic cell types differentiated from human pluripotent stem cells. These include adipocytes, hepatocytes, as well as the cells tasked with transport of insulin from the bloodstream to underlying organs, namely endothelial and vascular smooth muscle cells. The differentiated cells are comprehensively analyzed via RNA-seq, metabolomics profiling and insulin stimulation assays to generate a global picture of the effects of altered insulin resistance on the behavior of metabolically important cells. The results of these studies will be the subject of our poster presentation. Ultimately we hope to illuminate novel mechanisms and identify targets in the insulin signaling pathway that would be of value in understanding and treating insulin resistance in the setting of human metabolic disease.

F-1393

BETA-CATENIN REGULATES PRIMITIVE STREAK INDUCTION THROUGH COLLABORATIVE INTERACTIONS WITH SMAD2/3 AND OCT4

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Canonical Wnt and Nodal signaling are required for primitive streak (PS) formation during vertebrate embryogenesis and in vitro models of this process. However, whether β-catenin's role is reduced to simply regulating Nodal signaling remains unclear. We analyzed the genome-wide binding of β-catenin in hESCs induced to differentiate by GSK-3-inhibition. We show that β-catenin occupies regulatory regions in numerous PS and neural crest genes. Chromatin binding and loss-of-function studies show that PS gene expression requires direct interaction between β-catenin and SMAD2/3 at regulatory regions in PS genes. Furthermore, OCT4 binds in proximity to these sites and is required for PS induction, suggesting a collaborative interaction between β-catenin and OCT4. In contrast, β-catenin-induced neural crest gene expression is repressed by SMAD2/3 activity. Our study provides novel mechanistic insights into how Wnt signaling, via β-catenin-mediated transcriptional responses regulate early cell lineage choices through cooperative interactions with OCT4 and SMAD2/3 at the chromatin level.

F-1394

MEGAKARYOCYTE-PLATELET INDUCTION FROM MOUSE EMBRYONIC STEM CELLS BY ENFORCED EXPRESSION OF GATA2 IN LATE HEMOGENIC ENDOTHELIAL CELLS

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Hematopoietic stem cells emerge from aortic walls of developing embryos via endothelial to hematopoietic cell transition (EHT). To explore functions of Gata2 in EHT, we generated A2Lox-iGata2 mouse ES cell line, which carries doxycycline (Dox)-inducible Gata2 expression cassette. When A2Lox-iGata2 ES cells were differentiated on OP9 cells for 3 days followed by 2-day culture with or without Dox, we observed 3-fold increase of EHT of c-Kit$^+$CD41$^+$ cells from Tie2$^+$c-Kit$^+$CD41$^-$ hemogenic endothelial cells by Gata-2 overexpression. In contrast, when the hemogenic endothelial cells were isolated after 5 days of differentiation and cultured in the presence or absence of Dox and thrombopoietin for 4 days, we observed predominant differentiation of c-Kit$^-$CD41$^+$ cells by Gata-2 overexpression. These c-Kit$^-$CD41$^+$ cells were positive for acetylcholine esterase and possessed CFU-Mk, suggesting that they are megakaryocytic cells. When the culture was continued for 4 more days, they produced platelets expressing CD41, CD42b, CD42d, and CD61 on surface. Upon stimulation with thrombin, these platelets adhered to fibrinogen-coated cover glass and displayed actin stress fibers. Electron microscopic analysis revealed that they have discoid shape with alpha granules and open canalicular system, but are relatively larger in size than circulating platelets in adult mice. We often observed nucleated platelets as well in the A2Lox-iGata2 ES culture. These platelets could be related to primitive platelets present in yolk sac of E8.5-10.5 mouse embryos. Our data demonstrated that enhanced expression of Gata2 in late stage of ES-derived hemogenic endothelial cells makes them to produce megakaryocytes. This finding is also important for developing a new method of ex vivo platelet production from human ES and iPS cells.

F-1395

SUPPRESSOR OF FUSED PLAYS AN IMPORTANT ROLE IN REGULATING MESODERMAL DIFFERENTIATION OF MURINE EMBRYONIC STEM CELLS IN VIVO

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The hedgehog (Hh) signaling pathway plays fundamental roles during embryonic development and tumorigenesis. Previously, we have shown that ablation of the tumor suppressor and negative regulator, Suppressor of fused *(Sufu)*, within this pathway causes embryonic lethality around E9.5 in the mouse. Here, we examine how lack of Sufu influences early cell fate determination processes. We established novel embryonic stem cell (ESC) lines from pre-implantation *Sufu*$^{-/-}$ and wild-type mouse embryos and show that these ESCs express the typical pluripotency markers, alkaline phosphatase, SSEA-1, Oct4, Sox2, and Nanog. We demonstrate that these ESCs express all core Hh pathway components, and that *Gli1* mRNA levels are increased in *Sufu*-$^{-/-}$ ESCs. Upon spontaneous differentiation of *Sufu*$^{-/-}$ ESCs, into embryoid bodies (EBs) in vitro, the Hh pathway is strongly upregulated as indicated by an increase in both *Gli1* and *Ptch1* gene expression. However, developing *Sufu*$^{-/-}$ EBs were smaller than their wild-type counterparts. In vivo teratoma formation revealed that *Sufu*$^{-/-}$ ESCs have a limited capacity for differentiation as the resulting tumors lacked the mesodermal derivatives, cartilage and bone. However, *Sufu*$^{-/-}$ ESCs were able to develop into chondrocytes in vitro, which suggests a differential response of stem cells compared to in vivo conditions. Our findings further emphasize the role of *Sufu* as a key molecule in the Hh

signaling pathway, and suggest a function for *Sufu* in the mesodermal lineage differentiation process.

F-1396

INCREASED ACTIVITIES OF DRUG METABOLIZING ENZYMES IN HEPATIC SPHEROIDS DERIVED FROM HUMAN ES CELLS BY REPEATED EXPOSURES TO XENOBIOTICS

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Highly homogeneous and functional stem cell-derived hepatocyte-like cells (HLCs) are considered a promising option in the treatment of liver disease and the development of effective in vitro toxicity screening tool. However, the purity of cells and expression and/or activity of drug metabolizing enzymes in stem cell-derived HLCs are usually too low to be useful for clinical or in vitro applications. Here, we describe a highly optimized differentiation protocol, which produces more than 90% albumin-positive HLCs with no purification process. In addition, we show that hepatic enzyme gene expressions and activities were significantly improved by generating three-dimensional (3D) spheroidal aggregate of HLCs. The 3D differentiation method increased expressions of nuclear receptors that regulate the proper expression of key hepatic enzymes. Furthermore, a significantly increased hepatic functions such as albumin and urea secretion were observed in 3D hepatic spheroids and HLCs in the spheroid exhibited morphological and ultrastructural features of normal hepatocytes. Importantly, we show that repeated exposures to xenobiotics facilitated the functional maturation of HLC, as confirmed by increased expression of genes for drug metabolizing enzymes and transcription factors. In conclusion, the 3D culture system with repeated exposures to xenobiotics may be a new strategy for enhancing hepatic maturation of stem cell-derived HLCs as a cell source for in vitro high-throughput hepatotoxicity models. *This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (No. 2012M3A9B4028636 and 2012M3A9C7050139). School of Life Science and Biotechnology for BK21PLUS for Jong-Hoon Kim, Korea University.*

F-1397

PEROXIREDOXIN SUPPRESSES ROS/JNK-MEDIATED STEMNESS LOSS DURING NEUROGENESIS FROM EMBRYONIC STEM CELLS

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Redox balance has been suggested as an important determinant of "stemness" in embryonic stem cells (ESCs). In this study, we demonstrate that peroxiredoxin (Prx) plays a pivotal role in maintenance of ESC stemness during neurogenesis through suppression of reactive oxygen species (ROS)-sensitive signaling. During neurogenesis, Prx I and Oct4 are expressed in a mutually dependent manner and their expression is abruptly downregulated by an excess of ROS. Thus, in Prx I−/− or Prx II−/− ESCs, rapid loss of stemness can occur due to spontaneous ROS overload, leading to their active commitment into neurons; however, stemness is restored by addition of an antioxidant or an inhibitor of c-Jun N-terminal kinase (JNK). In addition, Prx I and Prx II appear to have a tight association with the mechanism underlying the protection of ESC stemness in developing teratomas. These results suggest that Prx functions as a protector of ESC stemness by opposing ROS/JNK cascades during neurogenesis. Therefore, our findings have important implications for understanding of maintenance of ESC stemness through involvement of antioxidant enzymes and may lead to development of an alternative stem cell-based therapeutic strategy for production of high-quality neurons in large quantity.

F-1398

IGF-1 SIGNALLING PLAYS AN IMPORTANT ROLE IN THE FORMATION OF THREE DIMENSIONAL LAMINATED NEURAL RETINA AND OTHER OCULAR STRUCTURES FROM HUMAN EMBRYONIC STEM CELLS

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We and others have previously demonstrated that retinal cells can be derived from human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC) under defined culture conditions. Whilst both cell types can give rise to retinal derivatives in the absence of inductive cues, this requires extended culture periods and gives lower overall yield. Further understanding of this innate differentiation ability, the identification of key factors that drive the differentiation process and the development of clinically compatible culture conditions to reproducibly generate functional neural retina is an important goal for clinical cell based therapies. We now report that insulin-like growth factor 1 (IGF-1) can orchestrate the formation of three dimensional ocular-like structures from hESCs which, in addition to retinal pigmented epithelium and neural retina, also contain primitive lens and corneal-like structures. Inhibition of IGF-1 receptor signalling significantly reduces the formation of optic vesicle and optic cups, whilst exogenous IGF-1 treatment enhances the formation of correctly laminated retinal tissue composed of multiple retinal phenotypes that is reminiscent of the developing vertebrate retina. Most importantly, hESC-derived photoreceptors exhibit advanced maturation features such as the presence of primitive rod- and cone-like photoreceptor inner and outer segments and phototransduction-related functional responses as early as 6.5 weeks of differentiation, making these derivatives promising candidates for cell replacement studies and in vitro disease modelling.

F-1399

DIFFERENTIATION OF HPSC-DERIVED NEURAL PROGENITOR CELLS INTO NEURONS AND ASTROCYTES

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Mature neural cell lineages derived from human pluripotent stem cells (hPSCs) are important tools for modeling human central nervous system (CNS) development and neurodegenerative disorders. However, published differentiation protocols for the

consistent and efficient generation of neurons, astrocytes and oligodendrocytes are often difficult to reproduce in laboratories, and as such rigorous optimization of these protocols is highly desirable to advance the field. We have previously demonstrated that the STEMdiff™ Neural Induction Medium (NIM) and AggreWell™800 can be used to efficiently produce CNS-type neural progenitor cells (NPCs) from multiple hPSC lines. Here we present preliminary results for downstream differentiation of NPCs to forebrain neurons and astrocytes that integrate with the STEMdiff NIM workflow for hPSC neural induction. NPCs were generated from hPSCs using STEMdiff NIM and AggreWell™800 embryoid body protocol and then used for downstream differentiation as described below. To terminally differentiate hPSC-derived NPCs to mature neurons and astrocytes, a sequential 2-phase induction and maturation approach was employed. For forebrain neurons, a Forebrain Neuron Induction Medium (FbIM) was applied shortly following re-plating and selection of neural rosettes. After 5 - 7 days, FbIM medium was replaced with Forebrain Neuron Maturation Medium and neuronal progenitors were allowed to mature for 2 - 4 weeks. Similar to published protocols, we could obtain 80 - 90% FOXG1+/TuJ-1+ forebrain neurons with 50% GABAergic neurons (n=2). Next, for the differentiation of hPSC-derived NPCs to astrocytes, Astrocyte Induction Medium (AIM) was added for 3 weeks after neural rosette selection to induce gliogenesis. The AIM was then replaced and glial precursors were differentiated to astrocytes for 30 - 60 days in Astrocyte Maturation Medium. The majority of the cells (80 - 90%; n=2) obtained were GFAP+ and exhibited the star-shaped astrocytic morphology. In summary, our results demonstrate efficient protocols for the differentiation of hPSC-NPCs into forebrain neuron and astrocytes. Studies are underway to further improve efficiency and reproducibility of the protocols to provide researchers with a robust integrated workflow for modelling neurological disease using human ES or iPS cells.

F-1400

A NOVEL HUMAN EMBRYONIC STEM CELL-DERIVED TROPHOBLASTIC SPHEROID IMPLANTATION MODEL FOR THE STUDY OF EARLY HUMAN IMPLANTATION

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Assisted reproduction technology offers the infertile couples with successful pregnancy over the past few decades though the pregnancy rate remains low. Failure in implantation remains as the rate-limiting factor. The exact mechanism of implantation failure is still poorly understood and investigations are constrained by ethical issues. The recent developments of informative animal models and novel in vitro human models have shed some lights on this implantation "black box" though choriocarinoma cell lines were used in these models. In this study, we aimed at generating a novel model of human embryonic stem cells (hESC) derived trophoblastic spheroid to mimic the three-dimensional structure of human blastocyst during the early implantation stage. To this end, we have generated hESC-derived trophoblastic spheroids which resembled early implanting human embryo in size and with blastocoel like cavity structure. Those spheroids expressed various trophoblastic markers and secrete βhCG. Interestingly, spheroids that have been differentiated for 3 days were able to attach and invade; while little or no attachment was found on those that have been differentiated for 2 days. The attachment was specific to the endometrial epithelial cells, as little or no attachment was found when they were ocultured with a number of different cell lines. The present modified differentiation protocol provides a novel approach to study trophoblastic cell differentiation, implantation, and trophoblastic invasion into human endometrial cells. *This work is supported by General Research Fund (HKU17111414)*

F-1401

THE EFFECTS OF FOLLISTATIN ISOFORMS ON SURVIVAL OF DIFFERENTIATING MOUSE EMBRYONIC STEM CELLS

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Follistatin (fst) is a protein that is able to bind and antagonize members of TGF-β superfamily, including activins and bone morphogenetic proteins (BMP). As activins and BMPs play an important role in embryonic stem (ES) cell self-renewal, pluripotency and differentiation, it is likely that follistatin is also involved in these important characteristics of ES cells. Follistatin isoforms arise from alternative pre-mRNA splicing and proteolytic cleavage and exist as three isoforms (i.e., fst288, fst303 and fst315) differing in length at the C terminus. In this work, we try to delineate the effects of follistatin isoforms on survival of differentiating mouse ES cells. We constructed four fst isoform-specific transgenes (fst288, fst303, fst315 and fst-myc), which are drived by the EF1 promoter and can consistently overexpress follistatin isoform 288, 303 and 315, respectively. The fst-myc was fst315 with a myc-tag linked at its C terminus, which design may be able to prevent fst315 from proteolysis into fst303 in vivo. Mouse ES cells were transfected with these four transgenes individually by electroporation, and then stable clones were screened. To test the influence of follistatin isoforms on survival of mouse ES cells during differentiation, the ES cell clones were cultivated on gelatin for 7 days and in suspension medium for 27 days. Assessments of cell proliferation and apoptosis were performed by flow cytometry for cell counting, BrdU incorporation, Annexin V-7 ADD staining and TUNEL for the various samples after the different periods of cultivation. The mRNA levels of apoptosis-related genes were evaluated by qRT-PCR, including Aifm1, Apaf1 Bcl2, Casp3, Casp8, Casp9 and Parp1. Fst isoforms did not block the endodermal, mesodermal, or ectodermal commitments, however, with different extents of diverse gene expressions during EB differentiation. Further, different fst isoform clones show the distinct characteristics in apoptosis-related gene expressions as well as the percentages of BrdU incorporation and positive TUNEL at the specific times during differentiation.

F-1402

ACTIVATION OF PROHGF BY ST14 INDUCES MOUSE EMBRYONIC STEM CELLS DIFFERENTIATION

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In mouse embryonic stem cells (mESCs) growth factors and cytokines (such as HGF) play pivotal roles in pluripotency and differentiation. They are usually synthesized as latent forms and require activation by the protease system located on the stem cells membrane. Thus, the interaction between these latent extracellular

regulators and "activators" on stem cell surface are extremely important for the fate decision of mESCs. In the current study, we studied the function of a transmembrane serine protease named St14 in mESCs. Whether the latent form of its well-known substrate, hepatocyte growth factor (proHGF) could be activated to bioactive HGF by St14 to induce differentiation was explored. Our result showed that at the presence of proHGF, over-expression of St14 resulted in decrease of pluripotent markers and increase of differentiation genes in mESCs. Similar results were observed in mESCs with knock-down of hepatocyte growth factor activator inhibitors 1 and 2 (HAIs) which are known inhibitors of the St14, as differentiation genes were up-regulated while pluripotent markers were decreased. This suggesting that under the control of HAIs, St14 plays important roles in mediating the collaboration between mESCs and stem niche by functioning as an upstream "activator" to activate proHGF and thus induced mESCs differentiation.

F-1403

EFFECTS OF LOW OXYGEN TENSION AND PROLYL-4-HYDROXYLASE INHIBITION ON DIFFERENTIATION OF HESCS TOWARD CHONDROCYTES

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Articular cartilage injury presents a major challenge in orthopaedics especially in young patients. A number of strategies to deal with this problem have been developed including cell-based methods for cartilage repair. Human embryonic stem cells (hESCs), which are pluripotent and can differentiate into all cell lineages are a potential cell source for such treatments. As the oxygen concentration in the human stem cell niche and in the articular joint are much lower than ambient, we have investigated stem cell growth and chondrogenic differentiation at lowered oxygen tension and in the presence of dimethyloxalylglycine (DMOG), a cell permeable prolyl-4-hydroxylase inhibitor, which mimics low oxygen effects by stabilizing hypoxia-inducible factor (HIF) proteins. An established 14 days differentiation protocol for chondrogenesis of hESC was set up to generate chondrocytes. We tested preconditioning of hESCs in 3 sub-ambient O_2 levels, followed by directed differentiation towards chondrocytes in parallel in ambient oxygen (20%) and low oxygen (3%). In addition we also investigated the effect of chondrogenic differentiation with DMOG under ambient oxygen (20%) in order to mimic low oxygen effects by up-regulating HIFs. The cells were characterised by qRT-PCR of chondrocyte-related genes, immunofluorescence staining, safranin O staining and western blotting. Our results showed that hESCs after preconditioning had a small but significantly favourable response to directed differentiation under lowered oxygen (3%) with up-regulation of *COL2A1* ($P<0.05$) and down-regulation of *COL1A1* ($P<0.001$) compared to 'normoxic' conditions (20%). However, clear increases in HIFs proteins were not detected in these low oxygen culture conditions. Differentiation with DMOG under ambient oxygen level (20%) also gave some increases of *SOX5*, *SOX9*, *ACAN* and *COL2A1* ($P<0.05$) with decrease of *COL1A1* ($P<0.001$). These results suggested that the effect of low oxygen (3%) in enhancing chondrogenic differentiation resulted from effects other than the direct activation of the HIF pathway. However, DMOG inhibition of prolyl-4-hydroxylase showed that specific activation of the HIF pathway could result in beneficial effects on chondrogenesis, but it remains to be established if these effects are sustainable.

F-1404

GENERATION OF STOMACH TISSUE CELLS FROM PLURIPOTENT STEM CELLS.

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Progress in developmental studies has led to establish various methods for differentiating mouse and human pluripotent stem cells into endodermal lineages, including liver hepatocytes, pancreatic β cells, and intestinal cells. A three-dimensional (3D) intestinal organoids composed of epithelium and mesenchyme mimics intestinal development and is useful for understanding the development as well as intestinal diseases. However, little is known about stomach lineage specification from pluripotent stem cells. Very recently, human Gastric Organoids (hGO) were successfully generated from pluripotent stem cells, but these Organoids only included antrum cell types and their functions have been unquestioned in detail. A major hurdle in in vitro functional stomach lineage specification from pluripotent stem cells is the requirement of mesenchymal-epithelial signaling in vivo. One of the essential factors for stomach development from the gut endoderm in vivo is mesenchymal Barx1. Barx1 is strongly expressed in the embryonic stomach mesenchyme and specifies the entire region of the stomach primordium. In murine stomach development, disruption of mesenchymal Barx1 expression results in intestinalization by expansion of intestinal Cdx2 expression in the stomach epithelium. In contrast, ectopic mesenchymal Barx1 expression causes gastrulation with mirroring gastric Sox2 expression in the region of the intestinal epithelium. We hypothesized that in vitro induction of mesenchymal Barx1 could also specify gut endoderm differentiation into the functional stomach lineages by mimicking the conditions of in vivo stomach-intestine specification. In this study, we determined the appropriate culture conditions for mesenchymal Barx1 induction from pluripotent stem cells. By combining several growth factors, we specifically differentiated pluripotent stem cells into a stomach primordium composed of a Sox2+ foregut endoderm and a Barx1+ mesenchyme, which could mature into stomach tissue cells including functional gastric chief cells, parietal cells, and muscularis-like layer in vitro.

F-1405

MICRORNAS GOVERN MOUSE TROPHOBLAST STEM CELL FATE VIA REPRESSION OF EMBRYONIC GENE REGULATORY NETWORKS

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The first cell fate specification resulting in the emergence of

trophoblast and embryonic stem cells (TSCs, ESCs) occurs as a consequence of an intricate balance between transcription factor regulatory networks. However, cell fate regulation may also occur post transcriptionally. We provide evidence of microRNA involvement in the commitment of TSCs versus the simultaneously specified embryonic lineage. High throughput analyses of ESC transcriptome and TSC microRNAome data was performed to identify TSC-enriched miRNAs targeting the ESC pluripotency gene network. miRNAs were cloned into doxycycline-inducible vectors and introduced to naïve ESCs. Transgenic ESCs were characterized by fluorescence microscopy, flow cytometry and miRNA-Taqman qPCR. miRNAs were induced for 6 days in the presence of an HDAC2 inhibitor and morphology was evaluated. Gene and protein expression was assessed by qPCR, microarray, and western blots respectively. We identified 3 TSC-miRNAs predicted to target 5 mRNAs essential to ESC lineage identity. Ectopic expression of these miRNAs in ESCs caused a downregulation of pluripotency genes and their corresponding proteins, accompanied with an upregulation of TSC markers Cdx2 and Elf5. Upregulated genes were strongly enriched in gene ontology terms associated with placenta/trophoblast development. Moreover, we observed multiple pathways required for sustaining ESC and TSC fate were affected by upregulation of these miRNAs, including signaling and epigenetics. Principle component analysis reveals a unifying trend, as all miRNA-induced lines and a Cdx2-iTSC line more closely resemble naïve TSCs rather then ESCs. Lastly, these lines are stable and self-renewing as they retain geno- and phenotypic resemblance to TSCs even following removal of the transgene-inducing agent and passaging. Using this stem cell conversion model, we show that TSCs contain miRNAs targeting genes essential for sustaining ESC pluripotency, but also that expression of these miRNAs in ESCs is sufficient to drive trans-differentiation to TS-like cells. We are assessing in vivo potential and performing AGO-ChIP and CRISPR-mediated deletions of the miRNAs. Lastly, RNA-seq is being performed on miR15b and Cdx2- lines to elucidate pathways leading to transcription factor- and microRNA- mediated conversion.

F-1406

DISSECTION OF THE REGULATORY NETWORK GOVERNING CARDIAC DIFFERENTIATION IN HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESCs) can be differentiated into beating cardiomyocytes with prospects for disease modeling, regenerative medicine and developmental biology. However, a precise understanding of the complex temporal changes in gene expression patterns that have to unfold during this process is missing. By manipulating (knockout/overexpression) putative cardiac differentiation related genes we aim to dissect this network. As a starting point, we chose the T-box transcription factor Eomes (Tbr2) that plays a pivotal role in multiple developmental processes including trophoblast differentiation, gastrulation, and epithelial-mesenchymal transition. Moreover, in mouse, Eomes has recently also been implicated in cardiac development. However, whether this function is conserved in human and if it might be cell-autonomous is not clear. We therefore generated EOMES knockout hESC lines (EO-KO) using the CRISPR/Cas9 method. EO-KO cells formed neuronal structures indicating a failure to differentiate along the mesendodermal lineage (spontaneous differentiation). Next, we used a directed 2D cardiac differentiation protocol that allows the generation of near-homogeneous CTNT positive, beating cardiomyocytes within 7 days. This protocol, in contrast to EB based methods, does not lead to possible signaling gradients and spatial differences across the cells and therefore non-cell-autonomous processes are highly unlikely. Morphological differences between hESCs and EO-KO cells were apparent from as early as day 2 and the EO-KO cells completely failed to differentiate into cardiomyocytes. Further, most genes that display pronounced upregulation during the first days of normal cardiac differentiation, including T (Brachyury), Mesp1, Mixl1 and Cer1, failed to become induced in the absence of EOMES. Interestingly, after 6-8 days of subjecting EO-KO cells to our cardiac differentiation protocol we observed the expression of marker genes that are characteristic of subtypes of placental cells, such as trophoblast giant cells. With these data we demonstrate for the first time that, while its ablation is tolerated for the induction of some lineages, EOMES is, besides its well-known role in endoderm induction, absolutely required for cardiac specification in human in a cell-autonomous manner.

F-1407

A NEW ES CELL LINE AS AN ALTERNATIVE SOURCE FOR NEURAL CREST DERIVED STRUCTURES IN TISSUE ENGINEERING

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Understanding the embryonic development and mineralization of maxillofacial structures is crucial for developing innovative techniques for tissue engineering therapeutic approaches. Neural crest cells (NCC) are a multipotent and migratory cell population unique to vertebrates that gives rise to diverse cell lineage including melanocytes, smooth muscle, peripheral and enteric neuron, glia and the mesenchymal tissues of the tooth. In the context of tissue engineering applications, the proof of concept of the generation of a tooth has been made in the mouse, using ED14 dental epithelium and ectomesenchymal neural crest-derived cell reassociations, including tooth implantation in the jaw. However, for obvious ethical reasons, a transfer of these data to the human absolutely required the identification of alternative cell sources. Pluripotent stem cells differentiated towards a dental ectomesenchymal program would represent an attractive alternative in the context of tissue engineering. For now, the best system for efficiently testing a cell odontogenic potential is set up in the mouse and thus murine pluripotent stem cell have to be used as a start. In view of obtaining such a cell population, we have derived an ES line from blastocysts of a cross between Wnt1-CRE and Rosa^{Tomato} mice. Since these ES cells expressed the fluorescent Tomato protein as they acquire the neural crest identity (NC), which allows for easily following their differentiation as well as its homogeneity. We then have set up an efficient differentiation protocol towards a NC phenotype

(2D culture and defined medium). qPCR, immunofliuorescence and histological analyses have allowed to show that 1) this new ES Wnt1Cre Rosa[Tomato] cell line displays all the classical characteristics of an ES cell i.e. normal karyotype, capacity to differentiate in vitro towards derivatives of the 3 embryonic layers and to form teratoma. 2) upon induction in our NC differentiation protocol, it acquires the NC characteristics markers in parallel with the tomato fluorescence. This new cellular tool should help in unravelling the signals involved in the acquisition of the odontogenic potential by the dental ectomesenchyme, as well as, to carry out fate analysis in NC development and tissue engineering.

F-1408

DIFFERENTIATION OF NOTOCHORDAL-LIKE CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Exhaustion of notochordal-like cells (NCCs) plays an essential role in the development of intervertebral disc degeneration (IDD). Cell-based therapy has emerged as a novel strategy of regenerative medicine for many tissues and organs, including IDD. Human pluripotent stem cells (ESC/iPSCs) offer the possibility of generating individual-specific NCCs. This study was to define strategies to derive NCCs from human ESC/iPSC. hESC7 and hESC9 were used for a two-step protocol to differentiate into NCCs. Activin A was used in step 1 for 3 days. Activin A, DKK1, Noggin, FGF2 and AGN193109 were used in step 2 for further 5days. Immunofluorescence staining was performed after differentiation. Our results showed that NCCs were successfully given rise from above protocol induced differentiating human ESC confirmed by immunofluorescence staining of Noto, Brachyury and Foxa2. The differentiation efficiency was only around 5%. Moreover, PI3-kinase inhibitor didn't enhance the differentiation efficiency of NCCs from human ESC. Co-culturing NCCs with Light2 cell line, a luciferase-based reporter responsive to Hedgehog (Hh) secreted protein by NCCs, was examined. Further differentiation of NCCs into nucleus pulpous progenitor cells (NPCs) was detected by expression of Tie2 (Tie2) and disialoganglioside 2 (GD2). Notochordal-like cells could be induced from human pluripotent stem cells through regulation of retinoic acid, BMP and Wnt signaling. Moreover, NCCs differentiation from human pluripotent stem cells is functional in Shh signaling and NPCs differentiation.

F-1409

INVESTIGATING THE CARDIAC PROGENITOR STAGE DURING CARDIOMYOCYTE INDUCTION OF HUMAN PLURIPOTENT STEM CELLS

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During embryogenesis two different lineages of cardiac progenitors originate from mesoderm and contribute to heart development: the First Heart Field (FHF), which gives rise to left ventricle and parts of atria, and the Second Heart Field (SHF), which gives rise to parts of atria, right ventricle and outflow tract. LIM-homeodomain Isl1 protein is a highly conserved component of the transcription factor network driving cardiac development during embryogenesis. Isl1 was for long considered a SHF marker, and in vivo studies show that this transcription factor is required for completing cardiac chamber formation, in part by promoting survival, proliferation and migration by SHF progenitors. Recent evidence, from several animal models, however, suggests that Isl1 may be a more general factor involved in cardiogenesis. In human, the role of ISL1 during cardiac development is not clear. Here we generated an ISL1 knockout model, using the CRISPR/Cas9 system, and an overexpression model, using the Tet-On system, on a human embryonic stem cell (hESC) background. Using a novel 2D cardiac differentiation protocol, through which we are able to differentiate hESCs into cardiomyocytes with high efficiency under defined conditions, we show that ISL1 knockout cells present a delay in terminal differentiation and a delay in cardyomyocyte maturation. Moreover the observation of different features in cardiomyocytes obtained from wild-type, ISL1 knockout and ISL1 overexpressing cells may account for different cardiac populations depending on ISL1 activity. These findings highlight the role of ISL1 during human cardiogenesis using hESCs as a model.

F-1410

NOVEL FUNCTIONS OF CX26 AND CX30.3 IN MOUSE ES CELLS

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Pluripotent embryonic stem (ES) cells are capable of maintaining a self-renewal state and have the potential to differentiate into derivatives of all three embryonic germ layers. Despite their importance in cell therapy and developmental biology, the mechanisms whereby ES cells remain in a proliferative and pluripotent state are still not fully understood. Overexpression vector for *Cx30.3* was introduced into ES cells and cultured in LIF(-) medium for 6 days. The colony became a differentiated shape. On the other hand, when overexpression vector for *Cx26* was introduced into ES cells and cultured in LIF(+) medium, the colony maintained at an undifferentiated shape. These suggest that *Cx30.3* and *Cx26* were not effective to the differentiation dependent morphological changes. Then the effects of overexpression of respective Cxs on *Oct3/4, Rex1, Cdx2* and *Gata4* expressions were investigated. *Oct3/4* and *Rex1* are undifferentiated state markers and *Rex1* is a downstream gene of *Oct3/4. Cdx2* is a trophectodermal marker and *Gata4* is an endodermal marker. We expected that the effects of *Cx30.3* and *Cx26* should be contradictive. In fact, however, both effects were similar. The expression level of *Oct3/4* decreases sharply when the medium was exchanged by LIF(-) medium. The overexpression of Cxs resulted in a more gradual decrease of its expression but no effect on *Rex1* expression. These suggest that the influence if any was limited on the signaling to *Oct3/4* but not to further downstream. On the other hand, the expression level of *Gata4* is no higher than 0.2 versus that of *β-actin* in LIF(+) medium and it increases gradually after exchanging the medium by LIF(-) medium. The overexpression of Cxs caused a marked increase at 4th day and then decreased to the control level at 6th day. A drastic effect on the *Cdx2* expression level was also observed only around 4-5th days in a similar manner to that of *Gata4*. The differentiation into the endoderm and the trophectoderm was thought to be decided around 4-5th days after the initiation of LIF(-) culture. The overexpression of these Cxs was effective just to this decision

step. After that the *Gata4* and *Cdx2* expressions were thought to be unnecessary any more. That was why the *Gata4* and *Cdx2* expression levels became lowered to the control level at 6th day.

F-1411

TARGETED DIFFERENTIATION OF PLURIPOTENT STEM CELLS TO HEPATOCYTES

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Pluripotent stem cells possess the ability to differentiate into virtually any cell type whilst also retaining the capacity to self-renew indefinitely. There is a current unmet need from the biotechnology industry for an inexhaustible supply of hepatocytes, the metabolically active cells of the liver, to perform drug toxicity and metabolism screens on early stage drugs. Pluripotent stem cells therefore hold potential as a resource to generate mature hepatocytes for pharmaceutical testing. To realise this potential, there is a requirement to faithfully recapitulate hepatocyte cell fate specification in vitro. The signalling pathways and key regulators involved in modulating the major metabolic cytochrome p450 family of enzymes are poorly understood and consequently currently in vitro derived hepatocyte-like cells (HLCs) show low functionality, making them unsuitable for application in drug toxicity screens. We have utilised Plasticell's award winning technology, CombiCult®, to screen thousands of combinations of media components to find efficient and robust protocols for the differentiation of human embryonic stem cells into functional hepatocytes. We have specifically aimed to derived protocols which are serum and feeder-free, do not require embryoid body formation and where small molecule agonists and inhibitors have been used in place of growth factors and cytokines to make protocols more reproducible and less costly. From these screens we have derived a number of protocols which give rise to hepatic-like cells which have inducible CYP activity. We are currently in the process of validating these protocols in 2D and 3D systems, and characterising the cells produced to optimise the protocols and investigate further the various stages of differentiation specifically the final maturation required to produce fully functional cells.

F-1412

EVIDENCE BASED THEORY FOR AN INTEGRATED GENOME REGULATION IN ONTOGENY

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Ontogeny of a multicellular organism from a single cell is regulated by a plethora of TFs and requires the coordinated regulation of multi-gene programs. Integrative modules for the Cell Cycle and Pluripotency gene programs have been described and our studies have revealed a pan-ontogenic Integrative Nuclear FGFR1 Signaling (INFS) which controls and integrates the Pluripotency, cell cycle and cell differentiation programs. We will present RNAseq and ChIPseq based evidence for a global and direct gene programing by the nuclear form of FGFR1 (nFGFR1), ensuring that pluripotent embryonic stem cells (ESCs) differentiate into neuronal cells (NCs) in response to Retinoic Acid (RA). nFGFR1 alone and with its partner nuclear RA and orphan receptors, targets thousands of active genes and controls the expression of pluripotency, cell cycle, ectodermal, morphogenic, neuronal and mesodermal genes. nFGFR1 targets genes in major developmental pathways and consensus sequences of transcription factors (TFs) encoded by nFGFR1 targeted genes. This dual-level of regulation, with nFGFR1 controlling the generation of ontogenic TFs as well as their downstream actions, suggests a feed-forward mechanism that fine tune complex ontogenic gene networks. Genetic experiments have positioned FGFR1 at the top of the developmental hierarchy. The present studies reveal the basic components for an integrated control of the master ontogenic networks mediated by nFGFR1.

F-1413

IDENTIFICATION OF CARDIAC PROGENITORS DIFFERENTIATED FROM PLURIPOTENT HUMAN EMBRYONIC STEM CELLS USING MUSCLE-SPECIFIC LAMININ MATRICES

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Cardiomyocytes (CM) undergo cell cycle arrest after birth. Therefore cardiac muscle injury is often permanent and results in high mortality rates in myocardial infarction (MI) patients. Recent progress in stem cell research has opened up possibilities for new cell therapy approaches for the treatment of cardiac muscle injury. However, significant limitations remain concerning the reproducibility, efficiency and effectiveness of these cells for heart repair. 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 CM 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 injury to the ventricles is created and different fluorescently labeled CM progenitors are injected directly into the damaged myocardium or delivered through the tail vein.. Survival, proliferation and tracking of the injected cells are monitored by bioluminescence combined with cardiac CT

analysis in IVIS Spectrum imaging system. Before we sacrifice the mice at 4 weeks, heart function is assessed by echocardiography. Tissue histology using human specific antibodies are utilized to stain the damaged heart muscles and look for cellular integration and neovascularization. This study will shed insights into the signature profile for the 'right' cells to be used for future clinical trials.

F-1414

DEVELOPMENT OF DOPAMINERGIC NEURONS FROM NEURONAL PROGENITOR CELLS DERIVED FROM FELINE EMBRYONIC STEM-LIKE CELLS

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Differentiation of stem cells into neuronal lineage plays an integral role in derivation of neuronal specific cells for further use in cell-based therapy. This study attempted to establish neuronal progenitor cells (NPCs) from embryonic stem (ES) like cells derived from in vitro produced feline embryos. In addition, we also tested whether these NPCs could differentiate into tyrosine hydroxylase (TH) producing dopaminergic neuronal subtype. The inner cell masses (ICMs) were isolated from feline blastocysts (n=40) and then plated onto mitomycin-treated mouse embryonic fibroblasts (MEF). After mechanical passage, the ES like colonies at passage 2-3 were allowed to spontaneously differentiate into neuronal-rosette structure (NRSs). These NRSs were plated onto Matrigel™ coated dish with NPC medium. The NPC like cells were passaged and characterized by means of doubling time, immunofluorescence, karyotyping and RT-PCR. Five ES like colonies were collected to demonstrate the expression of pluripotent genes (as a control). The derived NPC cell lines were chemically induced into dopaminergic neurons. Of 30 embryos used, 25 (83.3%) ICM outgrowths were formed around 4-7 days after ICM plating. The ICM outgrowths demonstrated round-to-oval shaped colony with well-delimitation from remaining trophectoderm and MEF. Following passage, a total of 10 ES like cell lines were successfully passaged to passage 2. The tubular-like structure of NRS was formed following spontaneous differentiation. After characterization, four NPC lines were established (4 of 10, 40%). They had bipolar phenotype with one or two large and prominent nucleoli. These cells expressed mRNA for *NANOG*, *NESTIN* and *NCAM*. They also expressed NESTIN, PAX6, NeuN and TUJ-1 at protein levels. The NPC morphology was changed from bipolar to multipolar phenotypic cells after culture in dopaminergic producing condition. These differentiated cells expressed *NR4A2* and tyrosine hydroxylase at mRNA and protein levels, respectively. This study concluded that NPCs can be generated from feline ES like cell lines. Further study is still required to further examine the potential of these NPCs to differentiate into other neuronal lineages and also the function of the derived neurons following transplantation.

F-1415

PDX1 GFP REPORTER HUMAN ES CELL LINES

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Pancreatic and Duodenal Homeobox 1 (*PDX1*) encodes a homeobox transcription factor that is expressed at the earliest stages of human embryonic pancreatic development. Lineage tracing studies in the mouse have conclusively shown that $Pdx1^{+}$ early pancreatic progenitor (ePP) cells are multipotent, giving rise to the exocrine, endocrine and ductal components of the adult organ. Consistent with this, loss of Pdx1 results in complete pancreatic agenesis both in mice and in man. This catastrophic phenotype clearly indicates that Pdx1 sits high atop the gene regulatory network that orchestrates the morphogenesis of this indispensible endocrine organ. To simulate human pancreatic development in vitro, we developed a human embryonic stem cell (hESC) differentiation protocol that tightly adheres to developmental logic and yields abundant $PDX1^{+}$ ePP cells whose molecular signature closely approximates the incipient pancreatic primordium in vivo. To better study both the biology and developmental potency of human ePP cells, we developed a series of *PDX1* reporter hESC lines using TALEN gene editing technology. We introduced EmGFP by homologous recombination into intron 1 of *PDX1*, which results in a null mutation designated $PDX1^{(1\text{-}136)\text{-}EmGFP}$, and constructed a second *PDX1* reporter line using the P2A self-cleaving peptide whereby the dosage of *PDX1* is maintained with concomitant expression of EmGFP - an allele we desigante as $PDX1^{P2A\text{-}EmGFP}$. Homozygous $PDX1^{(1\text{-}136)\text{-}EmGFP}$ hESC clones were also obtained, and consistent with in vivo loss of function studies, *PDX1* null cells are able to differentiate into definitive endoderm lineage but fail to activate the pancreatic transcriptional program in vitro. Overlapping microarray studies of wild-type and *PDX1* null mutant hESC cells line with in-house PDX1 ChIP-Seq data has revealed a novel list of candidate PDX1 transcriptional targets. In addition, the $PDX1^{P2A\text{-}EmGFP}$ reporter cells provide us with a powerful new tool for the purification and expansion to homogeneity of $PDX1^{+}$ ePP cells in chemically defined conditions. We anticipate that stable ePP cell culture will mitigate the need to differentiate ePP cells from pluripotent hESC and will provide a valuable platform for the screening of endocrine-inducing factors.

F-1416

DIRECTED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO MOTOR NEURONS BY USING COMBINATION OF DORSOMORPHIN, A8301 AND XAV939

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Human embryonic stem cells (hESCs) have the ability to self-renew and the potential to form all cell lineages in the body. Directed differentiation of hESCs into specific cell types is a challenge in developmental biology and small molecules are new idea for dominance this challenge. Under this approach and according to signaling pathways, we used small molecules Dorsomorphin, A8301, XAV, Retinoic acid and Purmorphamine in this protocol to generate motoneurons from hESC. HESC-derived neural cells characterized

with Immunofleoroscent, flow cytometry, qRT-PCR and whole cell patch clamp.Immunofleoroscent staining and flow cytometry analysis of hESC-derived neural ectoderm (NE) showed these structures are 92.68 NESTIN$^+$, 64.40% PAX6$^+$ and 82.11% SOX1$^+$ in a chemically defined adherent culture. After replating of hESC-derived NE, the differentiated cells are Tuj1$^+$, MAP2$^+$, HB9$^+$ and ISL1$^+$. Efficiency of motor neuron generation was 39% according to HB9/ISL1 expression. Level of gene expression evaluated with qRT-PCR in different stages of differentiation protocol. Electrophysiological properties of differentiated cells recorded by whole cell Patch clamp technique. HESC-drived motor neurons displayed voltage gated delay rectifier K^+ and Na^+/Ca^{2+} inward currents. Also rebound action potential was detected in these cells.Our findings suggested that, hESC-derived neurons expressed specific motor neuron proteins such as HB9 and ISL1 but electrophysiological properties showed that these cells were not mature because the resting membrane potential was more positive than mature motor neuron. Besides, Na^+/Ca^{2+} inward currents were not sufficient for firing action potentials. It seems that the cells should be more developed for functional maturation.

F-1417

DETERMINING THE EFFECT OF POST TRANSLATIONAL MODIFICATION ON HNF4A BIOLOGY IN STEM CELL-DERIVED HEPATOCYTES

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Hepatocyte nuclear factor 4α (HNF4α) is a member of the nuclear receptor superfamily. It is known as a master regulator of liver-specific gene expression and critical for maintaining hepatocyte phenotype. HNF4α is a modular protein, consisting of two activation domains (AD1 and AD2), a DNA binding domain, a ligand binding domain, a dimerization domain and a repression domain. Of interest in our studies, is the AD2 transactivation domain, which contains a SUMO post-translational modification site. We recently reported that SUMOylation of HNF4α takes place at the consensus motif found in AD2 leading to decreased protein stability, mediated by the proteasome 26S complex. In addition to regulating protein stability, SUMO modification at this site was coincident with an increase in mature hepatocyte gene expression, for example, albumin synthesis. Therefore, it is conceivable that SUMOylation of the AD2 domain of HNF4α may modulate HNF4α transcriptional activity, leading to improved hepatocyte phenotype. The aim of this study is to investigate whether SUMOylation of the AD2 domain regulates HNF4α transcriptional activity and if it is applicable to harness the potential of this modification to improve stem cell-derived hepatocyte phenotype.

F-1419

MITOCHONDRIAL DYNAMICS IS CORRELATED WITH NOTCH SIGNALING RELATED SELF-RENEWAL AND DIFFERENTIATION IN ESCS

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Mitochondria are dynamic organelles and have double membrane, they play essential roles in cellular biological processes, e.g. energy metabolism, apoptosis, differentiation, calcium homeostasis, etc. In adult cells, to accomplish their functions, mitochondria frequently remodel their morphology, by a balance between mitochondrial fission and fusion, referred to as mitochondrial dynamics. Dysfunction of mitochondrial dynamics has been implicated in many disorders such as neurodegenarative diseases, diabetes, muscle atrophy and cancer. Embryonic stem cells (ESCs) have sparse and immature mitochondria and preferentially rely on non-oxidative glycolysis as a major source of energy. Their differentiation is followed by dynamic changes in mitochondrial mass, ATP and ROS production, accompanied by mitochondrial maturation. The Notch signaling pathway is proved crucial for the development of most tissues. A lack of Notch signaling has been reported to induce early neuronal differentiation of ESCs and destroy the maintenance of neural rosettes, and proper cardiomyocyte differentiation also involves mitochondrial dynamics linked to Notch signaling. In this study, we found that mitochondrial morphology and distribution in mouse ESCs is associated with the Notch signaling pathway and the expression of mitochondria-shaping proteins was increased following Notch signaling-related differentiation of mouse ESCs. Inhibition of Drp1, a key protein in mitochondrial fission, partially changed the mitochondrial morphology in Notch signaling-blocked mouse ESCs and arrested the neuronal differentiation both in wild-type and Notch signaling-deficient mouse ESCs. In conclusion, mitochondrial dynamics and its regulatory proteins participate in the whole process from self-renewal to neuronal differentiation in mouse ESCs.

EMBRYONIC STEM CELL PLURIPOTENCY

F-1422

TERATOSCORE: ASSESSING THE DIFFERENTIATION POTENTIAL OF HUMAN PLURIPOTENT STEM CELLS BY QUANTITATIVE EXPRESSION ANALYSIS OF TERATOMAS

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Teratoma formation is the gold standard assay for testing the capacity of human pluripotent stem cells to differentiate into all embryonic germ layers. Although widely used, little effort has been made to transform this qualitative assay into a quantitative one. By analyzing the trascriptome of more than 25 tissues we generated a scorecard of cell-specific genes representing tissues from the three embryonic germ layers as well as the extra-embryonic placenta. TeratoScore, a new methodology based on this scorecard, shows that only teratomas express tissues-specific genes from all lineages in an even manner, while the tissue-specific tumors express only genes of their respected origin. Importantly, we transformed the expression values into a unified score, translating cell potency into a quantitative measure. The TeratoScore analysis is based on open-source code, allowing a rapid assessment of teratoma microarray data in a publicly available website created by us (http://benvenisty.huji.ac.il/teratoscore.php). The new database of teratomas used in this study also allowed us to examine the differences between tumors initiated from cells

with a diploid karyotype and those initiated by aneuploid cells. We found that while teratomas originating from trisomy 21 (Down syndrome) cells pass the TeratoScore benchmark for pluripotency at relatively high scores, teratomas from trisomy 12 show significantly lower scores. Since the latter aberration is embryonic lethal, this result implies on their aberrant differentiation capacity. An in-depth analysis of cell-specific genes demonstrates that both trisomy 12 and trisomy 21 teratomas exhibit aberrant gene expression congruent with human chromosomal syndromes. This gene expression signature is significantly different from that of teratomas originating from diploid cells, particularly in central nervous system-specific genes, suggesting aberrant teratomas may be beneficial for disease modeling.

F-1423

KLF5 REGULATES NAIVE PLURIPOTENCY DURING DEVELOPMENT THROUGH SUPPRESSION OF THE FGF-FGFR-ERK SIGNALING PATHWAY

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Inhibition of extracellular signal-regulated kinase (ERK) signaling is critical for attaining and maintaining a naïve state of pluripotency in cultured stem cells. ERK inhibition also facilitates the emergence of Nanog-positive naïve pluripotent stem cells in the blastocyst during murine development. However, it remains unclear whether a specific physiological mechanism exists that mediates ERK inhibition in vivo to promote pluripotency. Here, we show that the transcription factor Klf5 is necessary and sufficient for the emergence of Nanog-positive naïve pluripotent stem cells during development and that this occurs through inhibition of the Fgf4-FgfR-ERK signaling axis in a context-dependent manner. Klf5 knock-out embryos phenotypes can be rescued by inhibition of FgfR-ERK pathway. Analysis of Klf5 overexpressing embryos revealed that Klf5 overexpression mimics MEK inhibition in early embryonic development such as increased Nanog positive cell and expression in inner cell mass of blastocyst and suppression pf primitive endoderm lineage marker. Together, our results linking Klf5 to ERK inhibition provides new mechanistic insights into how this transcription factor regulates pluripotent development and cell reprogramming.

F-1424

METABOLIC CONTROL OF PLURIPOTENT STEM CELL FATE AND FUNCTION BY SIRTUINS

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Similar to cancer cells, diverse stem cells such as human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) primarily use an energetically inefficient glycolytic pathway instead of energetically efficient oxidative phosphorylation (OXPHOS), for ATP production. Thus, somatic reprogramming from somatic tissues to iPSCs accompanies the metabolic switch from OXPHOS to glycolysis. This metabolic reprogramming is also known as the "Warburg effect" and appears to be a fundamental aspect of the somatic reprogramming and stem cell fate control. However, the molecular mechanisms underlying the link between energy metabolism and stem cell fate are poorly understood. To address our general hypothesis that protein acetylation control is critical for the metabolic reprogramming, we investigated the expression pattern and the potential functional roles of modulators of protein acetylation such as histone acetyl transferases, class I, II, and III histone deacetylases (HDACs). Among these factors, the class III HDACs, termed sirtuins, are NAD-dependent protein deacetylases and represent potential candidates because their activities are highly dependent on NAD, a critical co-factor of metabolism. We will discuss our initial data regarding the potential functional roles of these acetylation modulators in metabolic reprogramming and stem cell fate control.

F-1425

A HUMAN SINGLE NUCLEOTIDE POLYMORPHISM CONTROLS SPLICING OF THE PLURIPOTENCY GENE NODAL: EXPLAINING THE FEMALE BIAS IN HUMAN EMBRYONIC STEM CELL LINES

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The potential use of pluripotent cells for personalized regenerative medicine necessitates an improved understanding of how germ-line genetic variation may affect pluripotency. Given the female bias in established human embryonic stem cell (hESC) lines, sex-specific differences must also be considered. Using SNP data from 98 hESC lines, we discovered that the minor alleles of a single nucleotide polymorphism (SNP) linkage group in proximity to the NODAL gene locus are drastically underrepresented in male relative to ancestry-matched female hESCs. This association is specific to hESCs as it is not present in ancestry-matched reference populations. These alleles are positively correlated with XIST expression in female hESCs, suggesting that aberrant X chromosome inactivation in male cells may be driving this sex bias. Mechanistically, one SNP allele in this linkage group specifically and directly enables the alternative splicing of a novel human NODAL transcript, that we have termed "variant Nodal". Treating hESCs with recombinant variant NODAL protein (that differs from annotated NODAL only in its C-terminus) results in decreased cell proliferation, decreased expression of pluripotency genes, and increased cell death. Collectively, these findings suggest that a genetically defined subset of male embryos is being negatively selected during the derivation of hESC lines, creating a female bias in this cell type. Furthermore, we show, for the first time, that a specific genetic polymorphism has a drastic functional impact on the NODAL gene, controlling the splicing of a previously unknown protein variant that can signal to and adversely affect hESC pluripotency. Nodal sustains pluripotency, initiates mesendoderm, and promotes cancer progression in a concentration and context dependent manner; hence the genetic regulation of variant Nodal could dramatically alter personalized regenerative medicine protocols and may also have ramifications in cancer biology.

F-1426

MOLECULAR MECHANISMS BY WHICH HUMAN EMBRYONIC STEM CELLS ARE PRIMED TO UNDERGO RAPID APOPTOSIS

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During development, cells of the inner cell mass (ICM) proliferate rapidly and differentiate into every cell precursor in the organism, while maintaining a delicate balance between division, multipotency and apoptosis. Achieving this balance is likely dependent on crosstalk between these fundamental stem cell signaling pathways not only in development but also in the context of cancer where cancer stem cells are implicated as drivers of tumor progression. The main mediator of cell death is Bax, a pro-apoptotic protein that is regulated by direct activators, anti-apoptotic proteins and sensitizers (inhibitors of anti-apoptotic proteins). While most mitotic cells maintain Bax in an inactive conformation in the cytosol, we found Bax to be constitutively active at the Golgi in undifferentiated human embryonic stem (hES) cells. Upon DNA damage Bax rapidly translocates to the mitochondria by a p53-dependent mechanism (p53 knockdown cells maintained Bax at the Golgi after DNA damage), with all cells dying by six hours after damage. Remarkably, after only two days of differentiation, hES cells significantly increase their resistance to apoptosis. We found this increased resistance to be accompanied by loss of constitutive Bax activation as well as striking global changes in the levels of various apoptotic proteins. Our data suggests that modulation of these apoptotic components affects not only the thresholds of apoptosis but also the ability of ES cell to maintain self-renewal. This is particularly interesting since several apoptotic proteins have been shown to be either inactivated or overexpressed in cancers. We are currently investigating the molecular basis of these changes, which were thought to only reflect the increased resistance of these cells to apoptosis, to examine whether they also modulate the ability of cancer stem cells to self-renew and/or differentiate.

F-1427

FLUCTUATIONS IN BMP SIGNALING REGULATE PLURIPOTENCY AND EARLY LINEAGE CHOICES IN NAIVE MOUSE EMBRYONIC STEM CELLS

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Naïve mouse embryonic stem cells (mESC) represent a heterogeneous population, fluctuating between inner cell mass- and epiblast-like phenotypes. The regulation of this metastable state is still not completely understood. Here we report the generation of a BMP-SMAD signaling reporter mESC line, and we describe a heterogeneous and metastable activation of this pathway in naïve mESC. We associate high expression of pluripotency genes and 5-methylcytosine hydroxilases Tet1/2 with transient activation of the BMP-SMAD pathway, while lineage-associated genes and DNA methyltransferases Dnmt3a/b have lower levels of expression. Consequently, naïve mESC with active BMP signaling were less prone to differentiation when compared to mESC without BMP signaling. This was further confirmed by using double Smad1/Smad5 knockout mESC. Surprisingly, these mESC showed pluripotency characteristics and continue self-renewing, but showed higher predisposition to differentiation. We conclude that the heterogeneous transcriptional and epigenetic state of naïve mESC is not stochastic, but it's a response to divergent signaling cues existent in cell culture and that, in some extent, BMP signaling activation acts to prevent differentiation in the presence of undefined conditions.

F-1428

CELL PENETRATING PEPTIDES CAN BE EFFICIENTLY USED FOR MIRNA DELIVERY INTO HUMAN EMBRYONIC STEM CELLS

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Cancer stem cells are believed to be involved in cancer metastasis and also in resistance to chemo- and radiotherapy. They express different stemness factors, as do normal stem cells, but their differentiation ability is hampered. Therefore, one therapy method could be to attack the stemness of cancer stem cells and guide them to differentiation. It is known that different miRNAs have important regulatory roles in these processes. For example miR-145 targets many pluripotency factors and is upregulated in differentiation. Thus, we plan to study miR-145 mediated differentiation. In our experiments, we use human embryonic carcinoma cells and their normal equivalents human embryonic stem cells (hES). Embryonic stem cells are however difficult to transfect and sensitive to manipulations, compared to many other cell lines. Hence we are at first, testing the applicability of cell penetrating peptides, which are thought of as relatively non-toxic and safe transporters. For now, we have tested three different cell penetrating peptides: PF6, PF14, and CADY. To assess the efficiency of miRNA transport with these peptides, fluorescently labeled miRNA which has no function in human was used. Experimental work revealed that these peptides differed considerably in terms of influence on cell viability as well as in miRNA transport efficiency. PF14 had significant toxic effects and was excluded from following experiments. PF6 had mild toxic effects and CADY did not have any negative influence on cell viability. To verify whether the cells were retained in pluripotent state after transfection, the presence of OCT4 and NANOG pluripotency markers was detected with immunofluorescence microscopy. The results showed that the cells remained in pluripotent state with both CADY and PF6 cell penetrating peptides. Difference was seen when assessing the efficiency of the microRNA transport. Namely, compared to PF6, CADY could deliver miRNA to the cells more efficiently. Thus, from the three peptides used, CADY was the most efficient and could be used on hES without loss of their viability and pluripotency. The functionality of transfected miRNA will however be evaluated in future studies.

F-1429

STRUCTURE ACTIVITY RELATIONSHIP ANALYSES OF YM155 TO DEVELOP ITS STEM-TOXIC EFFECT

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Human pluripotent stem cells (hPSCs) are capable to differentiate into all cells in human body owing to the pluripotency, which makes them promising sources for future regenerative medicine. However, teratoma formation, caused by incomplete removal of residual undifferentiated PSCs after differentiation, has been considered to be one of the serious technical hurdles for clinical application. Our group previously demonstrated that targeting hPSC-specific antiapoptotic factor with the small molecule inhibitor (e.g., quercetin or YM155) induces selective cell death of hPSCs and inhibits teratoma formation. Herein, we analyzed the structure-activity relationship (SAR) of YM155 toward hPSCs, based on the diverse derivatives of YM155 by determining effective dose 50 (EC50) of each derivative. We showed the evidence that the positions of two nitrogens in the pyrazine moiety appeared to be critical for stem-toxic activity of YM155, implying that formation of hydrogen bond of nitrogen with a target molecule would be required for the stem-toxic action. These data provide important clue to develop potent novel stem-toxic small molecules in future.

F-1430

OPTIMIZED SCALABLE SUSPENSION CULTURE OF HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) hold tremendous promise for diverse biomedical application, such as cell replacement of damaged tissue, artificial organ engineering and high-throughput screening system for novel pharmacological drug or toxicological test. However, large scale production of hPSCs in a robust, convenient, and inexpensive manner is still a major huddle in current translational application of hPSC technology. In this work, we developed an efficient scalable suspension culture of 3D hPSC spheroid under defined condition using a new simple clump passaging method. For clump passaging, the fully grown hPSC spheroids were dissolved by enzymatic or mechanical treatments into smaller-sized spheroid clumps, and continued to be grown in fresh culture medium. By testing various medium conditions, we optimized the medium for 3D hPSC spheroid culture. Our clump passaging and expansion method allowed high survival and expansion rate of hPSC spheroids, compared to conventional method. The pluripotency characteristic of hPSC spheroids were confirmed by standard assays such as expression of pluripotency markers and in vitro and in vivo differentiation potential. Our results may help to further optimize the hPSC culture condition intended for clinical use by facilitating long-term mass production required less labor and expense.

F-1431

FUNCTIONAL SCREEN IDENTIFIES NME6 IS CRITICAL FOR MAINTENANCE OF PLURIPOTENCY IN EMBRYONIC STEM CELL AND EMBRYONIC DEVELOPMENT

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In contrast to the somatic cells, embryonic stem cells (ESCs) are characterized by its immortalization ability, pluripotency, and oncogenicity. Revealing the underlying mechanism of ESC characteristics is important for the application of ESCs in clinical medicine. We performed systematic functional screen in mouse ESCs with 4801 shRNAs that are against 929 kinases and phosphatases. 132 candidate genes which regulate both ESC expansion and stem cell marker expression were identified. 27 out of the 132 genes were regarded as most important since knockdown of each gene induces morphological changes from undifferentiated to differentiated state. Among the 27 genes, we chose non-metastatic cells 6 (Nme6, also named as Nm23-H6) and non-metastatic cell 7 (Nme7, also designated as Nm23-H7) to study in-depth. Nme6 and Nme7 both belong to the members of nucleoside diphosphate kinase (NDPK) family. We demonstrate Nme6 and Nme7 are important for the regulation of Oct4, Nanog, Klf4, c-Myc, telomerase, Dnmt3B, ERas and Nanog expression. Either knockdown of Nme6 or Nme7 reduces the formation of embryoid body and teratoma. The overexpression of either Nme6 or Nme7 can rescue the stem cell marker expression and the embryoid body formation in the absence of leukemia inhibiting factor (LIF). This implies that Nme6 and Nme7 can regulate several critical factors in ESC pluripotency and renewal. In order to further investigate the physiological roles of Nme during embryogenesis. We generated Nme6$^{-/-}$ mice, and found that no viable double knockout mice were born and these embryo died at embryonic day (E)6.5 to E7.5. The double knockout embryo look small and morphologically abnormal. Thus, Nme6 is also essential for embryonic viability. We are attempting to reveal the cause of the embryonic lethality for embryos at E6.5 to E7.5 day. To sum up, we identify a novel pathway, Nme6/Nme7 that modulate ESC renewal and Nme6 is essential for the survival of early embryo.

F-1432

IMPROVING DERIVATION OF HAPLOID MOUSE EMBRYONIC STEM CELLS FROM PARTHENOGENETIC MOUSE BLASTOCYSTS BY SIMULTANEOUS SUPPRESSION OF TGF-BETA AND ERK SIGNALING PATHWAYS

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Nowadays, application of embryonic stem cells is crucial in developmental biology, cell therapy and drug screening. Although

diploid trait is known as of the challenges in developmental genetic studies, derivation of haploid embryonic stem cells from parthenogenetic embryos is a useful way to solve this problem. In this project, Feasibility of haploid parthenogenetic embryonic stem cells production has been studied in presence of the ERK and TGF-β signaling pathway inhibitors. At the initial point, Parthenogenetic blastocyst- produced with chemical activation of oocytes- was cultured in R2i culture medium condition (containing PD0325901 and SB431542 which inhibit ERK and TGF-β signaling pathways respectively). Parthenogenetic embryonic stem cell lines was subsequently expanded and preserved at the same culture medium .Using Immunofluorescent staining and qRT-PCR, expression of the pluripotent markers were evaluated. Moreover, differentiation potential of these cells was assessed by applying spontaneous differentiation and teratoma formation assay. Degree of haploid in this cell lines was determined by flowcytometry methods. In R2i culture condition, about 50% of parthenogenetic embryos produce cell lines, out of which 10-13% are haploid. Established parthenogenetic stem cell lines demonstrate pluripotent stemness characteristics including morphology, passagability as well as expression of pluripotent specific genes in mRNA and protein levels. In addition, these cells are able to form embryonic germ layers and teratoma. Inhibition of ERK and TGF-β signaling can provide are an appropriate condition for producing haploid stem cells from parthenogenetic embryos.

F-1433

CONVERSION OF THE PLURIPOTENT STATE IN COMMON MARMOSET ES CELLS

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Although both primate and mouse embryonic stem (ES) cells are pluripotent stem cell lines derived from inner cell mass (ICM) of blastocyst stage embryos, they show distinct characteristics. Currently, primate ES cells have been considered as counterpart of mouse epiblast stem cells (EpiSCs) which is derived from postimplantation embryo. The pluripotent state of mouse EpiSCs is termed primed state to distinguish from naïve state pluripotency of mouse ES cells. Therefore, primate ES cells have also been regarded as primed state pluripotent stem cells and the naïve state pluripotency in primate has not been well characterized. Using ES cells of common marmoset, a small non-human primate, we have carried out the conversion from primed state to naïve state pluripotency. Doxycycline-inducible (DOX-inducible) reprogramming factors transgenes were introduced into common marmoset ES cells by piggyBac transposon transgenesis, and the cells were selected by drug-resistance. After expanding of the clones, cells were cultured in a medium containing LIF, Dox and cocktail of small molecules. Resulting cells formed mouse ES cell-like dome-shaped colonies and maintained expression of pluripotency markers. Furthermore, these cells showed similar gene expression pattern to mouse ES cells and ICM cells of marmoset blastocyst. These phenotypes were lost by addition of JAK inhibitor to culture, suggesting maintenance of this pluripotent state require LIF/STAT3 signal. These properties of the common marmoset ES cells closely resembled to mouse ES cell, suggesting that the cells acquired naïve state pluripotency.

F-1434

STOCHASTIC TRANSCRIPTIONAL REGULATION - HETEROGENEITY OF NANOG AND REPORTERS

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The transcription factor Nanog is part of the core gene regulatory network for pluripotency and it displays heterogeneity at the transcriptional and protein level. Variability of transcriptional regulators can provide an insight into the balance of stochastic and deterministic influences on pluripotency and differentiation. There have been conflicting reports concerning the degree of Nanog heterogeneity observed as well as varying conclusions regarding allelic regulation. We use stochastic modelling and experiment to investigate the role played by fluorescent Nanog reporters in the study of stem cell heterogeneity. Reporters can affect feedback loops involved in regulation and we experimentally demonstrate their affect on Nanog levels at the population level as well as potential mismatch between Nanog and reporter status at the single cell level. We also demonstrate a novel, analytical stochastic model that shows how synchronicity of two uncoupled genes is determined by the time-variance of a joint upstream regulator. This study provides mechanistic insight into the variety of heterogeneity observations in this field.

F-1435

NETWORK-BASED ANALYSIS OF PROTEOMIC PROFILE UPON REX1 KNOCKDOWN IN HUMAN EMBRYONIC STEM CELLS

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Previous reports have demonstrated that REX1 plays an important role in pluripotency, proliferation and differentiation. However, the molecular mechanisms underlying the functions of REX1 and its downstream targets remain largely unclear. In the present study, we performed quantitative proteomic analysis of control and REX1 knockdown (KD) human embryonic stem cells (hESCs) to elucidate the cellular events which are directly or indirectly regulated by REX1. Analysis of the proteome via nano-LC-MS/MS identified 140 differentially expressed proteins (DEPs) displaying a >2-fold difference in expression level between control and REX1 KD hESCs. The major identified proteins were validated by quantitative real-time RT-PCR and western blotting. To determine the molecular functions of the proteins and pathways regulated by REX1, DEPs were analyzed by using different bioinformatic tools, such as gene ontology, pathway and functional clustering analyses. Functional network analysis revealed a highly interconnected network among these DEPs and indicated that these interconnected proteins are predominantly involved in translation and the regulation of mitochondrial organization, and specific differentiation. To identify the cluster of closely co-regulated proteins within the interactome, a core interconnected network among the translation-associated proteins was also identified, such as ribosomal proteins (RPs) including RPL6, RPL9, RPL10A, RPL13, RPL30, RPS7, RPS20 and RPS21, via the Cytoscape plugin MCODE analysis. Further, we compared the results of the proteome analysis to those of the transcriptome analysis. These findings regarding REX1-mediated

network have revealed the contributions of REX1 to maintaining the status of hESCs and extended our understanding of the molecular events that underlie the fundamental properties of hESCs. *This work was supported by grants through the KRIBB/KRCF research initiative program (NAP-09-3) and the National Research Foundation of Korea [NRF; 2010-020272(3), and 2012M3A9C7050224] funded by the Ministry of Science, ICT and Future Planning.*

F-1436

LINEAGE COMMITMENT OF PLURIPOTENT STEM CELLS AS A PHASE TRANSITION

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In the developmental context of the pre-implantation embryo, only a small proportion of cells in the inner cell mass of the blastocyst possess the capacity to contribute to all embryonic germ layers. However, during a brief preparatory phase, these cells multiply greatly to form a founder tissue, the epiblast, from which the germ layers emerge and subsequently mature cellular identities are established. Cells reflective of the in vivo pluripotent state can be isolated and propagated in vitro. In conditions devoid of external differentiation cues, the pluripotent state may be maintained indefinitely in vitro by expression of a self-sustaining network of pluripotency regulatory factors. We sought to determine whether the developmental progression away from the pluripotent state could be compared to a phase transition in physics, which occur when a system switches rapidly from one stable state to another. Commonly, such transitions are characterised by certain generic phenomena including flickering (an increase in noise), increased autocorrelation, and critical slowing down. Using a well-established protocol to direct mouse embryonic stem cell (mESC) differentiation towards a neuronal identity, we assessed if these characteristics also attend the early stages of mESC lineage commitment. Over the course of seven days, changes in global mRNA and protein expression were recorded in parallel with mRNA expression at the single cell level. Integrated analyses of these datasets were conducted, including the construction of machine learning classifiers, and comparison with a large custom curated database of literature-derived gene expression patterns. This analysis revealed that many features characteristic of phase transitions in physical systems are also recapitulated during stem cell differentiation, suggesting that the tools of statistical mechanics may be usefully applied to understanding of stem cell dynamics.

F-1437

THE EVOLUTION AND CONSERVED ROLES OF VERTEBRATE POUV PROTEINS IN REGULATING AND INDUCING PLURIPOTENCY

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Oct4, a transcription factor belonging to class V POU protein, plays essential roles in the maintenance and induction of pluripotency, differentiation and somatic cellular reprogramming. Here we have used evolution to decipher distinct Oct4 networks. Oct4 homologues are found among vertebrates in two forms: POU5F1 and POU5F3 proteins. Eutherian mammals and some other vertebrates retain only single one of those. Other species including axolotl, coelacanth, turtle, and tammar wallaby retain both genes. We investigated the functional significance of POU5F1 and POU5F3 split in these species by using a combination of in silico sequence analysis and Oct4-null mouse embryonic stem cells (ESC). We found that all POU5F1 proteins are better able to rescue ESC-self-renewal than their paralogs, both at a quantitative level and with respect to maintaining normal naïve ESC phenotypes. Microarray-based global gene expression profiling of coelacanth POUV-rescued ESC lines shows that POU5F1 supports conserved activities in developmental germ cell specification. In contrast, ESC rescued by POU5F3 exhibit the upregulation of various cell differentiation programs and cell adhesion regulators (e.g E-cadherin and N-cadherin). Thus, POU5F1 appears linked to the evolution of pluripotency in the germ line while POU5F3 is related to gastrulation stage or primed pluripotency. However, there is one POU5F3 factor that rescues ESCs like Oct4, the Xenopus gene xlpou91 (pou5f3.1). In Xenopus, a further duplication of POU5F3 gene enabled specialization, and Xlpou91 appears specifically in the primodial germ cells. Xlpou25 (Pou5f3.2) exhibits epiblast-specific activities lacking of capacity to maintain naïve pluripotency, similar to other POU5F3 proteins; whereas, Xlpou91 is a germ-cell specific protein that has the capacity to rescue ESC self-renewal, similar to other POU5F1 proteins. The epiblast versus germline (naïve) activities of these proteins were confirmed in both ESC rescue and reprogramming. Taken together, these observations suggest that gene duplication enables segregation of Oct4 activity and allows the study of cell type specific roles of this pivotal transcription factor. Only through this evolutionary approach have we been able segregate these two clear distinct Oct4-dependent regulatory programs.

F-1438

THE ACTIVATION OF CBP/BETA-CATENIN SIGNAL PATHWAY IS INVOLVED IN THE TRANSITION FROM PRIMED- TO NAïVE STATE ON MICE PLURIPOTENT STEM CELLS

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Wnt/beta-catenin signaling pathway is conserved in numerous animal species, and greatly involved in homeostasis mechanism as well as various body developments. Recently, it was reported that the pluripotent stem cell exhibited the presence of distinctive two types of stem cells such as naïve and primed states. These divergent characteristics are similar to the distinction between the property of mouse ESC and human ESC. However, the comparison with two-pluripotent stem cell states have not yet been revealed. In the present study, we hypothesized the involvement of Wnt/beta catenin, especially CBP/beta catenin signaling pathway is deeply associated with the transition of the state, and we attempted to induce the conversion of primed into naïve state on the mouse model by their activation. In this study, to specifically activate CBP/beta catenin signaling pathway, we used a small molecule: IQ1. The analysis was performed by Realtime-PCR, Westernblot, Immunofluorescence and the production of chimeric mice. Firstly, to elucidate the distinct expression pattern of beta catenin signaling pathway between mESC and mEpiSC, we confirmed the protein expression pattern for comparison. The mESC as naïve state exhibited predominantly CBP/beta catenin than p300/beta catenin. Next, we tried to convert from mEpiSCs to mESCs by adding the small molecule. Their converted cells were obtained and these cells expressed ESC marker genes such as Klf4 and Stra8. On the contrary, the expression of Epiblast specific marker genes were decreased. Thus, we succeeded in the conversions of two-pluripotent states. The present study provides evidence that the activation of CBP/beta catenin signaling pathway plays an important role for the promotion and stabilization of mouse ESC as the naïve pluripotent state. Determination of the factors and signal cascade enhancing the conversion or increasing mESC- or mEpiSC-state stabilities in future studies are essential to correctly understanding the developmental stages and properties of pluripotent stem cells, which may lead to a more ideal model system for studying human development.

F-1439

TRAF2 RECRUITMENT VIA T61 IN CD30 DRIVES NFKB ACTIVATION AND ENHANCES HESC SURVIVAL AND PROLIFERATION

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CD30 (TNFRSF8), a tumor necrosis factor receptor family protein, and CD30 variant (CD30v), a ligand-independent form encoding only the cytoplasmic signaling domain, are concurrently over-expressed in transformed human embryonic stem cells (hESCs) or hESCs cultured in the presence of ascorbate. CD30 and CD30v are thought to increase hESC survival and proliferation through NFκB activation, but how this occurs is largely unknown. Here we demonstrate that hESCs that endogenously express CD30v and hESCs that artificially over-express CD30v, exhibit increased ERK phosphorylation levels, activation of the canonical NFκB pathway, down-regulation of the non-canonical NFκB pathway, and reduced expression of the full-length CD30 protein. We further find that CD30v, surprisingly, resides predominantly in the nucleus of hESC. We demonstrate that alanine substitution of a single threonine residue at position 61 (T61) in CD30v abrogates CD30v-mediated NFκB activation, CD30v-mediated resistance to apoptosis and CD30v-enhanced proliferation, as well as restores normal G2/M-checkpoint arrest upon H2O2 treatment while maintaining its unexpected subcellular distribution. Using an affinity purification strategy and LC-MS, we identified TRAF2 as the predominant protein that interacts with WT CD30v but not the T61A-mutant form in hESCs. The identification of Thr61 as a critical residue for TRAF2 recruitment and canonical NFκB signalling by CD30v reveals the substantial contribution this molecule makes to overall NFκB activity, cell cycle changes and survival in hESCs.

F-1440

ROLES OF LONG NONCODING RNAS IN PLURIPOTENCY MAINTENANCE OF MOUSE EMBRYONIC STEM CELLS

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Mammalian genomes encode thousands of long noncoding RNAs (lncRNAs), which are a class of transcripts longer than 200 nucleotides, usually transcribed by RNA polymerase II, 5' capped, spliced, and polyadenylated, but with little/no protein-coding potential. Recently, lncRNAs have been recognized as new regulators of pluripotency maintenance in embryonic stem cells (ESCs), such as ROR and TUNA. Yet the functions of majority lncRNAs are still unknown. Here, we studied the expression profiles of lncRNAs in mouse ESCs, and identified some new lncRNAs with ESC specific expression patterns. Large scale loss of function screen experiments discovered that a lncRNA as an essential factor for the maintenance of mouse ESC pluripotency. Mechanism studies revealed that the lncRNA and *Nanog* transcript both have the binding site of a same miRNA, and the lncRNA can compete with *Nanog* transcript for miRNA binding, therefore release the repression of *Nanog* transcript by the miRNA. We thus termed this lncRNA as *NAP* (*Nanog* protector). The expression of *NAP* is activated by core pluripotency factors OCT4 and SOX2. Knocking down *NAP* induces mouse ESCs to differentiate, together with decreased expression of pluripotent marker genes. In vivo developmental evaluation of *NAP* knockdown ESCs results in reduced teratoma formation ability and chimera contribution potential, knocking down *NAP* in mouse blastocysts also induces embryonic lethality. These findings reveal the functional mechanism of *NAP*, and add a new layer of lncRNAs in regulating the functions of *Nanog* and pluripotency maintenance of mouse ESCs.

F-1441

ASCORBIC ACID IMPROVES THE PLURIPOTENCY OF HUMAN PARTHENOGENETIC EMBRYONIC STEM CELLS THROUGH MODIFYING IMPRINTED GENE EXPRESSION IN THE DLK1-DIO3 REGION

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Human parthenogenetic embryonic stem cells (hpESCs) are

generated from artificially activated oocytes. To date, the issue of whether hpESCs have equivalent differentiation ability to human fertilized embryonic stem cells (hfESCs) remains controversial. Comparison of five hpESCs with different differentiation abilities (including threethat significantly develop teratoma and two that do not form teratoma easily) revealed that levels of paternal genes in the Dlk1-Dio3 expression gene cluster on chromosome 14 in the former are enhanced, but strictly methylated and silenced in the latter. Treatment of hpESC-2 cells with ascorbic acid, a histone deacetylase inhibitor, rescued their ability to support teratoma formation and altered the expression profiles of paternally expressed genes in cells that could not form teratoma easily. To confirm the roles of paternally expressed genes in the Dlk1-Dio3 gene cluster in hpESC differentiation ability, other imprinted genes were identified and characterized. No differences in gene expression were evident between hpESCs with higher and lower differentiation potential, except for those in the Dlk1-Dio3 region. Based on data from the present study, we conclude that the Dlk1-Dio3 imprinting gene cluster distinguishes the differentiation ability of hpESCs. Moreover, modification by ascorbic acid may facilitate application of hpESCs to clinical settings in the future by enhancing their pluripotency.

F-1442

IMAGE-BASED QUANTIFICATION AND MATHEMATICAL MODELING OF SPATIAL HETEROGENEITY IN ESC COLONY FORMATION

Zerjatke, Thomas[1], Herberg, Maria[1], de Back, Walter[2], Glauche, Ingmar[1], Roeder, Ingo[1]

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Pluripotent embryonic stem cells (ESCs) have the potential to differentiate into all cell lineages of the body. This unique property has been mainly studied on the intracellular, transcriptional level. However, cultured ESCs form cell clusters of distinct shape and size and establish spatial structures that are vital for the maintenance of pluripotency. Even though it is recognized that the cells' arrangement and local interactions play a role in fate decision processes, correlations between transcriptional and spatial patterns have not yet been studied. We present a systems biology approach that combines a quantitative analysis of time-lapse live-cell imaging data with a dynamic multi-scale mathematical model of ESC growth. In particular, we develop quantitative measures on the morphology and the spatial clustering of ESCs with different transcription factor expression levels. Applying these measures to time-lapse movies of cultured ESCs we can quantify the spatial heterogeneity and patterning in the colony formation process under self-renewal as well as differentiation conditions. The image analysis is complemented by a mathematical modeling approach that combines an intracellular model describing the regulation of key pluripotency transcription factors, with a spatial model of ESC colony formation. Using the same quantitative measures on simulated and experimental time-lapse movies, we are able to directly compare distinct model scenarios with different assumptions on cell-cell adhesions and intercellular feedback mechanisms between single ESCs with experimental data. This integrated approach combining image analysis and mathematical modeling allows us to reveal potential transcription factor related cellular and intercellular mechanisms behind the emergence of observed patterns that cannot be derived from images directly.

F-1443

DETERMINE MOLECULAR CHANGES OF STEM CELL DIFFERENTIATION WITH SINGLE-CELL TRANSCRIPTOMES

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Stem cell therapy has the potential to restore, repair and even replace aging or diseases organs in human. Such clinical applications of stem cells depend on our ability to manipulate pluripotency and direct stem cell differentiation toward specific cell types. Single-cell molecular profiling has the potential to reveal the molecular mechanism of stem cell pluripotency and provide us the ability to manipulate stem cells for regenerative medicine. A human embryonic stem cell (hESC) colony contains spontaneously differentiated cells at the edge of the colony with pluripotency cells at the center. Here, we report the sequential changes of transcriptomes for the initial human stem cell differentiation. Enabled by microfluidic devices, we performed single-cell transcriptome analysis on individual cells of H9 hESC colonies. Each hESC colony contains individual cells at various differentiae stages from pluripotent (center) toward differentiated stages (edge). Single-cell transcriptomes of individual cells at consecutive differentiation stages are obtained with a microfluidic device. Because cells at consecutive stages are more similar to each other than that from cell at disparate stages, clustering these transcriptomes by similarity reveals the stepwise transcriptome changes from pluripotent stages toward initial differentiated cells. This molecular map of H9 differentiation reveals the early sequential molecular steps of H9 stem cell differentiation, and provides gene targets for more effective manipulating stem cell pluripotency. Manipulating the target genes with small molecular will enable the development of safe and effective stem cell applications for regenerative medicine.

EMBRYONIC STEM CELL CLINICAL APPLICATION

F-1421 *This poster board is located in a different topic area*

30 CLINICAL-GRADE HUMAN EMBRYONIC STEM CELL LINES GENERATED IN MTESR1 SYSTEM

Ma, Yanlin[1], Jiang, Weiming[1], Xiao, Lu[2], **Wei, Yanxing**[2], Guo, Lei[2], Li, Qi[1], Zhao, Xing[1], Li, Linjiang[1], Xu, Wen[1], Lu, Weiying[1], Quan, Song[2], Huang, Yuanhua[1], Yu, Yanhong[2]

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Dendritic cell (DC) based cancer vaccines have been developed to initiate and promote antigen-specific immune responses to target tumor cells. We have generated DCs derived from human embryonic stem cells (hESCs) that are electroporated with mRNA encoding telomerase/lysosome-associated membrane protein 1 (hTERT/LAMP-1) chimeric tumor antigen. Telomerase is an attractive

tumor antigen, as high levels of hTERT expression are associated in more than 85% of all human cancers. Our hESC-derived DC product, AST-VAC2, is being developed as an off-the-shelf allogeneic cancer vaccine for clinical testing in partially HLA matched patients with non-small cell lung carcinoma. We have previously shown AST-VAC2 expresses CD83, migrates in response to chemokines, and can elicit hTERT-specific T cell responses from partially HLA-matched donor PBMCs in vitro. In preparation for clinical testing, the manufacturing process for AST-VAC2 has been scaled up from T-flasks to large surface area CellSTACKs. This has required the development of in-process controls to monitor the efficiency of hESC differentiation toward the DC lineage and novel process modifications including large scale embryoid body filtration and flow-through electroporation. As a safety precaution, the final product will be exposed to gamma irradiation to mitotically arrest any residual hESCs. The radiation dose required for halting replication while still preserving antigen presentation was determined using hESC colony forming and DC functional assays. Irradiation reduced hESC colony formation, but maintained the capacity of AST-VAC2 cells to stimulate T cells and migrate in the presence of chemokines. It is anticipated that the proposed Phase 1/2a clinical trial will enroll approximately 30 patients and will include both a dose escalation and a broadening of inclusion criteria to permit an assessment of safety, toxicity and immunogenicity in patients with advanced disease. Asterias has recently partnered with Cancer Research United Kingdom (CRUK) and the University of Southampton for the GMP manufacturing and initial clinical testing of AST-VAC2 in the UK.

F-1445

THE DEVELOPMENT OF A NOVEL DENDRITIC CELL-BASED VACCINE DERIVED FROM HUMAN EMBRYONIC STEM CELLS FOR CLINICAL USE AS A CANCER IMMUNOTHERAPY

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Dendritic cell (DC) based cancer vaccines have been developed to initiate and promote antigen-specific immune responses to target tumor cells. We have generated DCs derived from human embryonic stem cells (hESCs) that are electroporated with mRNA encoding telomerase/lysosome-associated membrane protein 1 (hTERT/LAMP-1) chimeric tumor antigen. Telomerase is an attractive tumor antigen, as high levels of hTERT expression are associated in more than 85% of all human cancers. Our hESC-derived DC product, AST-VAC2, is being developed as an off-the-shelf allogeneic cancer vaccine for clinical testing in partially HLA matched patients with non-small cell lung carcinoma. We have previously shown AST-VAC2 expresses CD83, migrates in response to chemokines, and can elicit hTERT-specific T cell responses from partially HLA-matched donor PBMCs in vitro. In preparation for clinical testing, the manufacturing process for AST-VAC2 has been scaled up from T-flasks to large surface area CellSTACKs. This has required the development of in-process controls to monitor the efficiency of hESC differentiation toward the DC lineage and novel process modifications including large scale embryoid body filtration and flow-through electroporation. As a safety precaution, the final product will be exposed to gamma irradiation to mitotically arrest any residual hESCs. The radiation dose required for halting replication while still preserving antigen presentation was determined using hESC colony forming and DC functional assays. Irradiation reduced hESC colony formation, but maintained the capacity of AST-VAC2 cells to stimulate T cells and migrate in the presence of chemokines. It is anticipated that the proposed Phase 1/2a clinical trial will enroll approximately 30 patients and will include both a dose escalation and a broadening of inclusion criteria to permit an assessment of safety, toxicity and immunogenicity in patients with advanced disease. Asterias has recently partnered with Cancer Research United Kingdom (CRUK) and the University of Southampton for the GMP manufacturing and initial clinical testing of AST-VAC2 in the UK.

F-1446

SYNAPTIC INTEGRATION OF TRANSPLANTED HUMAN EMBRYONIC STEM CELL DERIVED NEURONS IN THE ADULT RAT BRAIN

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The use of human embryonic stem cells (hESC) derived neurons for cell replacement therapy in Parkinson's disease, is hastily moving forward towards clinical translation. Transplanted hESC-derived neurons patterned towards dopaminergic fate have been shown to survive long-term, release dopamine and to extensively innervate correct host structures in vivo. However, mainly due to previous technical limitations, little is known on the extent and pattern of synaptic connectivity established between grafted and host neurons. A better comprehension on graft-host neuronal communication might be fundamental towards bringing cell replacement therapy towards successful clinical trials. We have established a technique aimed at assessing synaptic connectivity between transplanted cells and host neurons using modified rabies virus as a transsynaptic tracer. This technique allows for analysis of specific host-to-graft and graft-to-host connectivity by using EnvA-pseudotyped rabies vector to restrict initial infection only to cell populations that have been modified to express the avian TVA receptor. In this work, 6-OHDA lesioned rats received intrastriatal transplants of hESC-derived dopaminergic neurons. Then, to assess for synaptic connectivity, animals were injected with rabies vector either 5 or 23 weeks after transplantation and perfused one week later for histological analysis. Using this strategy we show that host neurons from either local striatum or distant striatal afferent structures such as prefrontal cortex, thalamus and substantia nigra, are able to establish synaptic connections with transplanted neurons. Surprisingly, such graft integration was observable at an early survival time point such as 6 weeks post grafting. Moreover, this pattern of connectivity was maintained at 24 weeks. Graft to host integration is also present in this transplantation context, as we demonstrate that grafted cells are able to synapse both with local striatal medium spiny neurons and prefrontal cortical neurons. Overall, modified rabies.based monosynaptic tracing provides a promising tool to study both host to graft and graft to host integration of transplanted stem cell derived neurons of diverse phenotypes and in different animal and disease models.

F-1447

BANKING OF CLINICAL GRADE HUMAN EMBRYONIC STEM CELL LINES

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Good Manufacturing Practice (GMP) compliant banks of human embryonic stem cell (hESC) lines have been established at Roslin Cells as a cell therapy resource. These were derived: i) from ethically procured voluntarily donated surplus to assisted conception requirements preimplantation embryos by outgrowth on human neonatal fibroblasts using media, reagents and substrates warranted as suitable for this purpose; ii) with certification and licensing warranting compliance with pertinent standards, notably ISO 9001:2008 accreditation by BSI Management Systems Ltd and licenses from the United Kingdom Human Fertilisation and Embryology Authority and Human Tissue Authority and iii) in facilities operating to provide a detailed and traceable compilation of cell line history, including donor consent, medical history and health screening, implementation of standard protocols and batch recording of cell procurement, processing, and quality control characterization. As we evidenced here for three lines (RC9, 11, & 17) the latter includes: RT-PCR confirmation of absence of mycoplasma (i), and viruses (CMV, HTLV1, HIV1, HCV, HBV, EBV) (ii), a normal karyology by G-banding (iii) and whole-genome SNP analysis (iv), confirmation of undifferentiated cell identity by flow cytometry (OCT3/4, Tra-1-60, SSEA-4, SSEA-1) (v), genetic identity by microsatellite PCR (vi), HLA (vii) and blood type (viii), lineage potency by teratomas in NOD/SCID mice (ix) and/or undirected differentiation in vitro (x), and post thaw viability by flow cytometry for apoptotic and dead cells (Guava ViaCount) (xi). These lines have been deposited in the UK Stem Cell Bank and are available for licensing and use as a resource for production of clinical grade cell products for use in allogeneic cell transplantation to repair diseased or damaged tissues.

F-1448

A NEW APPROACH TO FOR THE TREATMENT OF PARKINSON'S DISEASE - AN UPDATE

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In 2011, our team overcame one of the biggest challenges of stem cell therapy for Parkinson's Disease (PD) - the lack of cells suitable for transplantation. Such cells, if manufactured at scale, in defined conditions and at high purities would bring relief to those no longer responding to conventional PD therapy. Importantly, a fundamental characteristic of PD is the progressive, severe and irreversible loss of specific dopamine-producing neurons in the midbrain, resulting in disabling motor dysfunction. Cell therapy therefore maybe a superior treatment when compared to pharmacological or electromechanical therapies. Fetal cell grafts have been used to restore the function of the lost cells with mixed outcomes. Some patients are living without drug therapy for more than 15 years, while others have seen no benefit - or worse, developed side effects knows as dyskinesias. Despite these problems, cell therapy for PD remains attractive due to the possibility of restoring actual dopamine (DA) neurons capable of integration into the host circuitry. Our major discovery enabled the derivation of nearly unlimited numbers of transplantable dopaminergic neurons from human pluripotent stem cells (hPSCs). Building on extensive efforts in labs across the world, our technology opened the door for a truly novel PD therapy and one of the first therapies based on human pluripotent stem cell technology. With the help of an award by New York State (NYSTEM), we are able to develop this therapy over the next 4 years. Today, we are in year 2 of our award and after our first meeting with the FDA we are now planning and performing pilot studies to inform our pre-IND meeting. We anticipate that we will have completed safety and toxicology testing by the summer of 2017, at which time we will seek IND approval for this new therapy. I will highlight some of product optimizations, namely cell sorting for the cell surface marker CD142 and cell freezing, that we believe will have a large impact on our ability to bring a safe and efficacious therapy to the PD community. Further, I will discuss in detail the challenges we encountered in moving this cell therapy from the lab to the clinic. These challenges are not only scientific, but also financial, legal and logistic. We believe that sharing our experience with the stem cell community will be of great value to all attendants.

F-1449

ENVIROMENTAL FACTORS THAT MAY AFFECT MUTATION RATES IN HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) are an attractive cell source for regenerative medicine. Recent advances in in vitro culturing methods have allowed generation and propagation of hPSCs under xeno-free and chemically defined conditions. Human embryonic stem cells (hESCs) can be derived from a single blastomere cell without a need to destroy the parental embryo addressing the ethical concern of many. Such methods may facilitate the development of cell lineages for therapeutic purposes. Propensity to genetic mutations and tumorigenicity are common features of all types of hPSCs that are major remaining hindrances to hPSC-based therapies. Prolonged culturing in vitro often leads to adaptive genetic changes in hPSCs, which resemble those observed in tumor cells. It is largely unknown if certain environmental cues affect the rate of genetic mutations in the cells. In this study, hESCs and human induced pluripotent cells (hiPSCs) were cultured for 3 months side-by-side in different wells of multi-well plates in the same medium to ensure that majority of the environmental variables were fixed. Passaging of cells in single cell suspensions versus cellular clumps; use

of enzymes versus mechanical passaging; culturing on Matrigel versus defined substrata such as laminin-521 and vitronectin; use of a hPSC culturing medium with or without ROCK inhibitor Y-27632 were compared to reveal the safest method of hPSC culturing in vitro. Influence of temperature and pH shocks on the rate of mutagenesis in the cells was also analyzed. High-resolution DNA analysis using Illumina OmniExpress whole genome genotyping microarrays containing 750,000 probes for single nucleotide polymorphisms was performed to reveal small genetic aberrations in the cultured cells at the beginning of the experiment and 3 months later (12 to 15 passages). Quantitative RNA sequencing of transcriptomes was used to reveal changes that were might have resulted from culture adaptations. Levels of tumor-suppressor and anti-apoptotic gene expression in the cells before and after prolonged culturing in vitro were compared to detect cancer-related adaptation in cultured hPSCs. One hundred samples of seven independent hESC and hiPS cell lines were analyzed using both methods. The results may reveal ways to increase safety of hPSCs for cell therapy.

TISSUE ENGINEERING

F-1450

TARGET EDITING OF THE WISKOTT-ALDRICH SYNDROME PROTEIN (WASP) USING ZINC FINGER NUCLEASES IN WAS PATIENT'S INDUCED PLURIPOTENT STEM CELLS

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Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder characterized by thrombocytopenia with small-sized platelets, recurrent infections, eczema, and increased susceptibility to autoimmune diseases and hematologic malignancies. It is caused by mutations in the gene encoding the WAS protein (*WASP*), a regulator of actin cytoskeleton and chromatin structure. The *WASP* is expressed in all hematopoietic lineages. Patients with severe WAS mostly die from infection or bleeding within the first decades of life. Hematopoietic stem cell transplantation (HSCT) remains the only curative therapy for WAS. The difficulty in finding HLA-matched donors and complications related to transplantation urge the development of gene therapy strategies for WAS. With the improvement in techniques for generating clinical-grade hematopoietic stem cells from induced pluripotent stem cells (iPSCs) and advances in gene targeting strategies, gene-corrected hematopoietic stem/progenitor cells derived from the patient iPSCs could be an effective alternative for treatment of severe immunodeficiency disorders. Here we reported on the successful generation of the genetically-corrected WAS-iPSCs using zinc finger nuclease-based strategies. A total of eight corrected WAS-iPSC clones were obtained and revealed no random integration. Western blot analysis revealed the WAS protein was expressed from platelets derived from the corrected WAS iPS cells. These corrected WAS-iPSCs could be further validated prior to use in a clinical setting. This study is one of the most important steps in using gene targeting strategies to precisely correct the *WASP* mutation in patient-derived iPSCs.

F-1451

OSTEOGENIC DIFFERENTIATION FROM HUMAN DENTAL PULP STEM CELLS (HDPSCS) USING MELATONIN ON COLLAGEN SPONGE: A NOVEL APPROACH FOR TISSUE ENGINEERING

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Stem cell differentiation toward an osteogenic phenotype represents a core problem in bone tissue engineering research. Recent research indicates that human dental pulp stem cells (hDPSCs) are adult mesenchymal stem cells that can differentiate into osteoblasts in vitro, which suggests that they may become a new kind of seed cells for bone tissue engineering. The aim of this study was to isolate, characterize and evaluate the osteogenic differentiation of hDPSCs in vitro and bone-like tissue formation using also melatonin as enhancer for differentiation and the behavior of these cells on three-dimensional collagen scaffolds generally used in dentistry clinical practice as hemostatic in the post extractive alveolus. We obtained hDPSCs from dental pulp tissue extracted from human third molar during orthodontic treatment by mechanical and enzymatic digestion using collagen III. After expansion, the cells were immunoselected by MACS columns using CD117 antibody, analyzed by flow cytometer (FACSaria) for mesenchymal markers CD29, CD44, CD31 and CD45. Seeded collagen scaffolds (CONDRESS) and cultured in osteogenic medium with or without melatonin. After 7,14 and 21 days in culture, the cells in the collagen scaffolds were processed for Alizarin and for qPCR analysis of the osteogenesis-specific genes Osteocalcin (OCN), Runt-related transcription factor 2 (RUNX2), KLF9, stanniocalcin (STC), zbtb16, NR4A3 and of the pluripotency-related genes OCT3/4, SOX2, NANOG. Western blotting was avail for confirmed gene expressions analysis. Scanner Electron Microscopy (SEM) was used to examine interaction of cells among them and with the scaffold. In vitro studies revealed that hDPSCs possess osteogenic differentiation potential bearing the ability to develop an organized structures when cultured in the presence of specific scaffolds. The presence of melatonin in the medium increase the osteogenic induction and developed mineralization nodules (clusters). These findings suggested that dental pulp is a peculiar stem cells source easily available, that could improve tissue regeneration; in addition, the presence of melatonin in the osteogenic medium increases the yield of differentiation.

F-1452

LIGHT OR DARK - A MATTER OF MESENCHYMAL CELLS IN BIO-ENGINEERED HUMAN SKIN

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Mesenchymal factors derived from human palmoplantar fibroblasts have been shown in vitro to regulate melanocyte proliferation and function. We wanted to investigate in vivo the influence of mesenchymal-epithelial interactions in human tissue-engineered skin substitutes containing fibroblasts from palmar- or nonpalmoplantar skin sites. Tissue-engineered dermo-epidermal analogs based on collagen type I hydrogels were populated with either human palmar or nonpalmoplantar fibroblasts and seeded with human nonpalmoplantar-derived melanocytes and keratinocytes. The bioengineered dermo-epidermal skin substitutes were then transplanted to full-thickness skin wounds of immuno-compromised rats. The development of skin color was measured four weeks after transplantation. The skin analogs were excised and analyzed in general with regard to epidermal characteristics, in particular to melanocyte number and function. Our skin substitutes containing palmar-derived fibroblasts in comparison to nonpalmoplantar-derived fibroblasts showed a significantly lighter pigmentation, a reduced amount of epidermal melanin granules and a distinct melanosome expression. Importantly, the number of melanocytes in the basal layer remained similar in both transplantation groups. These findings demonstrate that human palmar fibroblasts regulate the function of melanocytes in human pigmented dermo-epidermal skin substitutes after transplantation. This underscores the influence of site-specific stromal cells and their importance when constructing skin substitutes for clinical application.

F-1453

STANDARDIZED AND CLINICAL GRADE HUMAN PLATELET LYSATE HYDROGEL (HPLG) FOR THE OPTIMIZATION OF LARGE-SCALE EXPANSION OF HUMAN MESENCHYMAL STEM CELLS

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The amplification of stem cells, such as human Bone-Marrow Mesenchymal Stem Cells (MSC), in standardized and xeno-free conditions represents a major challenge for the production of safe and reproducible cell therapy products in clinical applications. Recently, human platelet lysate has emerged as a safe and efficient substitute for Fetal Calf Serum (FCS), a traditionally-used animal product showing risks of pathogens transmission and xenogeneic immune reactions. We evaluated the use of a standardized HPLG manufactured under GMP conditions as a 3D-supportive environment for large-scale expansion of MSC. In culture on HPLG, MSC were progressively degrading the gel, releasing the growth factors entrapped (VEGF, PDGF-AB, IGF-1, TGF-β1, bFGF) with specific kinetic profiles. These profiles were different in absence of cells. HPLG thickness studies (from 50 to 200µL/cm²) identified the 100µL/cm² condition as the more potent on MSC's expansion. Fluorescently labeled MSC seeded on HPLG surface were shown to migrate inside the gel during culture. Cells harvesting from HPLG was quicker and easier than on plastic using a clinical grade enzymatic solution. Comparing MSC's expansion on plastic with a 10%FCS-bFGF supplemented-medium to a 10% HPLG without any medium supplementation, we demonstrated 1) On HPLG, on 3 consecutive passages, the maintenance of MSC proliferation rate around 90% when a 60% decrease was observed at passage 3 on plastic; 2) no modification for CD13, CD29, CD44, CD45 and CD73 expression; 3) an increase expression of CD90 and CD105 on HPLG; 4) a similar osteogenic and adipogenic differentiation potential. Interestingly, without any medium change on a 10-days period, we observed that the total number of cells continued to efficiently increase on HPLG, while on plastic proliferation stopped after D5. Taken together, the presented data clearly demonstrate that HPLG represents a new, secured and efficient way to amplify MSCs for cell therapy.

F-1454

A NOVEL WOUND REPAIR ELECTROSPUN COMPOSITE WITH HEMOSTATIC AND ANTIBACTERIA PROPERTY

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Wound repair is one of the most complex biological processes in trauma care. Compared with babies, the adults wound repair process commonly leads to a non-functioning mass of fibrotic tissue known as a scar, and treatment for chronic wound disabling conditions remains limited and largely ineffective. In this study, we prepared a novel electrospun nanofibrous composite which has the multiple functions, such as hemostasis, bacteriostat, stimulating wound heal and promoting tissue regeneration. The first layer is made from two kinds of natural polymers. One is oxidized regenerated cellulose (ORC) microfibers, the diameter is 3µm±1.2µm, and the other one is collagen (Col) from bovine tendon, the diameter is 600nm±450nm. There is more specific surface area from this kind of micro-nano structure which has the ability to absorb the liquid from blood rapidly and block the capillaries and small veins quickly. Both the Col and ORC have the ability to adhere and activate the platelets from the SEM images, then they start the intrinsic coagulation pathway and stop bleeding from the ELISA data and hemostatic text. As there are a mass of negative charges on ORC, this layer also has the ability to suppress bacteria. The second layer is a special core-shell structure. The core is Col/PCL nanofibers (mCol/mPCL, 1.5/1) with mouse transforming growth factor beta 1 (mTGF β1), and the shell is hydrogel with mesenchymal stem/stromal cells (mMSCs) cells and connective tissue growth factor (mCTGF). The mCTGF-stimulated mMSCs lost their surface mesenchymal epitopes, expressed broad fibroblastic hallmarks, and increasingly synthesized collagen type I. And the release amount of mTGF β1 from Col/PCL in PBS at 37 °C is 12.37% for 7 days, which plays an important role to promote fibroblast to myofibroblast in 5 days from fluorescent microscope images and PCR results, so as to decrease fibrosis and scarring.

F-1455

APPLICATION OF COLLAGEN SCAFFOLDS AND POLYPEPTIDE MULTILAYER FILMS FOR DIFFERENTIATION OF MOUSE IPSCS INTO CARDIOMYOCYTES

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Biomaterial scaffolds for tissue engineering are rapidly being developed to mimic physiological microenvironment and physical properties. Well-designed scaffolds, which have similar features of extracellular matrix (ECM), can guide stem cell differentiation and serve as functional cell-substrate environments for tissue regeneration. Physical factors including the elasticity and dimensionality of the matrix have been identified as important factors to direct stem cells fate. In this study, mouse induced pluripotent stem cells (iPSCs) were cultured on two types of gelatin-coated biomaterials: type-I collagen scaffold and polypeptide multilayer films. The mechanical property of freeze-dried collagen scaffolds was manipulated by maintaining the calf skin type-I collagen concentration at 6 mg/mL while varying the concentrations of cross-linkers, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-Hydroxysulfosuccinimide (NHS). Polypeptide multilayer films, also EDC/NHS cross-linked, were built up by the alternate adsorption of cationic poly-L-lysine (PLL) and anionic poly-L-glutamic acid (PLGA). These materials allow for modulation of thickness, stiffness, and adhesiveness. The cross-linked collagen scaffolds demonstrated better interconnectivity than the native collagen scaffolds without cross-linking. In addition, the Young's moduli of collagen scaffolds, measured by an AR2000ex rotational rheometer, increased with the elevating concentrations of cross-linkers. After embryonic bodies of iPSCs were cultured on scaffolds for 6 days, cardiomyocyte beating was observed. The cardiac differentiation level of iPSCs was evaluated by Quantitative PCR analysis of cardiac specific gene markers, especially the expression of cardiac troponin T (cTnT) increased. In the experimental group of polypeptide multilayer films, the characteristics of film thickness, surface roughness, and shear modulus were measured by AFM and QCM-D. Furthermore, cardiomyocyte beating was monitored by polypeptide multilayer-coated electrodes using impedance sensing technique. Our observations indicate a possible way to promote iPSCs toward cardiomyocyte differentiation both on type-I collagen scaffolds and polypeptide multilayer films by regulating the concentration of cross-linkers.

F-1457

MUSCLE PRECURSOR CELL INTERACTION WITH A DECELLULARIZED EXTRACELLULAR MATRIX IN A TISSUE ENGINEERED SKELETAL MUSCLE FOR IN VIVO REGENERATION

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The purpose of this work is to study the interaction between muscle satellite cells (SCs) and extracellular matrix (ECM)-derived scaffolds with the final aim of developing a mature and functional skeletal muscle construct for muscle regeneration in vivo. To date, the majority of engineered muscles have been developed delivering muscle cells into synthetic polymers or natural components for a 3D culture. However, these systems are far from reproducing the complex network and function of a skeletal muscle. Decellularization is a technology that removes the cell compartment from tissues and organs, creating scaffolds that retain the architecture of the native tissue, including vasculature and biofactors of the ECM. For this study, primary murine SCs were seeded into decellularized muscles in combination with other muscle interstitial cells, and cultured in vitro for 7-14 days. Acellular muscles were produced comparing three decellularization protocols: detergent-enzymatic treatment (DET), anti-polimerizing agent-enzymatic treatment (Lat B) and detergent alone (SDS). Efficient decellularization was achieved after 3 cycles of DET, 1 cycle of Lat B and 3 cycles of SDS as evidenced by histology and DNA quantification. Matrices showed distinct ultra- and nano-architecture, pro-angiogenic properties and ECM composition, with different levels of decrease in collagen (50-60%), elastin (100-40%) and glycosaminoglycans (75-60%) content in respect to the native tissue. SCs seeded into the 3 scaffolds displayed differences in proliferation, migration and differentiation. When cultured in the 3 muscle-derived matrices, they showed distinct self-renewal capacity due to specific arrangement and composition of the scaffold. Co-seeding with interstitial cells improved cell differentiation and migration within decellularized muscles with an overall amelioration of cell invasion and new myocyte maturation. Our results strongly suggest that acellular muscles support myogenesis and muscle stem cell maintenance in vitro, and show great potential for their application in muscle reconstruction in vivo for regenerative medicine. This study also highlights the active role of the ECM in affecting cell migration and differentiation of a heterogeneous population seeded into decellularized scaffolds.

F-1458

EFFECTS OF CO-EXPRESSION OF C-MYC AND BCL-2 ON PROLIFERATION, APOPTOSIS AND SURVIVAL OF DENTAL PULP STEM CELLS

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The development of cell lines with high cell density, controlled proliferation, apoptosis resistance, and easy adaptation into cultures of serum free media is essential for the success of tissue engineering strategies. This study aimed to enhance the proliferation and survival of dental pulp stem cells (DPSCs) via over-expression of oncogenes, bcl-2 and c-Myc. Dental pulp stem cells isolated from freshly extracted third molars were trasnfected with premade lentiviral particles (GenTarget Inc, San Diego, CA) of bcl2-GFP, cMyc-RFP or both. Vector-only viral particles with green or red fluorescent genes (GFP or FRP) were used to achieve control groups. Bcl-2-GFP, GFP, cMyc-RFP, RFP and Bcl2-GFP-cMyc-RFP over-expressing cell lines were selected using puromycin or/and blastomycin antibiotics. Gene and protein over-expression were determined using quantitative polymerase chain reaction (qPCR) and western blotting. Cell proliferation rate, apoptosis and necrosis of different cell groups were determined using cell counting kit-8 (cck-8) (Sigma-Aldrich), cell death detection ELISA kit (Roche) and caspase-3 colorimetric assay (R&D systems) respectively. All experiments were conducted in triplicate. Western blot and qPCR results confirmed the over-expression of c-Myc or/and bcl-2 in respective cell lines. c-Myc over-expressing DPSC cultures showed an increase in proliferation rate and maximum cell number compared to the wild type cells and bcl-2 overexpressing cells as shown by cck-8 assay. Bcl-2 over-expressing cells demonstrated a lower apoptosis and higher cell survival compared to the other groups. Over-expression of both oncogenes resulted in a cell line that exhibited higher proliferation rates and maximum cell numbers, with a decrease in apoptosis when compared to the parental cell line. Over-expression of c-Myc and

bcl-2 increases the proliferation rate and reduces the apoptosis and cell death in DPSCs, enhancing its potential as a promising cell source in tissue engineering approaches.

F-1459

INJECTABLE HYDROGELS PROMOTE SURVIVAL AND INTEGRATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED OLIGODENDROCYTES AFTER TRANSPLANTATION INTO THE INJURED RAT SPINAL CORD

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Stem cells promise to offer suitable cell populations to provide methods of repairing, regenerating or protecting diseased or injured tissue when the body is unable to heal itself. However, survival of grafted cells is usually low and it is envisioned that increased survival and host tissue integration will promote improved tissue repair and functional recovery. The loss of function after traumatic spinal cord injury (SCI) is mainly due to the disruption of long distance projecting axons. The limited capacity to regenerate is due to inherent neuronal properties, the expression and/or release of axon-growth inhibitory/repulsive molecules at- and around the lesion site, glial scarring and cystic cavitation. Biomaterials can positively influence a number of these factors and provide grafted cells with an environment, including a cell-adhesive matrix and growth factors, to promote their survival.
We differentiated human induced pluripotent stem cells into oligodendrocyte precursor cells (hOPCs), as identified by the expression of OLIG2, NKX2.2, SOX10, and PDGF-receptor alpha. As a cell delivery vehicle a minimally invasive, injectable hydrogel comprised of hyaluronan and methylcellulose (HAMC) modified with adhesive peptides was used. The peptide modification increased cells survival and cell-substrate (HAMC) interaction in vitro compared to hOPCs grown in plain HAMC or tissue culture plastic. To test survival in vivo, hOPCs were transplanted into experimental animals receiving a moderate clip compression injury at level T2. Cells were transplanted with and without the modified HAMC 7 days after injury at 4 sites rostral and caudal to the lesion. Surviving cells were found at the injury/injection site 7 days after transplantation (14 days after injury). The modified hydrogel promoted greater survival of grafted cells compared to cell transplantation in media. These surviving hOPCs expressed SOX10, a marker for oligodendrocytes and oligodendrocyte precursor cells, but not NF200 for neurons or GFAP for astrocytes. While some cells expressed the proliferation marker Ki67, no Oct4+ cells where found. Furthermore, we found surviving cells 9 weeks after injury, albeit without an increase of functional recovery. These data indicate the feasibility of our approach to promote graft cell survival with hydrogels.

F-1460

ADVANCED BIOMATERIAL SYSTEMS FOR DIRECTING FATE IN MOUSE AND HUMAN PLURIPOTENT STEM CELL CULTURES

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The promise of regenerative therapies is currently hindered by the availability of large-scale, homogeneous populations of desired cell types. The in vitro differentiation efficiency of human pluripotent stem cell (PSC)-derived cell types could be enhanced with robust biomimetic stem cell culture systems that recapitulate the dynamic interplay between a cell and its extracellular matrix (ECM). We have therefore leveraged novel biomedical engineering technologies for improving the local and sustained delivery of bioactive molecules for i) efficiently and reproducibly guiding differentiation, ii) minimizing the risk of unwanted cell types, iii) and having the potential for scale-up production for industrial tissue engineering applications.
As cells precisely regulate matrix metalloproteinase (MMP) expression and activity, they spatially and temporarily control ECM remodelling and degradation when transitioning from a pluripotent phenotype. Here, we studied in vitro MMP expression profiles in human and mouse PSCs cultured in feeder-free conditions. MMP2 is upregulated and released directly to the ECM during spontaneous differentiation, but is minimally produced in OCT4 expressing cells, making it an ideal candidate for a differentiation-sensitive material. We report a new biomaterial for in vitro stem cell culture that is sensitive to differentiation and supports stem cell survival through a combined nano-vesicle biomolecule delivery system, exploiting natural enzymatic mechanisms, with 3D tissue design. We explore thermoresponsive polymersomes, composed of poly(ethylene glycol), poly(acrylic acid) and poly(N-isopropylacrylamide) block copolymer, ranging in diameter from 150-200 nm, that can be cross-linked with various peptide linkers and reliably encapsulate large molecules. Incorporating MMP-degradable peptide cross-links within these particles affords site-directed burst-controlled release of cell-instructive morphogens. This enzyme-activated reciprocal response system is illustrated by ELISA-based platforms, achieving nano-molar detection. In both 2D and 3D, we can initiate local delivery of soluble cues to highly heterogeneous pluripotent stem cell-derived populations, enhancing differentiation efficiencies for a wide variety of regenerative strategies.

F-1461

TREATMENT OF CURCUMIN LOADED POLY(LACTIC CO GLYCOLIC ACID) NANOPARTICLES ON OXIDATIVE STRESS INDUCED RETINAL DAMAGE IN STEM CELL DERIVED RETINAL PIGMENT EPITHELIUM CELLS

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Age-related macular degeneration (AMD) is the leading cause of blindness among the elderly. Recent studies suggest that the pathogenesis of AMD is associated with oxidative stress and inflammation in the retina (retinal pigment epithelium cells). In this study, we synthesized poly(lactic-co-glycolic acid) (PLGA) nanoparticle as a sustained release system for AMD therapy. In such of effective therapeutic agents, we used curcumin (CUR) for therapy, a natural phenolic compound that possesses anti-apoptosis, anti-inflammation and neuroprotection properties in several ocular diseases. Retinal pigment epithelium cells (RPE) derived from human induced pluripotent stem cells are similar to adult human RPE and can be expanded in culture to a theoretically infinite degree. Thus, we used hiPSC-RPE as model to evaluate the possible therapeutic effects of curcumin-loaded PLGA nanoparticles (CUR-PLGA NPs) on oxidative stress induced retinal damage. In the results, cell cytotoxicity test demonstrated the optimal concentration of CUR-PLGA NPs to treat RPE cells without cytotoxicity was 15µM. The chemiluminescence assay indicated that CUR-PLGA NPs could eliminate the oxidative stress induced by hydrogen peroxide (H2O2). The results of cell viability and TUNEL assay showed that the developed CUR-PLGA NPs might have anti-apoptosis effects in RPE cells under oxidative stress. These results suggested CUR-PLA NPs may have promising therapeutic effects on stress-induced retinal damage. Overall, we believe that the therapy of CUR-PLGA NPs on stem cell derived RPE cells have a great potential for patient-specific clinical study in the future.

F-1462

ORTHOTOPIC TRANSPLANTATION OF A NEWLY DEVELOPED 3-D PRINTED TRACHEAL GRAFT IN RATS

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Transplantation of tissue engineered tracheal grafts has recently become clinical reality. However, the creation of the scaffold is time consuming and hence not applicable in urgent clinical scenarios. We here investigated a new method of rapid processing by using 3-D technology in order to create a synthetic customized tracheal scaffold that can be seeded with stem cells and orthotopically transplanted into rats. The 3D tracheal graft was fabricated by a self-made projection stereolithography with XY resolution of 37 µm and layer thickness of 30µm. Light intensity of 7000 µW/cm2 and curing time of 25s for each layer were used for fabrication. The scaffolds were investigated for their biocompatibility using cell viability and live/dead assays and structural properties prior to implantation. Allogeneic mesenchymal stromal cells (MSCs) from Sprague Dawley rats were used to seed both surfaces of the scaffolds. A tracheal segment of 0.8cm was replaced with either a non-seeded (group I) or MSC-seeded 3-D printed scaffold (group II) and animals were observed for 14 days. The 3-D printed graft showed well-suitable in vitro and in vivo biocompatible and biomechanical characteristics. MSC seeding via the bioreactor allowed a complete surface coverage of both tracheal surfaces and the initial detection of extracellular matrix specific proteins (e.g., laminin, collagen). Animals receiving non-seeded scaffolds were sacrificed at day 2±2 due to significant breathing difficulties whereas all animals receiving seeded scaffolds reached the study end-point (14 days) and were asymptomatic. All harvested scaffolds in group I showed airway obstruction due to granulation tissue and inflammatory processes. In contrast, scaffolds of group II showed no signs of adverse foreign body response and epithelialization of their internal scaffold surface (pan-keratin positive cells). The harvested grafts were entirely patent and cross section dimensions maintained equal across the anastomotic site. Our data suggest that the developed 3-D printed tracheal scaffolds are completely biocompatible, maintained their biomechanics and allow regeneration of epithelium on their internal surface. However, for successful in situ regeneration pre-operative MSC seeding is necessary.

F-1463

ENGINEERING A HIGH-BIOFIDELITY, MECHANICALLY AND ELECTROPHYSIOLOGICALLY FUNCTIONAL 3D HUMAN VENTRICULAR PUMP FROM PLURIPOTENT STEM CELLS: A HUMAN CELL-BASED BIOARTIFICIAL HEART PROTOTYPE

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Self-renewable human pluripotent stem cells (hPSC) can be effectively specified into ventricular cardiomyocytes (VCM) whose single-cell properties of fundamental importance have been extensively characterized. Although hPSC-CMs have been organized as multi-cellular monolayers or patches for electrophysiological (EP) assessment and grafting, such configurations are not amenable to contractile characterization; by contrast, engineered muscle strips are suitable for force measurements (not pressure) but have a highly restricted capability for EP due to significant boundary effects. Here we report a novel approach for engineering hPSC-VCM into human ventricular cardiac organoid chambers (hvCOC), by mixing ~10^7 hPSC-VCM, type-I collagen and Matrigel in a custom-made bioreactor that houses a spherical gap between an inner silicone balloon and an outer agarose cup. After 10-12 days, the resultant hvCOC displayed a hollow spheroidal geometry with diastolic volume ~135 µL, diameter ~6.5 mm and can spontaneously beat. An intact Frank-Starling mechanism was evident from an increase in developed pressure as luminal pressure was increased. The developed pressure also increased with increasing concentrations of free Ca^{2+}. High-resolution optical mapping revealed that as pacing frequency increased from 0.5 to 3.0 Hz, chamber conduction velocity decreased from 8.35±0.49 cm/s to 0.45±0.05 cm/s

and action potential duration at 50% maximum decreased from 215±14ms to 95±28ms. hvCOC also accurately reproduced the pharmacological responses for drugs such as isoproterenol, verapamil and flecainide. We conclude that hvCOC function as fluid-ejecting pumps as a result of coordinated electro-mechanical coupling, uniquely enabling the simultaneous assessment of such physiological parameters as ejection fraction, passive pressure volume and stress strain relationships that better describe cardiac functions at the organ level, reproducing key physiological and pharmacological features of a natural ventricle. Not only do hvCOC present a novel, versatile in vitro tool of high-biofidelity for facilitating drug discovery, cardiotoxicity screening and disease modeling that cannot be matched by simpler 2D/3D systems, they also offer important insights into the engineering of future cell-based human hearts.

F-1464

EXTRACELLULAR MATRIX HYDROGEL SCAFFOLDS FOR SPINAL CORD INJURY TREATMENT

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Spinal cord injury (SCI) often results in a loss of motor and sensory function as a consequence of the inability of axons to regenerate across the lesion. To bridge the lesion and re-establish damaged connections after SCI, modern biomaterials, hydrogels made from tissue specific extracellular matrix (ECM), have been prepared and evaluated in vitro in order to be later implanted in vivo into the acute model of SCI. We prepared three types of ECM extracted from porcine tissue. Mesenchymal stromal cells (MSCs) from different sources (human bone marrow, human adipose tissue, human Wharton jelly) were cultured on the ECM hydrogels and evaluated in vitro in terms of cell adhesion, proliferation and migration. ECM scaffolds were prepared by the decellularization of porcine brain tissue (BM), spinal cord (SCM) and urinary bladder (UBM). After decellularization, collagen and glykosaminoglycans in the scaffolds were evaluated. Cell viability and migration were evaluated. In an in vivo study, a hemisection was performed at the Th8 level, and SCM and UBM hydrogels were injected into the spinal cord defect and allowed to gelate in situ. The effects were evaluated via histological analysis and real-time qPCR. The structure of ECM hydrogels was similar to that of the native ECM. In terms of composition, BM ECM contained the highest amount of glycosaminoglycans, while UBM ECM contained the highest amount of collagen. All types of ECM hydrogels supported the proliferation of MSCs which was comparable with cell proliferation on tissue culture plastic. All type of ECM hydrogels also promoted MSC migration. In vivo, SCM and UBM hydrogels integrated into the host tissue and stimulated neovascularization and nervous tissue ingrowth into the lesion. No significant differences were found between SCM and UBM hydrogels with regards to ingrowth of neurofilaments and blood vessels at all observed time points. ECM hydrogels were biocompatible, promoted cell proliferation in a similar way as TCP and revealed chemotactic properties. Both ECM hydrogels modulated the innate immune response and provided the benefit of a stimulatory substrate for in vivo neural tissue regeneration. In conclusion, ECM scaffolds are a promising material for the repair of injured neural tissue. *This project was supported by GAUK 1846214, TG01010135, LO1309 of the MEYS CR.*

F-1465

ORDER AND DISORDER: MODELING NEURAL DEVELOPMENT AND DISEASE USING HUMAN CEREBRAL ORGANOIDS

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The development of the human cerebral cortex is a complex process governed by an array of intrinsic and extrinsic programs. However, the underlying mechanisms regulating the early human cortical development and disorder remain elusive. Here, we adopted a recently reported three-dimensional in vitro culture system to derive cerebral organoids from human pluripotent stem cells. Using this method, we show that human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) differentiate into neural and glial cell types of the cortical lineages, and self-organize into three-dimensional neural tissue with key features of the early developing human cerebral cortex. Combining the human cerebral organoid culture system with CRISPR/Cas9-mediated genome editing, we seek to investigate the molecular mechanisms that regulate the proper development of the cerebral cortex, as well as the pathogenesis of neural developmental disorders. Human cerebral organoids generated from human ESCs bearing loss-of-function allele(s) of the DCX gene allowed us to model aspects of pathological cortical development reminiscent of lissencephaly and double cortex syndromes. Furthermore, by modulating growth factor signaling pathways, we are investigating their influences on the size of the neural progenitor pool, and the effects on cortical development.

F-1466

STIFFNESS CONTROL OF COLLAGEN SCAFFOLDS FOR HUMAN MESENCHYMAL STEM CELL GROWTH AND CARDIAC DIFFERENTIATION

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Mechanical force is known to be a critical regulator of cell behavior, such as growth, migration, and differentiation. Recently, the stiffness of the matrix was identified as an important factor to direct developmental pathways of human mesenchymal stem cells (hMSCs). In this study, 7 mg/ml rat tail-derived type I collagen solution were freeze-dried and cross-linked with two different concentrations of cross-linkers, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS). The Young's modulus of scaffolds was measured by rheometer. After hMSCs seeding on the scaffolds, cell viability was assessed by alamarBlue assay. Subsequently, hMSCs were induced to differentiate into cardiac lineage. Quantitative polymerase chain reaction and immunocytochemistry were used to confirm the cardiac differentiation. In order to observe the interaction between hMSCs and scaffolds, we used the scanning electronic microscope (SEM)

and confocal microscope. The results showed that controlling the concentration of EDC/NHS resulted in collagen scaffolds with two different moduli: 15, and 31 kPa. However, the diameters and thicknesses have no difference between these scaffolds. The alamarBlue results showed that the stiffer scaffolds allow a better viability of hMSCs. Moreover, the induced hMSCs cultured on 31 kPa scaffolds could interact with collagen scaffolds, and express the cardiac specific markers at both of gene and protein level. The overall results suggest that three dimensional type I collagen scaffolds with suitable cross-linking to adjust the stiffness can affect hMSCs fate and direct the differentiation of MSCs into cardiac lineage cells.

F-1467

FUNCTIONAL MESENCHYMAL STEM CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS ARE ABLE TO REPAIR CARTILAGE DEFECTS

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Since its derivation in 2006, induced pluripotent stem cells (iPSCs) have been considered as a possible avenue to achieve an ideal patient-specific cell source for regenerative medicine. Although there have been some reports on iPS-derived mesenchymal stem cells (iPS-MSCs), the most promising stem cells for clinical application, there is no step-wise protocol to fully recapitulate the developmental stages of MSCs, little is known about the signaling and molecular events of MSC development. For clinical use, the negative pluripotent effects of iPSCs can be attenuated by deriving iPS-MSCs before transplantation. We report here a step-wise, highly efficient, serum-free, feeder-free, chemically-defined and reproducible protocol to derive MSCs from human iPSCs through primitive streak and mesoderm. iPSCs undergo epithelial-mesenchmal transition (EMT) to derive MSC. iPS-MSCs lose their pluripotent-associated markers, displayed similar surface antigen profile to bone marrow-derived MSCs showing negative for CD14, CD34, CD45, but positive for CD29, CD44, CD49b, CD73, CD90, CD105, CD151 and CD166. The iPS-MSCs had the ability to differentiate into adipogenic, chondrogenic and osteogenic lineages in vitro. Most importantly, iPS-MSCs were able to fully repair cartilage defects after transplant into cartilage defects for 12 weeks. Histologic analyses demonstrated de novo cartilage repair and regeneration in the defects. Moreover, iPS-MSCs were confirmed to participate in cartilage regeneration in the neo-tissue. These findings demonstrate that iPSCs can offer a functional patient-specific MSC source for personalized regenerative medicine.

F-1468

FORMATION OF VASCULAR NETWORK STRUCTURES WITHIN CARDIAC CELL SHEETS FROM MOUSE EMBRYONIC STEM CELLS

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Bioengineered cardiac tissues represent a promising strategy for regenerative medicine. However, methods of vascularization and suitable cell sources for tissue engineering and regenerative medicine have not yet been established. In this study, we developed methods for the induction of vascular endothelial cells from mouse embryonic stem (ES) cells using three-dimensional (3D) suspension culture, and fabricated cardiac cell sheets with a pre-vascularized structure by co-culture of mouse ES cell-derived endothelial cells. After induction, isolated CD31+ cells expressed several endothelial cell marker genes and exhibited the ability to form vascular network structures similar to CD31+ cells from neonatal mouse heart. Co-culture of ES cell-derived CD31+ cells with ES cell-derived cardiomyocytes and dermal fibroblasts resulted in the formation of cardiac cell sheets with microvascular network formation. In contrast, microvascular network formation was reduced in co-cultures without cardiomyocytes, suggesting that cardiomyocytes within the cell sheet might enhance vascular endothelial cell sprouting. Polymerase chain reaction array analysis revealed that the expression levels of several angiogenesis-related genes, including fibroblast growth factor 1 (FGF1), were up-regulated in co-culture with cardiomyocytes compared with cultures without cardiomyocytes. The microvascular network in the cardiac sheets was attenuated by treatment with anti-FGF1 antibody. These results indicate that 3D suspension culture methods may be used to prepare functional vascular endothelial cells from mouse ES cells, and that cardiomyocyte-mediated paracrine effects might be important for fabricating pre-vascularized cardiac cell sheets.

F-1469

HUMAN NEURAL STEM CELL-MEDIATED TARGETING OF STIMULI RESPONSIVE NANOPARTICLES TO SOLID TUMORS

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Despite the identification of effective tumor-toxic compounds, carriers are needed that selectively target these compounds to tumors so that healthy tissue remains undamaged. While nanoparticle carriers (NPs) succeeded in protecting cargo from rapid clearance and degradation, they still cannot provide the required tumor-selectivity, particularly for marker-negative tumors. Here, we hypothesize that coupling NPs to tumor-tropic neural stem cells (NSCs) can improve NP biodistribution to metastatic triple negative breast cancer. We test this hypothesis utilizing the clinically relevant HB1.F3 neural stem cell (NSC) line that inherently migrates to primary and metastatic breast tumor foci, and can even cross the blood-tumor-barrier (BTB) and penetrate to hypoxic tumor regions.

The NSCs are coupled first with model polystyrene NPs, then with three different stimuli-responsive NP preparations: 1) surface conjugated drug-loaded polymeric nanoparticles, and internalized 2) Gold or 3) Iron Oxide NPs that can thermally ablate tumors upon stimulation with either near infrared light or alternating magnetic field respectively. We provide evidence that NSCs NSCs improve retention and distribution of stimuli-responsive NPs within solid tissue tumors. In all three scenarios, NSC-NP conjugates improve the anti-tumor therapeutic efficacy relative to free NP injections. This research serves as the foundation for translational preclinical studies of major impact.

F-1470

IMPACT OF THE ADIPOSE TISSUE PROCUREMENT AND ADIPOSE STEM CELLS EXPANSION AND DIFFERENTIATION TO OBTAIN A SCAFFOLD-FREE 3D OSTEOGENIC GRAFT

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The reconstruction of a large bone defect using stem cells remains limited by the size of the engineered implant. We assessed the impact of different conditions of native adipose tissue (AT) procurement and adipose stem cells (ASC) expansion/differentiation on the development of a scaffold-free 3D osteogenic autologous ASC to remain compatible with the clinical surgery timing and GMP guidelines. Several conditions were tested: (i) the preservation of native AT for ASCs isolation: deep freezing (-80°C) vs. fresh AT (4°C, n=4); variable quantities of AT (<2g vs. >20g, n=17); blood removal of AT prior digestion (n=3), (ii) the conditions of ASC proliferation (P1 to P3): foetal bovine serum (FBS) vs. human platelet lysate (HPL, n=3); crude trypsin vs. trypLE (n=4) and subculturing at 50/75/100% of confluency (n=4), (iii) the osteogenic differentiation procedure at P4: direct or sequential (n=4), in normo- (21%) vs. hypoxia (5%O2, n=8) and (iv) the 3D production by different combinations of ASC with demineralized bone matrix (DBM, n=25). The impact of each factor on the 3D graft was studied in terms of: ASC proliferation/survival, phenotype expression and osteogenic differentiation capacity (alizarin red, osteocalcin, von Kossa staning). The preservation of AT (prior ASCs isolation), the quantity of harvested tissue, the blood washing, the confluency rate and the type of trypsin did not modify the course of ASC proliferation, the rate of ASC survival, the CD44/CD45/CD73/CD90/CD105 expression and the osteogenic differentiation. A significant reduction (by 59%, $p < 0.05$) of the time, to achieve P4, was obtained for ASC incubated in media supplemented with HPL in comparison to FBS. The direct differentiation reduced significantly (-11%) the time to obtain the osteogenic phenotype ($p < 0.05$). No advantage of hypoxia was demonstrated on both ASC proliferation/differentiation. Finally, the 3D structure was only obtained by a sequencing of ASC pre-differentiation and DBM exposition. The manufacturing of a 3D osteogenic graft (derived from autologous ASCs) can be improved (to be full in line with GMP) for ASC expansion/differentiation following: (i) the preservation of native tissue at -80°C, (ii) the use of free-animal origin HPL and TrypLE and (iii) a direct osteogenic differentiation at P3-P4.

F-1471

EFFECTS OF SYNTHETIC HIGH POLYMERS USED AS MATERIALS OF BLOOD-CONTACTING MEDICAL DEVICES ON THE GENE EXPRESSION PROFILES OF HUMAN MESENCHYMAL STEM CELLS AND HUMAN MONOCYTIC LEUKEMIA CELLS

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In this study, we examined the effects of poly (2-methoxyethyl acrylate) (PMEA) and poly (2-hydroxyethyl methacrylate) (PHEMA) used as materials for blood-contacting medical devices on the gene expression profiles of human mesenchymal stem cells (hMSCs) and a human acute monocytic leukemia cell line THP-1. By spin coat method, polyethyl acrylate or polycarbonate sheets were coated with a polymer mixture that was composed of PMEA and PHEMA at a ratio of 100:0 (PMEA), 75:25 (M75H25), 50:50 (M50H50), 25:75 (M25H75), or 0:100 (PHEMA). After hMSCs and THP-1 cells were cultured on the polymer-coated sheets for 24 hours, DNA microarray experiments and pathway analyses were performed to compare gene expression profiles between the cell culture conditions. The DNA microarray experiments and the subsequent pathway analyses showed that the expression levels of genes associated with "Regulation of the Epithelial-Mesechymal Transition (EMT) Pathway" were significantly changed in hMSCs on the sheets coated with PMEA, M75H25, or M50H50, compared with those on the non-coated sheets. The pathway analyses also indicated that the expression levels of genes associated with TGF-β, FGF and EGF receptors were significantly altered in hMSCs cultured on the sheets coated with PMEA, M75H25, M50H50, or M25H75. Furthermore, the expression levels of genes associated with Notch signaling was also changed in hMSCs cultured on the PMEA-coated sheets. As TGF-β, Notch, Wnt, and tyrosine kinase play key roles in the initiation of EMT, our findings suggest that PMEA affects the stemness and cellular motility of hMSCs through EMT/MET by modulating the multiple signaling pathways. In contrast, hierarchical clustering analyses for the gene expression profiles of THP-1 cells cultured on the polymer-coated sheets indicated that the potency order of the gene modulatory effects of the polymers was PMEA> PHEMA> M25H75> M75H25 and M50H50. Among ontology clusters of physiological system development and function, "Hematological System Development and Function" and "Organismal Survival" showed the most significant changes in gene expression by PMEA and PHEMA coatings, respectively. PMEA and PHEMA showed effects opposite to each other on a number of genes associated with diseases and biological functions.

F-1472

DERIVATION OF CRISPR-CAS9 ENGINEERED RETINAL ORGANOIDS FROM HUMAN EMBRYONIC STEM CELLS FOR RETINAL REPAIR

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Stem cell therapy of retinal degeneration (RD) is a promising approach to ameliorate blindness yet it has several critical limitations. Photoreceptor (PR) replacement strategies need to add thousands of PRs, integrated specifically into the synaptic circuitry of the degenerating retina to alleviate blindness. To address these limitations, we derived 3D retina-like tissue from human embryonic stem cell (hESC) line WA01 (H1). We extended our published retinal differentiation protocol with noggin, FGF9, Dkk-1 and IGF-1 to derive 3D retina-like structures from hESCs and achieved generating reproducible pattern of 3D organization of primitive human retina-like tissue grown in adherent monolayer cultures in Neurobasal medium with N2 and B27 supplements. While both the retinal organoids (0- >6weeks) and multipotential retinal progenitors (mRPC) expanded according to our original protocol (0->12 weeks) demonstrated the robust upregulation of anterior neuroectoderm (OTX2, FOXG1) and eyefield markers PAX6, RAX, LHX2, SIX6, retinal organoids but not retinal progenitor cell showed strong upregulation of another eyefield marker SIX3, and typical retina-like organization. We find 3D growth of retina-like organoids in adherent monolayers, rather than retinospheres in suspension, allows to achieve flexible and long stretch of 3D retina-like tissue suitable for subretinal transplantation, while not compromising the dynamics of retinal maturation. All key neural retina and retinal pigment epithelium (RPE) markers typical for retinal cell lineages were found in a 6-week old 3D retinal organoids. We are doing bisulfite pyrosequencing to determine the percentages of 5-methylocytosine compared to unmethylated cytosine at each CG dinucleotide position in selected retina-specific genes as well as repetitive loci and using human retinal tissue as control to compare the state of retina-like tissue maturation to that of adult human retina. 3D retina-like tissue demonstrates striking similarity to fetal retina in the ability to produce stratified rows of early PRs, maturing second order INL neurons with dendritic arbors, developed processes and evidence of synaptogenesis, making it a suitable tissue for testing in subretinal grafting experiments aimed at treating blindness.

F-1473

TISSUE ENGINEERED CELL SHEETS FOR PREVENTION OF ESOPHAGEAL STRICTURES IN PATIENTS UNDERGOING ENDOSCOPIC INTERVENTIONS

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Superficial (pre-) cancerous lesions of the esophagus can effectively be removed using endoscopic interventions. However, the high incidence of stricture development after such therapy remains a major clinical concern. We have previously shown that cell sheets grown from autologous oral mucosa promotes esophageal healing in a Japanese patient cohort. The aim of this study was to transfer the technology and prove safety and feasibility in a European setting. Oral biopsies from patients (n=7) were enzymatically digested to create single-cell suspensions which were then seeded into thermoresponsive cell culture inserts. After 16 days, temperature reduction was used to harvest intact cell sheets, consisting of 4-5 layers of epithelial cells. In contrast to enzymatic harvesting, our technique enables retention of several extracellular proteins. Using electron microscopy and immunohistochemistry we found the bioactive proteins collagen, elastin, laminin and gap junction proteins present in the harvested cell sheets. Interestingly, we also found that the cell sheets exhibited pluripotency markers on gene- and protein level. The cells remained highly viable, metabolically active and proliferative after harvesting. Cell sheets (2-7 / patient) were then transplanted in an autologous fashion to patients after mucosal resection of Barrett's esophagus, adeno- or squamous cell dysplasia. The cell sheets readily adhered to the wound bed, without need of sutures or tissue glue. The resections stretched from 75-100% of the esophageal circumference and we found no adverse effects related to oral biopsies or transplantation of cell sheets. Although the aim of the study was to prove safety and feasibility, our findings suggest that cell sheet transplantation after endoscopic resections reduced incidence and severity of esophageal strictures. Further studies are ongoing to accurately evaluate the effect of cell sheet transplantations.

F-1474

HIPS DERIVED MESENCHYMAL CELLS - A NOVEL CELL SOURCE FOR LUNG TISSUE ENGINEERING

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Recent advances in whole lung regeneration provide unprecedented possibilities for treatment of lung diseases. Perhaps one of the most important hurdles that remain is the identification of cells with the capacity for long-term self-renewal and differentiation that can be reliably utilized for lung regeneration. The aim of the current study is to evaluate the utility of hiPS and hES derived mesenchymal cells (MSCs) to repopulate decellularized lung tissue. We have derived multiple MSC lines from hES and hiPS cells. The mesenchymal identity of derived cells was confirmed by flow cytometry (positive expression of CD90, CD73, CD105, negative expression of CD45, CD34), as well as successful differentiation into osteogenic, chondrogenic and adipogenic lineages. Further, cells were seeded on slices of decellularized rat lung in specialized epithelial growth media such as SAGM (small airway growth medium). In order to understand the progression of differentiation, reseeded lung slices were tested at day 1 and day 7 for various epithelial marker expression. Our preliminary data suggest a mesenchymal to epithelial differentiation in the presence of the microenvironment of the decellularized lung scaffold. Histological analyses revealed distinct cellular cuboidal morphologies. Epithelial marker expression was confirmed by PCR, and immunostaining for CCSP, and Pro-SPC. We will also perform PCNA and Tunel analysis to determine the state of the cells. We will utilize TEM to investigate ultra structures in the cells such as presence of lamellar bodies that are characteristic of type II epithelial cells. Further studies are underway to understand specific anatomical locations that cells bind preferentially and the involvement of signaling pathways that promote this differentiation. Results and conclusions from these studies have the potential to

lead to several key findings. They will identify a possible progenitor population of mesenchymal phenotype that is capable of epithelial differentiation thus providing new insights into the role of MSCs in the context of lung regeneration. More importantly, these cells could possibly form a novel renewable cell source that might aid in repopulating decellularised lungs and thus aid the field of lung tissue engineering.

F-1475

A NOVEL ADULT STEM CELL BASED 3-D DE NOVO VASCULARIZED CARDIAC MUSCLE CONSTRUCT FOR TREATMENT OF ISCHEMIC HEART DISEASES

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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. Engineering a tissue of clinically relevant magnitude requires the formation of extensive and stable microvascular networks within the tissue. Since most in vitro engineered tissue constructs do not contain the intricate microvascular structures of native tissue, the cells contained in scaffolds heavily rely on simple diffusion for oxygenation. In addition, the interaction of the cells of the host and construct has not been well characterized. The aim of this current study is to develop a 3-D model of vascularized cardiac tissue to study 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 this, we have developed an in vitro model of three-dimensional (3-D) vascularized cardiac muscle construct using ventricular embryonic cardiac myocytes (ECMs) and adipose-derived mesenchymal stem cells (MSCs). To generate the prevascularized scaffold, human cardiac microvascular endothelial cells (HMVEC-C) and MSCs were co-cultured on a 3-D collagen cell carrier (CCC) for 7 days, HMVEC-C underwent maturation and differentiation characteristic of microvessel morphogenesis. Next, the ECMs and MSCs were co-cultured onto this generated prevascularized CCCs for further 7 or 14 days. The phenotypic inductions were analyzed at the morphological, immunological, biochemical and molecular levels. The observed expression of transcripts coding for cardio myocyte phenotypic markers and the immunolocalization of cardiomyogenic lineage-associated proteins revealed typical expression patterns of neo-cardiomyogenesis. Our unique 3-D co-culture system provides an in vitro model and a prospect to elucidate various molecular mechanisms underpinning the integration and orderly maturation and differentiation of MSCs into neo-cardio myocytes in the context of postnatal de novo vasculogenesis during myocardial regeneration.

F-1476

SUB-REGIONAL DIFFERENCES IN METABOLIC ACTIVITY OF HUMAN AMNIOTIC MEMBRANE - A RICH SOURCE OF STEM CELLS

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The human amniotic membrane (hAM), the innermost fetal membrane, contains cells with stem cell characteristics with low or no immunogenicity, making it a suitable material for tissue engineering. For clinical application, profound knowledge of properties, differentiation capacity and quality of the applied material is a prerequisite. In previous studies, we have shown that hAM can be differentiated towards osteogenic, chondrogenic and Schwann cell-like lineages. Differentiation is a highly energy-consuming process. However, the hAM can be partitioned in different sub-regions, giving rise to the inevitable question, whether these sub-regions show differences in morphology and energy metabolism, particularly local distribution of mitochondrial quality and activity. Histological sections from placenta-associated and non-placental regions were stained with haematoxylin/eosin. Mitochondrial respiration was monitored by high resolution respirometry, Oroboros Instruments. Samples were incubated in DMEM+0.1% BSA and substrates/inhibitors of the electron transfer chain were applied. Epithelial cells of the placental region appear cylindrical with decentralized nuclei and cytoplasmic sub-compartments, whereas epithelial cells of the non-placental region show a rather flattened, homogenous morphology. The placental region shows a 4-fold higher mitochondrial respiration compared to the non-placental region. Interestingly, respiratory control ratio show no differences, reflecting similar quality of mitochondria in both regions, suggesting higher numbers of mitochondria in the placental region. Thus, the latter seems to be more capable in terms of energy production, but simultaneously also more oxygen-dependent than the non-placental region. The two different amniotic regions, placental and non-placental, despite being the same cell type, show distinct differences in morphology and mitochondrial activity. Since the latter is closely related to cell type and cell function, the obvious question is whether regional differences reflect different cellular functions. If so, may this also play a role in functionality, differential potential and cell fate, and may hence impact therapeutic properties when applied in different clinical settings.

F-1477

EFFECTS OF COMBINATORIAL TREATMENT OF TRIIODOTHYRONINE, ELECTRICAL STIMULATION AND ALIGNMENT-INDUCED ANISOTROPY ON THE MATURATION OF HUMAN PLURIPOTENT STEM CELL DERIVED-CARDIOMYOCYTES

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Human pluripotent stem cell (PSC) such as human embryonic stem cell (hESC) and induced PSC (iPSC) represent a promising unlimited source of cardiomyocytes (CMs) for therapeutic, disease modeling and other applications. However, hESC-CMs are both functionally and structurally immature. Here, we hypothesized a combination of our previously identified pro-maturation stimuli, namely triiodothyronine (T3), electrical stimulation (ES) and alignment-induced anisotropy would synergistically promote an even more mature electrophysiological phenotype. Microgroove substrates were prepared by molding shrink film on PDMS stamps and hESC-CMs were differentiated using a highly efficient ventricular specification protocol. The resultant hESC-CM monolayer would be treated with T3, the active form of thyroid hormone, followed by a step-wise electrical field conditioning protocol. High-resolution optical mapping was conducted to study action potential conduction properties such as the anisotropic ratio (AR), transverse (TCV) and longitudinal conduction velocities (LCV). A significantly higher AR was seen in hESC-CM monolayer on microgroove substrate when compared to that of random control group, as a result of reduced TCV and increased LCV in the alignment-induced group. Interestingly, electrical field stimulation selectively increased LCV in more so than TCV in both microgroove and random control group, contributing to the increased ARs in both groups compared to controls without ES. The combination of microgroove substrate, chronic T3 treatment and ES could further promote the AR closer to the physiological level. Additionally, immunofluorescence showed that T3 and electrical conditioning could promote cellular alignment and CM maturation. We conclude that structural and functional maturation is best achieved by a combination of but not single stimuli.

F-1478

HUMAN AMNIOTIC EPITHELIAL CELLS INHIBIT GRANULOSA CELL APOPTOSIS INDUCED BY CHEMOTHERAPY AND RESTORE THE FERTILITY

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Premature ovarian failure and insufficiency (POF/POI) represent significant repercussions of chemotherapeutic intervention for female cancer patients. Recently, stem cells transplantation has been identified as a promising treatment for POF/POI. Our previous investigation demonstrated that human amniotic epithelial cells (hAECs) can migrate into injured tissue and promote the recovery of ovarian function in chemoablated mice. However, the molecular mechanism guiding this process remains unclear. To further investigate the effect of hAECs on chemotherapy-induced apoptosis, cultured primary hAECs were injected into a POF/POI mouse model. hAECs significantly inhibited tumor necrosis factor-alpha (TNF-α)-mediated granulosa cells (GC) apoptosis induced by chemotherapeutics and reduced the inflammatory reaction in ovaries at 7 days after transplantation. In addition, 4 weeks post-transplantation, hAECs promoted the development of follicles and increased the number of cumulus oocyte complexes (COC) in chemoablated mice getted by superovulation. And hAECs improved ovary weight and increased the number of varied stage of follicles compared with chemoablated group. Furthermore, grafted hAECs partially rescued the fertility of chemoablated mice. hAECs transplantation aids the restoration of ovarian function by inhibiting TNF-α-mediated cell apoptosis and reducing the inflammatory reaction in chemotherapy-induced premature ovarian failure. These results suggest a potential molecular mechanism for the effective therapy of hAECs transplantation in chemotherapy induced POF/POI.

F-1479

MESENCHYMAL STEM CELLS ATTENUATED PLGA-INDUCED INFLAMMATORY RESPONSES BY INHIBITING HOST DC MATURATION AND FUNCTION

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The poly lactic-co-glycolic acid (PLGA) bio-scaffold is a biodegradable scaffold commonly used for tissue repair. However, implanted PLGA scaffolds usually cause serious inflammatory responses around grafts. To improve PLGA scaffold-based tissue repair, it is important to control the PLGA-mediated inflammatory responses. Recent evidence indicated that PLGA induce dendritic cell (DC) maturation in vitro, which may initiate host immune responses. In the present study, we explored the modulatory effects of mesenchymal stem cells (MSC) on PLGA-induced DCs (PLGA-DC). We found that mouse MSCs inhibited PLGA-DC dendrite formation, as well as co-stimulatory molecule and pro-inflammatory factor expression. Functionally, MSC-educated PLGA-DCs promoted Th2 and regulatory T cell differentiation but suppressed Th1 and Th17 cell differentiation. Mechanistically, we determined that PLGA elicited DC maturation via inducing phosphorylation of p38/MAPK and ERK/MAPK pathway proteins in DCs. Moreover, MSCs suppressed PLGA-DCs by partially inactivating those pathways. Most importantly, we found that the MSCs were capable of suppressing DC maturation and immune function in vivo. Also, the proportion of mature DCs in the mice that received MSC-PLGA constructs greatly decreased compared with that of their PLGA-film implantation counterparts. Additionally, MSCs co-delivery increased regulatory T and Th2 cells but decreased the Th1 and Th17 cell numbers in the host spleens. Histological analysis showed that MSCs alleviated the inflammatory responses around the grafted PLGA scaffolds. In summary, our findings reveal a novel function for MSCs in suppressing PLGA-induced host inflammatory response and suggest that DCs are a new cellular target in improving PLGA scaffold-based tissue repair.

F-1480

BRIDGING SCIATIC NERVE DEFECTS IN BEAGLES BY NEURONAL TISSUE ENGINEERED NERVE GRAFTS

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Tissue-engineered nerve grafts (TENGs), typically composed of neural scaffolds and support cells and/or growth factors, are considered as a potential alternative to autologous nerve grafts used for peripheral nerve repair. In the present study, we developed a new-designed TENGs, neuronal TENGs, which were constructed by acellular nerve graft seeded with neurons and schwann cells, followed by bridging long-distance gap in beagles sciatic nerves. A set of behavioral, functional, and histological examinations were carried out to evaluate the effects of neuronal TENGs on nerve regeneration and functional recovery of sciatic nerve defects in beagles. Neural tissue-committed stem cells from bone marrow were cultured and identified, and then were induced into neurons and schwann cells respectively. Neuronal TENGs were constructed by acellular nerve scaffold seeded neurons and schwann cells at a 1:1 ratio. A 65-mm sciatic nerve defect was created on the right hind limb in beagle sciatic nerve and bridged with neuronal TENGs.Schwann cells TENGs, which composed of acellular nerve scaffold seeded schwann cells only and autologous nerve grafts as control. The results showed average time of recovery of motor function was at 17 weeks in neuronal TENG group, which was earlier than the other two groups. Compound muscle action potentials, general morphology observation and Bradford protein assay in the gastrocnemius showed gastrocnemius atrophy in neuronal TENGs group was the lightest among the three groups. immunohistochemical staining, Toluidine blue staining also showed the quality of nerve fibers in neuronal TENGs group was better than the other two groups. At 28 weeks after neuronal TENGs implantation, both retrograde tracing and immunohistochemistry staining showed the implanted neurons and schwann cells still alive. By using transmission electron microscope, synaptic structures were observed in neuronal TENGs group and synapsin 1, was found by using immunohistochemistry staining. The recovery of nerve function in neuronal TENGs group was faster than the other two groups. Neurons in neuronal TENGs can survive and improve nerve regeneration, and neuronal TENGs may provide an alternative way to repair peripheral nerve defects.

REGENERATION MECHANISMS

F-1481

THE CARDIOPROTECTIVE ROLE OF THE AMNIOTIC FLUID STEM CELL SECRETOME IN A DOXORUBICIN-INDUCED CARDIOTOXICITY MODEL

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Doxorubicin (Dox) is an antineoplastic drug used for tumours such as lymphoma and breast cancer. Unfortunately, its clinical application is hampered by progressive cardiotoxicity leading to irreversible cardiomyopathy following chemotherapy. Despite several efforts being made to improve treatment, Dox-induced cardiomyopathy (DIC) remains a major health issue. Thus, accurate identification of novel therapeutic strategies is highly demanded. Human amniotic fluid stem cells (hAFS) have been described as immature progenitors with a distinct proteomic profile and paracrine potential. The aim of this study is to assess whether the hAFS secretome may protect cardiomyocytes against Dox toxicity. c-kit+ hAFS were isolated from leftover samples of prenatal diagnostic amniocenteses and cultured in normoxia (20% O_2) versus hypoxia (1% O_2) for 24h in serum-free medium to obtain the cell-conditioned medium (hAFS-CM) and stimulate the release of paracrine factors. Rat H9c2 cardioblasts were purchased from ATCC. Mouse neonatal cardiomyocytes (mnCM) were isolated from 1-2 days-old pups by enzymatic digestion. These cells were treated for 3 hours with the hAFS-CM and then incubated with pro-senescent (0.1uM) and pro-apoptotic (1uM) doses of Dox for 3 and 24 hours, respectively. Cell senescence and apoptosis were evaluated by β-galactosidase and cleaved caspase-3 staining. hAFS-CM antagonized the Dox-related senescent phenotype in H9c2 cardioblasts: normoxic hAFS-CM decreased the amount of β-galactosidase-positive cells by 39.5%, while the hypoxic hAFS-CM further lowered it down by about 51%. As well, the normoxic and hypoxic hAFS-CM reduced Dox-induced apoptosis of H9c2 cells by 30% and 50%, correspondingly. These results were confirmed on primary mnCM, with the hAFS scretome from cells stimulated by hypoxia being able to significantly decrease dox-induced senescent and apoptotic effects by 47% and 42%, respectively. Here we show for the first time that the hAFS secretome can limit Dox-derived cardiotoxicity by inhibiting cellular senescence and apoptosis. These findings open new perspectives by identifying DIC as a novel target disease for stem cell-based therapy while expanding the knowledge about the cardioprotective effects of the hAFS secretome. Bollini S. and Ameri P. equally contributed to this work.

F-1482

COMBINING MESENCHYMAL STROMAL CELL INFUSIONS WITH PULSED FOCUSED ULTRASOUND TO KIDNEYS INCREASES MSC PRODUCTION OF INTERLEUKIN 10 AND IMPROVES ACUTE KIDNEY INJURY

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Pulsed focused ultrasound (pFUS) enhances homing of iv mesenchymal stromal cells (MSC) to murine kidneys during cisplatin (CIS)-induced acute kidney injury (AKI). Furthermore, pFUS+MSC therapy improves prevention and enhances rescues AKI compared to MSC therapy alone. This study examined potential mechanisms for pFUS+MSC to treat AKI. C3H mice received CIS (15 mg/kg ip), kidney pFUS (4 MPa; 5% duty cycle) and/or MSC (106 human MSC iv). Groups included AKI only, AKI+pFUS, AKI+MSC, AKI+pFUS+MSC, and normal mice. Mice received CIS on Day (D) 0 and pFUS/MSC on D1. Mice were euthanized on D2 for molecular/cellular analyses and immune cell profiling. Other mice were euthanized on D4 to measure blood urea nitrogen (BUN), serum creatinine (SCr), Ki67, pAKT, and CD31. pFUS stimulated a molecular response in AKI kidneys consisting of numerous chemoattractants and interferon-γ (INFγ). pFUS increased MSC homing to AKI kidneys increased by ~3. pFUS+MSC-treated kidneys had significantly more human interleukin (IL)-10 compared to kidneys receiving MSC

alone ($p<0.001$). Consequently, tumor necrosis factor-α decreased and vascular endothelial growth factor increased in kidneys treated with pFUS+MSC compared to MSC alone ($p<0.01$). pFUS+MSC reduced the number of natural killer (NK) cells and proinflammatory dendritic cells (DC). pFUS+MSC increased cell proliferation markers Ki67 and pAKT and increased CD31 (vessel density) ($p<0.001$). By D4, pFUS+MSC lowered BUN and SCr compared to MSC alone ($p<0.05$). pFUS creates a "molecular zip code" to enhance homing of infused MSC where they produce IL-10. IL-10 has been shown to ameliorate AKI by reducing molecular and cellular inflammation. Stimulating MSC with INFγ increases IL-10 production and therapeutic effectiveness. Greater IL-10 seen with pFUS+MSC could be the result of potential stimulation of MSC by endogenous INFγ upregulated by pFUS. While this potential link requires additional investigation, the increased IL-10 leads to an anti-inflammatory immune cell profile, increased revascularization, increased expression of regenerative signals, and ultimately improved renal function. pFUS is a viable modality to improve MSC therapy during established AKI, which often has limited therapeutic options clinically.

F-1483

NRIP ACTIVATES SATELLITE CELLS FOR SKELETAL MUSCLE REGENERATION

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NRIP (nuclear receptor interaction protein) is a Ca^{2+} dependent calmodulin binding protein that can activate its downstream protein, calcineurin. Due to calmodulin can activate calcineurin-NFAT and CaMKII pathways those involve in muscle specific gene expression during muscle regeneration. We currently characterize that NRIP deficiency would delay muscle regeneration capacity using conventional NRIP knock out (KO) mice by revealing lower centralized nuclei and small myofiber size distribution compared to WT. Therefore, we would like to know whether NRIP could activate satellite cells' activation and proliferation. We examined and compared the expression patterns of Pax7 and MyoD between WT and NRIP KO mice at day 1, 3 and 6 after muscle cardiotoxin (CTX) injection by western blot and tissues immunofluorescence assay. The amount of NRIP can be enhanced after muscle injury and reached the peak at day 6, afterward NRIP expression equals to normal status. The Pax7 and MyoD expression is coupled increased to the peak at day 3 and then decreased in WT. However, the highest amount Pax7 and MyoD expression of KO mice is at day 6. IFA assays also reveal the less number of Pax7 and $Pax7^+BrdU^+$ cells in NRIP KO mice at day 3 post injury than WT. Additionally, the number MyoD and $MyoD^+BrdU^+$ cells is less in KO than WT mice at day 3 post injury, but NRIP KO mice at day 6 post injury would increase $MyoD^+BrdU^+$ cells. Besides, the number of $MyoD^+Pax7^+$ cells those indicate satellite cell activation in WT mice is higher than NRIP KO mice. Taken together, the delayed regeneration capacity of NRIP KO mice may derive from the late expression of Pax7 and MyoD resulted in postpone the speed of muscle regeneration.

F-1484

THE PERIPHERAL CHIMERISM OF BONE MARROW-DERIVED STEM CELLS AND REGENERATION OF TISSUES IN LETHALLY-IRRADIATED MICE

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Bone marrow-derived cells represent a heterogeneous cell population containing haematopoietic stem and progenitor cells. These cells have been identified as potential candidates for use in cell therapy for the regeneration of damaged tissues caused by trauma, degenerative diseases, ischemia and inflammation or cancer treatment. In our study, we examined a model using whole-body irradiation and the transplantation of bone marrow or haematopoietic stem cells (HSCs) to study the repair of haematopoiesis, extramedullary haematopoiesis and the migration of GFP^+ transplanted cells into non-haematopoietic tissues. We investigated the repair of damage to the bone marrow, peripheral blood, spleen and thymus and assessed the ability of this treatment to induce the entry of bone marrow cells or $GFP^+lin^-Sca\text{-}1^+$ cells into non-haematopoietic tissues. The transplantation of bone marrow cells or $GFP^+lin^-Sca\text{-}1^+$ cells from green fluorescent protein (GFP) transgenic mice successfully repopulated haematopoiesis and the haematopoietic niche in haematopoietic tissues, specifically the bone marrow (BM), spleen and thymus. The transplanted GFP^+ cells also entered the gastrointestinal tract (GIT) following whole-body irradiation. Our results demonstrate that whole-body irradiation does not significantly alter the integrity of tissues such as those in the small intestine and liver. Whole-body irradiation also induced myeloablation and chimerism in tissues, and induced the entry of transplanted cells into the small intestine and liver. This result demonstrates that grafted bone marrow cells or $GFP^+lin^-Sca\text{-}1^+$ cells are not transient in the GIT. Thus, these transplanted cells could be used for the long-term treatment of various pathologies or as a one-time treatment option if myeloablation-induced chimerism alone is not sufficient to induce the entry of transplanted cells into non-haematopoietic tissues. *This work was supported by grant GACR No 15-09161S.*

F-1485

CONNECTIVE TISSUE GROWTH FACTOR IS AN INDUCIBLE EXTRINSIC REGULATOR OF HEMATOPOIETIC STEM CELL CYCLE PROGRESSION

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We studied hematopoietic "stress" responses through the analysis of gene expression changes in an in-vitro model of regenerative stress: co-cultures of HSC-supportive UG26-1B6 stromal cells in contact with lineage- Sca-1+ Kit+ (LSK) cells. Analysis of stromal cells shows that LSK cells promote bone and tissue remodeling. Gene prioritization identified stromal connective tissue growth factor (Ctgf) to be induced by the presence of LSK cells. To validate this finding, we confirmed Ctgf induction in the bone marrow after total body irradiation. In experiments to find out whether HSC activity is modulated by Ctgf, LSK cells co-cultured on shCtgf-UG26-1B6 were found to show severely diminished long-term engraftment, particularly of the myeloid lineage, including myeloid progenitors and LSK cells. Defective engraftment was further confirmed in serial transplantations. To understand how extracellular Ctgf affects intracellular signaling, we validated a Ctgf signaling network model by sorting LSK cells from co-cultures with Ctgf deficient stroma. Loss of stromal Ctgf strongly increases Smad2/3 activation and inhibited Akt and canonical Wnt signaling, through upregulation of Pten and Gsk3b in LSK cells. Concomitantly, p27 (Cdkn1b) was increased, and decreased expression Cyclin D1 and E2f1 correlated with an expanded fraction of LSK cells in G0/G1. Conversely, single cell cultures in conditioned media of shCtgf stromal cells showed delayed cell cycle entry of CD34- CD150+ LSK (LT-LSK) cells which was rescued by addition of recombinant CTGF. interestingly, mature CD34+ LSK cells were not affected by changes in CTGF, showing that CTGF acts on the HSC-enriched LT-LSK cells. Our study represents the first systematic characterization of how stromal cells regulate the initial stages stem cell cell cycle recruitment and shows that rapid upregulation of Ctgf under these conditions is required for maintenance of long-term repopulating ability.

F-1486

SYSTEMIC ADMINISTRATION OF HMGB1 AMELIORATES BLEOMYCIN-INDUCED TISSUE FIBROSIS BY PROMOTING LESIONAL ACCUMULATION OF MESENCHYMAL STEM CELLS FROM BONE MARROW

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Bone marrow-derived cultured mesenchymal stem cells (MSCs) have been documented to show a variety of bioactivities, such as anti-inflammatory and regeneration-inducing activities, when inoculated into the injured tissues. However, the role of endogenous MSCs in bone marrow in tissue regeneration processes is still unclear. Previously, we reported that necrotic epithelia-derived HMGB1 (high mobility group box protein 1) in serum stimulated platelet-derived growth factor receptor α-positive (PDGFRα+) MSCs in bone marrow to mobilize into the circulation in the mouse skin graft model. The HMGB1-induced circulating MSCs were then shown to accumulate to the skin graft via SDF-1/CXCR4 axis, and then suppressed inflammation by releasing anti-inflammatory molecules including TSG-6 and induced regeneration of the injured skin graft by differentiating to the skin fibroblasts and keratinocytes. We further hypothesized that HMGB1-induced MSC mobilization may also ameliorate the bleomysin (BLM)-induced skin fibrosis by inducing accumulation of MSCs in the lesion. To attest this hypothesis, we injected BLM under the dorsal skin of C57/BL6 mice fifth a week for four weeks, and during the period, we injected HMGB1 intravenously twice a week for four weeks. As a result, BLM-induced skin fibrosis was significantly declined in the mice group treated with HMGB1 as compared to control group. The same result was obtained in the skin of the mice with prior green fluorescence protein (GFP)-transgenic bone marrow transplantation (GFP-BMT) following lethal dose irradiation, accompanied with significant accumulation of GFP+/PDGFRα+ MSCs in the lesion. Depletion of the PDGFRα+ MSCs in the BMT resulted in attenuation of the anti-fibrotic activities of HMGB1 administration, suggesting a pivotal role of the accumulated PDGFRα+ MSCs in the lesion for the anti-fibrotic activity. These results suggest HMGB1 as a medicine for fibrotic diseases, such as systemic sclerosis as well as scleroderma.

F-1487

DIFFERENTIAL ABILITIES OF DERMAL STEM CELLS DERIVED SCHWANN CELLS TO SUPPORT AXONAL REGENERATION AND REMYELINATION

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Schwann cells (SCs) play a key role in supporting axonal regeneration and remyelination following a peripheral nerve injury. It is well known that outcomes following delayed nerve repair are poorer. Data suggests that in the chronically denervated nerve, SCs progressively lose their capacity to support axonal regeneration and may be less robust for remyelination. We hypothesized that recapitulating the early denervation phenotype of SCs in chronic denervation may restore remyelination and regeneration support capacity. In this study, we compared SCs from adult rodent sciatic nerve with acute and chronic denervation, adult rodent dermal stem cells derived precursor SCs (aSKP-SCs), and nerve derived SCs from E16 embryonic nerve. SCs re-express key pro-myelinating transcription factors (Oct-6 and Krox-20) following acute (day 5) nerve injury, but lose this phenotype with chronic denervation (day 56) both in vivo and in cultured nerve SCs in vitro. We found that aSKP-SCs express Oct-6 and Krox-20, in vitro, to similar levels as the ones from acutely denervated nerve and significantly greatly than ones from chronically denervated nerve. We next tested and compared the various SCs for myelination both in vitro and in vivo and neurite outgrowth assay (DRG-SCs co-culture) in vitro. Additionally we compared aSKP-SCs and SCs for cellular proliferation. Adult SKP-SCs were comparable to acutely denervated nerve SCs or embryonic nerve SCs in terms of proliferation, survival in injured nerve, in vitro and in vivo myelination, and in vitro neurite outgrowth. Chronically denervated SCs were significantly poorer in all these capabilities. From this study we conclude that: 1) temporal delay following injury results in important phenotypic changes in distal Schwann cells within the nerve and 2) adult SKP-SCs can be used as an alternate therapy to restore myelination and promote axonal regeneration, in injured peripheral nerve, making these cells a favorable source of autologous cell transplantation.

F-1488

MAXIMAL STAT5 ACTIVATION IN THE DERMAL PAPILLA IMPROVES HAIR INDUCTIVE POTENTIAL AND IS ASSOCIATED WITH THE TRANSITION FROM THE RESTING TO THE GROWTH PHASE OF THE HAIR FOLLICLE

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Hair follicles are skin appendages that undergo periods of growth (anagen), regression (catagen) and rest (telogen) regulated by a mesenchymal component the dermal papilla (DP). Expression array analyses of the DP and STAT5 knockout studies suggest that STAT5 may play an important role in the regulation of hair follicle cycling by the DP. We aimed to investigate STAT5 activation within the DP during hair follicle development and cycling. We detected no activation of STAT5, identified by anti-Phospho-STAT5 staining, during embryonic and postnatal hair follicle development until P19, the first telogen. From that point and in adulthood STAT5 activation was almost exclusively identified in the DP, beginning in mid telogen and persisting until early anagen. Staining intensity increased from mid telogen through to early anagen. SOCS2, a downstream STAT5 transcriptional target, showed expression corresponding to the chronology of STAT5 activity. Skin derived precursors (SKPs) cultured from neonatal DPs and transduced with constitutively-active STAT5A or B adenovirus constructs were significantly better at forming hair follicle DP than control SKPs transduced with GFP alone. In contrast, STAT5A/B deletion in SKPs resulted in a significant reduction of new hair in regeneration assays. Conditional knock-out of STAT5A and STAT5B at P19 using Sox18-Cre/ER X STAT5A/B lox/lox mice resulted in the loss of STAT5 in all back skin hair type except guard and awl hairs (Sox2 dependent). This did not result in a significant change in hair follicle types compared to controls. Upon crossing with topflash mice, a canonical Wnt reporter mouse driving luciferase expression, conditional STAT5KO in the DP resulted in delayed anagen entry as characterised by a significant delay in bioluminescence. Microarray analysis of SKP transduced with constitutive active STAT5 versus STAT5KO or controls revealed key changes in BMP signalling possibly explaining this phenotype. We conclude that STAT5 activation may act as a mesenchymal switch to improve hair inductive potential and induce natural anagen entry following the first hair follicle cycle.

F-1489

EPENDYMAL CELLS HAVE HIGHER STEM CELL POTENTIAL IN JUVENILE MICE THAN ADULTS: IMPLICATION FOR SPINAL CORD INJURY

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The discovery of stem cells has raised great hope for regenerative medicine. It has been shown that stem cells transplantation can promote functional recovery in several diseases including spinal cord injury (SCI). However several limitations are associated with transplantation-based approaches for clinical use. Therefore recruitment of endogenous stem cells represents an attractive non-invasive alternative. Recently, we have shown that stem cell potential is confined to ependymal cells in the adult spinal cord. However, the knowledge about these cells during development is poorly understood. Using FoxJ1-YFP non-inducible transgenic line, we have studied ependymal cells genesis during development. We found, based on the expression of FoxJ1, that the first ependymal cells appeared at E16.5 and form all the central canal by birth time. We then studied the effect of age on the stem cell potential of ependymal cells. Using FoxJ1-CreERT2:YFP transgenic line, we show that self-renewal capacity of ependymal cells declined over time from P2 to P21 to adult mice in vitro by the neurosphere assay. However, this potential can be reactivated after SCI. Indeed, P21 neurospheres after SCI mimicked the self-renewal capacity of P2 neurospheres, and generate more oligodendrocytes significantly upon differentiation assay compared to those derived from adult mice. We also demonstrate that SCI activated ependymal cells in vivo in P21 mice in a similar way as in adults and we are currently investigating the difference of these cells' contribution to the scar in P21 mice and adults. Altogether, we here describe the generation of ependymal cells during spinal cord development and we also demonstrate that all stem cell potential is already confined within this cell population by birth. Moreover, we show that juvenile animals arbour a higher stem cell potential than adults which suggest that ependymal cells could be more efficiently targeted in order to improve regeneration upon SCI in young human adult.

F-1490

CD34+/CD45-DIM STEM CELL MOBILIZATION BY HYPERBARIC OXYGEN - CHANGES WITH OXYGEN DOSAGE

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Because hyperbaric oxygen treatment mobilizes bone marrow derived-stem/progenitor cells by a free radical-mediated mechanism, we hypothesized that there may be differences in mobilization efficiency based on exposure to different oxygen partial pressures. Blood from twenty consecutive patients was obtained before and after the 1st, 10th and 20th treatment at two clinical centers using protocols involving exposures to oxygen at either 2.0 or 2.5 atmospheres absolute (ATA). Post-treatment values of CD34 +, CD45-dim leukocytes were always 2-fold greater than the pre-treatment values for both protocols. Values for those treated at 2.5 ATA were significantly greater than those treated at 2.0 ATA by factors of 1.9 to 3-fold after the 10th and before and after the 20th treatments. Intracellular content of hypoxia inducible factors − 1, − 2, and − 3, thioredoxin-1 and poly-ADP-ribose polymerase assessed in permeabilized CD34 + cells with fluorophore-conjugated antibodies were twice as high in all post- versus pre-treatment samples with no significant differences between 2.0 and 2.5 ATA protocols. We conclude that putative progenitor cell mobilization is higher with 2.5 versus 2.0 ATA treatments, and all newly mobilized cells exhibit higher concentrations of an array of regulatory proteins [R01 DK09426002].

F-1491

ATORVASTATIN ENHANCES PARACRINE PROANGIOGENIC ACTIVITY OF HEMATOPOIETIC STEM/PROGENITOR DERIVED CELLS IN VITRO BUT NOT IN VIVO

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Circulating proangiogenic cells (PAC), sometimes named as endothelial progenitor cells (EPC), were described as bone marrow-derived cells that can contribute to angiogenesis and even de novo blood vessel formation. However, they are most probably myeloid cells able to exert their proangiogenic activity through release of various cytokines and growth factors leading to paracrine stimulation of local angiogenesis. Number and function of PACs are impaired in patients with diabetes or cardiovascular diseases. Both diseases can be accompanied with decreased levels of heme oxygenase-1 (HMOX1), cytoprotective, heme-degrading enzyme, which is crucial for PAC function in mouse models. Therefore, the aim of our study was to check whether pharmacological enhancement of HMOX1 expression in hematopoietic stem/progenitor (HSPC) derived PAC cells would improve their paracrine proangiogenic activity. We used GCSF-mobilized CD34+ cells, FACS-sorted from healthy donor peripheral blood mononuclear cells. Sorted cells were CD45dimCD90-CD105-CD181- and predominantly CD133+ and CD11b-. We used serum-free StemSpan ACF medium. CD34+ cells after 6 days in culture were stimulated with atorvastatin, acetylsalicylic acid, sulforaphane, resveratrol or metformin for 48 h. Conditioned media from such cells were then used to stimulate human aortic endothelial cells (HAoEC) to enhance tube-like structure formation in Matrigel assay. The only stimulant that enhanced PAC paracrine angiogenic activity was atorvastatin. On the other hand, the only one that induced heme oxygenase-1 expression was sulforaphane, known activator of HMOX1 inducer - NRF2. Moreover, none of the stimulants used changed the levels of 30 cytokines and growth factors tested with multiplex test. Then, we used atorvastatin-stimulated cells or conditioned media from them in the Matrigel plug in vivo angiogenic assay. Neither atorvastatin alone in control media nor conditioned media nor AT-stimulated cells enhanced host angiogenic response and number of endothelial cells in the plug. Concluding, atorvastatin can enhance paracrine angiogenic activity of human CD34+ HSPC-derived PAC cells in vitro, but the effect was not observed in vivo. Moreover, the enhancement of HMOX1 expression with sulforaphane does not influence PAC proangiogenic action in vitro.

F-1492

MECHANISM OF PLACENTA-DERIVED CELLULAR THERAPY IN MITIGATION OF ACUTE RADIATION SYNDROME

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Radiation-induced hematopoietic failure is a severe consequence of Acute Radiation Syndrome (ARS). PLX-RAD, 3D-expanded placenta-derived cells, have biological properties suggesting a profound capacity to protect and regenerate bone marrow, mitigating some of the lethal effects of ARS. PLX-RAD secrete a broad array of cytokines that contribute to post-radiation reconstitution of the hematopoietic and immune systems, such as G-CSF, IL-6, MCP-1, MCP-3 and GRO. Importantly, PLX-RAD can be safely administered without regard to HLA type, compatible for use in a nuclear disaster scenario. To assess this therapeutic potential, PLX-RAD cells were administered intramuscularly to C3H/HeN male mice one and five days following 7.7 Gy total body irradiation. Mice body weight and survival were monitored for 3 weeks when the animals were euthanized, and their BM and blood were evaluated. 10 out of 11 (91%) PLX-RAD-treated animals survived compared to only 4 out of 9 vehicle treated animals (44%), ($P<0.05$). Vehicle-treated irradiated mice suffered from loss of body weight reaching almost 20% peak average, compared to just up to 10% weight reduction of the PLX-RAD-treated mice. Consistent with the improved survival, BM and blood counts of all the three lineages in PLX-RAD treated mice were significantly increased as compared to vehicle treated mice and were close to normal levels. Moreover, the presence of critical, PLX-RAD-derived (human) cytokines as well as alterations in the equivalent murine cytokines were detected in the mouse plasma, suggesting a direct role of PLX-RAD for animal survival. Finally, in vitro studies demonstrated a 3.1 fold-increase in the number of migrating cells in a BM migration assay with PLX-RAD-derived conditioned medium vs. SDF-1-supplemented positive control medium and induction of the formation of all CFUs in a Methyl Cellulose Colony formation assay. These in vivo and in vitro assays revealed key factors likely contributing to the underlying mechanism. Our data suggest PLX-RAD fosters hematopoietic recovery after potentially lethal (LD70) total body irradiation, which markedly improves survival. PLX-RAD, a novel cell product, may be a highly effective therapy for radiation-induced BM damage in general and particularly in a nuclear disaster scenario.

F-1493

GROWTH DIFFERENTIATION FACTOR 11 SECRETED BY HUMAN MESENCHYMAL STEM CELLS ACCELERATES PROLIFERATION AND EXTRACELLULAR MATRIX PROTEIN EXPRESSION IN HUMAN DERMAL FIBROBLASTS

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Human mesenchymal stem cells-derived conditioned media (hMSC-CM) contains various growth factors and is used for cosmetic ingredient for the purpose of skin regeneration. Based on that growth differentiation factor 11 (GDF11) is known for rejuvenating factor for skeletal muscle and neurons, we investigated whether GDF11 is secreted by MSCs and affects to human dermal fibroblasts (hDFs). We found that GDF11 is secreted by hMSCs and GDF11 expression was higher in human umbilical cord blood-derived MSCs than adipose tissue- or bone marrow-derived MSCs. In hMSCs, inhibition of GDF11 diminished cell proliferation, colony forming ability and multipotency. Next, to confirm whether GDF11 affects to human dermal fibroblast, we treated GDF11 on hDFs and found that GDF11 ameliorates growth rate and extracellular matrix protein expression and diminishes expression of MMP1 in hDFs. Moreover,

hMSC-CM increased cell proliferation and ECM protein secretion in hDFs. On the other hand, these phenotypes were diminished by knock down of GDF11 in hMSC-CM. Taken together, we validate that GDF11 is one of the main growth factors secreted by hMSCs which regulates the skin aging and regeneration.

F-1494

CHARACTERIZATION OF AN INJURY-INDUCED SKELETAL PROGENITOR

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The postnatal skeleton undergoes continual phases of growth, remodeling, and repair. We hypothesized that skeletal progenitor cells active during these disparate phases are genetically and behaviorally distinct. By prospectively isolating osteogenic populations from fracture calluses of 8-week-old C57Bl6 mice, we identified a highly potent regenerative cell type that we term the f-BCSP (fracture-induced Bone, Cartilage, Stromal Progenitor). The f-BCSP possesses markedly higher skeletogenic potential than BCSP harvested from uninjured bone (u-BCSP). Furthermore, the f-BCSP recapitulates many skeletogenic gene expression patterns that characterize BCSPs isolated from mice at postnatal day 3. Our results indicate that the skeletal progenitor population is functionally stratified, containing distinct subsets responsible for growth, remodeling, and injury-induced regeneration. Furthermore, our findings suggest that injury-induced changes to the skeletal stem and progenitor microenvironment might activate these cells and enhance their regenerative potential.

F-1495

CARTILAGE REGENERATION AND CHONDROCYTE PROGENITOR CELL PROLIFERATION INDUCED BY PIGMENT EPITHELIUM-DERIVED FACTOR

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Osteoarthritis (OA) can be induced by age-related degeneration or by sport-related injury. The fundamental mechanism of OA is the destruction of articular cartilage. Pharmacologic treatment options for OA are few. Therefore, a treatment that could protect or regenerate cartilage and replenish chondrocytes will be of great practical value. Here we propose evidence that a peptide derivative of human pigment epithelium-derived factor (PEDF) may have such potential. Recently, in monosodium iodoacetate-induced (MIA) and collagenase-induced OA models in rat, we found that PEDF peptides joint injection can prevent arthritis and related joint pain. Histologically, extensive chondrocyte necrosis, articular cartilage degradation and destruction of joint structure can be prevented. BrdU incorporation assay revealed that the proliferation of chondrocytes were stimulated by PEDF peptide. In cell culture, chondrocytes progenitor cells isolated from cartilage can be expanded more efficiently by PEDF peptide. The expanded cells go through spontaneous chondrocyte differentiation and synthesize more aggrecan. These evidence indicated that PEDF peptide may be a potential therapeutic agent for osteoarthritis. Its mode of action may be cartilage regeneration through the expansion of remaining chondrocytes.

F-1496

REPLENISHMENT OF THE POST-NATAL BONE MARROW FOLLOWING INDUCTION OF MOUSE HIND-LIMB ISCHAEMIA

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Bone marrow (BM)-derived cells participate in regeneration processes following ischaemic disease, and in this work we aimed to investigate the response of bone marrow to severe hypoxic injury. In a model of hind limb ischemia at day two post-injury, we detected 98% cell death in the injured tibia; the majority of viable cells (approximately 84% of total cells) were characterized as non-haematopoietic PDGFR-α and Sca-1 positive cells (PαS; CD45-/CD31/Ter119-/PDGFRa+/Sca1+). Over the course of the first month we detected an increase in colony-forming Fibroblast units (CFU-Fs) indicating a gradual rise in the numbers of Mesenchymal Stromal Cells (MSCs) within the bone marrow of the injured tibia. However, we could not detect incorporation of nucleoside analogue EdU or expression of Ki-67 in PaS cells suggesting that the surviving PaS cells did not proliferate, but instead may be recruited from other tissues. During the first 2 weeks following ischemia we observed an increase in osteogenic progenitors in the bone marrow of the contralateral tibia, the blood and the periosteum of the ischemic limb. By 3-4 weeks we observed ossification of the bone marrow while by 7 months, haematopoietic cells were detected within the treated BM suggesting haematopoietic reconstitution of the treated limb. This suggests that following hind limb ischemia osteogenic progenitors may be recruited from other distant stem cell niches (periosteum or the contralateral limb, possibly via the blood) to the injured limb to promote ossification of the marrow space that precedes the regeneration of the BM niche.

F-1497

HIGH AND LOW OXYGEN TENSIONS INDUCE DIFFERENT SECRETOME PROFILES OF ADIPOSE TISSUE-DERIVED STEM CELLS

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Growing evidence from animal studies shows that adipose tissue-derived stem cells (ASCs) improve cardiac function of infarcted hearts. It is commonly accepted that therapeutic potential of ASCs may depend more on their paracrine effects than differentiation potential. The underlying mechanisms remain unclear. Most data regarding paracrine factors were obtained from ASCs cultured in normoxic condition (20%). The present study investigated how in vivo physiological oxygen (4%) tension influenced secretome of ASCs. ASCs were isolated from three 8-week-old BALB/c mice. ASCs were confirmed by the expression of stem cell markers (CD44 and CD90) and their capacity to differentiate into adipocytes and osteocytes. ASCs at passage 5 were cultured in normoxic (20%) and lower oxygen (4%) incubators and conditioned for 24 h

(3 cultures/group). The conditioned media (CM) from ASCs were subjected to trypsin digestion followed by analysis using automated nano-flow liquid chromatography tandem mass spectrometry. The collected LC/MS/MS data were searched against the rodent subset of the Uniprot database and the total proteomes were identified. The data were from 6 technical replicates. A total of 28 proteins were identified and 7 proteins were unique to normoxic CM. Of the 21 common proteins detected in both normoxic and lower oxygen CM, 9 were extracellular matrix proteins. The abundance of 6 of these proteins (e.g., collagen I and laminin) differed noticeably between normoxic and lower oxygen CM. In addition, a greater amount of cytokine CXCL5 and matrix metalloproteinase (MMP)-2 was detected in lower oxygen CM than in normoxic CM while tissue inhibitor of metalloproteinase (TIMP)-1 was only detected in normoxic CM. These results indicate for the first time that lower oxygen tension differentially regulates the secretome of ASCs. Extrapolating the results of this study to the in vivo setting, it would appear that injected ASCs may exert their anti-fibrotic and trophic effect by 1) directly regulating the balance of MMP/TIMP production and preventing collagen accumulation in ischemic hearts to decrease fibrosis, and 2) secreting trophic factors including CXCL5. These data suggest that proteomic analysis of CM is useful for elucidation of paracrine effect of ASCs in vivo.

F-1498

LOCALIZATION OF THE JUXTACRINE FACTOR EPHRIN/EPH IN THE RAT PITUITARY STEM PROGENITOR CELL NICHES

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The anterior lobe of the pituitary gland is a key endocrine tissue composed of five endocrine-cells and non-endocrine-cells. Among non-endocrine-cells, stem/progenitor cells exist and play a role in the regeneration of endocrine-cells in the adult pituitary. Recently, we have reported that Sox2-expressing pituitary stem/progenitor cells form a micro-environment (niche) via a homophilic tight-junction protein CAR in the marginal cell layer (MCL) and the parenchyma, and that a juxtacrine factor ephrin-B2 specifically exists in these niches. The ephrin and Eph are activated by cell adhesion, and its interaction raises bidirectional (forward and reverse) signaling. Especially, B-class ephrin/Eph signal is known to play a role in maintaining stem cell function in the various niches such as the subventricular zone, crypt and bone marrow. In the present study, we attempted to identify the pairing partner Eph for ephrin-B2 and their localization in the pituitary stem/progenitor niches. Immunohistochemistry was performed to determine localization of Ephs (A4, B1, B2, B3 and B4) in the pituitary, and demonstrated that EphB3 exists in the pituitary stem/progenitor cell niches. Double staining of ephrin-B2 and EphB3 in the adult pituitary revealed that they localize in same cells and mainly form inactive cis-interaction. On the other hand, on postnatal day 5, when cell proliferation and differentiation actively occur, ephrin-B2 single immuno-positive signals were observed beneath the MCL and in some niches in the parenchyma clusters. Further, immunohistochemistry demonstrated that GH- and ACTH-producing cells neighbor upon these pituitary niches at high frequency on P5, and that EphB2 specifically exists in ACTH-producing cells. Next, we performed double immunohistochemistry for ephrin-B2 and EphB2, and demonstrated that ephrin-B2-positive cells neighbor upon EphB2-positive ones beneath the MCL on P5. In addition, some of them are positive for a differentiated cell marker, PIT1. These results suggest that cis-interaction of ephrin-B2/EphB3 induces a quiescent state in the adult pituitary niches, and trans-interaction between ephrin-B2-expressing stem/progenitor cells and EphB2-expressing neighboring cells induces an active state for the differentiation.

TECHNOLOGIES FOR STEM CELL RESEARCH

F-1500

MONOCLONAL ANTIBODY KILLS HUMAN PLURIPOTENT STEM CELLS BY ONCOSIS VIA EXCESS ROS PRODUCTION

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Various studies have demonstrated the potential of human pluripotent stem cells (hPSC) for tissue engineering, regenerative medicine and drug testing. However, the risk of in vivo teratoma formation by residual undifferentiated hPSC remains a major safety concern. Previously, our group has demonstrated the ability to prevent teratoma formation using a cytotoxic IgM antibody, mAb84, which binds to and kills undifferentiated hPSC. However, penetration of the pentameric mAb84 into differentiated cell aggregates to kill residual hPSC may be impeded because of its size (MW~950kDa vs IgG ~150kDa). Intriguingly, we have successfully generated another cytotoxic antibody, TAG-A1 (A1). In this study, we benchmarked the characteristics of A1 against mAb84. Additionally, the death mechanism induced by A1 was investigated. We found that A1 kills hPSC as efficiently as mAb84 in a time and dosage-dependent manner. Most of the killing occurs within the first 5 minutes of incubation. Unlike mAb84 that targets podocalyxin (PODXL) on hPSC, A1 binds to multiple proteins on hPSC via O-linked glycan epitopes. Moreover, A1 exhibits better penetration efficiency than mAb84. Mechanistically, A1 do not kill hPSC by apoptosis i.e. absence of caspase activity and DNA fragmentation. Instead, formation of homotypic adhesion, cell swelling, loss of microvilli, and formation of membrane pores were observed under scanning electron microscope. All these features of hPSC killing by A1 are consistent with that of oncosis. We demonstrated that excess reactive oxygen species (ROS) production is critical for A1-induced hPSC death. A1-induced ROS production is mediated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, most likely Nox2 isoform. ROS production occurs downstream of homotypic adhesion (HA), upstream of mitochondrial depolarization, actin cytoskeleton reorganization and severely cellular damage. We therefore proposed the first mechanistic model for oncosis cell killing by a mAb on hPSC.

F-1501

A RAPID IMAGE CYTOMETRY METHOD FOR QUANTIFICATION OF CANINE STROMAL VASCULAR FRACTION CELLS

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In recent years, the lipoaspirate collected from adipose tissue has been seen as a valuable source of adipose-derived mesenchymal stem cells for autologous cellular therapy. For multiple applications, adipose-derived mesenchymal stem cells are isolated from the stromal vascular fraction (SVF) of adipose tissue. Because the fresh stromal vascular fraction typically contains a heterogeneous mixture of cells, determining cell concentration and viability is a crucial step in preparing fraction samples for downstream processing. Due to a large amount of cellular debris contained in the SVF sample, as well as counting irregularities standard manual counting can lead to inconsistent results. Advancements in imaging and optics technologies have significantly improved the image-based cytometric analysis method. In addition, fluorescence detection using novel fluorescent probes have improved sensitivity of detection methods. In this work, we validated the use of fluorescence-based image cytometry, Cellometer Vision, for SVF concentration and viability measurement. We compared the Cellometer Vision to the current methods, standard flow cytometry and manual hemocytometer. Five freshly collected canine SVF were analyzed using all three methods to measure concentration and viability. The results were highly comparable, which validated the image cytometry method for canine SVF analysis, and potentially for SVF from other species.

F-1502

IN VIVO MR IMAGING OF INTRAHEPATIC AUTOLOGOUS TRANSPLANTATION OF INSULIN-PRODUCING CELLS DERIVED FROM PANCREATIC PROGENITOR CELLS TO DIABETIC MONKEY MODELS

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Insulin-producing cells (IPCs) derived from patient's own stem cells provide great potential for autologous transplantation therapy in type 1 diabetes. However, cell death after transplantation is a major limitation for cell replacement therapy. To study the effect of the survival of autologous IPCs on therapy efficacy, we monitored the dynamic changes of the number of autologous IPCs and blood C-peptide level in the diabetic cynomolgus monkeys. Pancreatic progenitor cells (PPCs) from diabetic monkey models were labeled with superparamagnetic iron oxide contrast agent, Feridex, and differentiation capacity of labeled cells was assessed in vitro. Then, the IPCs generated from Feridex labeled PPCs were auto-transplanted into liver of diabetic monkeys, and one year follow-up serial MR imaging and mapping were performed in order to track transplanted IPCs. Meanwhile, daily blood glucose and C-peptide levels were also measured. Following euthanasia, immunostaining and prussian blue staining of liver tissue were performed to determine the survival and the immunogenicity of these autologous IPCs. SPIO labeled cells appeared as hypointense images in vitro and in vivo MRI scans. The labeling procedure did not disrupt the viability or differentiation capacity of PPCs. More importantly, the synchronous change trend was found between total area of hypointense spots and the blood C-peptide level after IPCs autotransplantiation. Immunohistochemical studies confirmed that all of the islet-like cell clusters were Prussian blue positive on liver sections one year after autologous transplantation. Collectively, this study identifies that SPIO can be used to label PPCs for the noninvasive and real-time monitoring of the effect of IPCs survival on therapy efficacy vivo.

F-1503

ROBOT AIDED MICROINJECTION TO DELIVER SYNTHETIC MODIFIED MRNAS INTO HUMAN PLURIPOTENT STEM CELL DERIVED VENTRICULAR CARDIOMYOCYTES

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Synthetic modified mRNAs have been demonstrated as a useful tool for reprogramming and directed differentiation. Conventional transfection techniques such as electroporation and chemical-based protocols often requires an excessive amount of mRNAs yet without a precise control over the amount received by the target cells, leading to significant heterogeneity and variability. A delivery method by which a precise amount of mRNAs can be delivered into each and every targeted cell is therefore highly desirable and would enhance the biofidelity. Microinjection has been sought for delivering a precise dosage of exogenous materials into adherent cells, but previous approaches often suffer from low productivity and operational difficulty. Here we attempted to employ a novel robotic microinjection system for efficient delivery of mRNAs into human embryonic stem cell-derived ventricular cardiomyocytes (hESC-VCMs). This system was capable of handling automated injection of 256 cells at a time. The amount of mRNAs to be injected could be controlled by the injection pressure and the concentration of the injected solution. As a case study, nuclear eGFP encoding synthetic modified mRNA (StemMASTM) was used to evaluate the system performance. The success rate of infection for hESC-VCM was 27.9% ($n > 900$), which was calculated based on the proportion of injected cells showing nuclear eGFP fluorescence 6 hours after injection. Of note, the required mRNA concentration was extremely low (1 ng/µL) with an injection volume of only 1400 fL. Therefore, only ~1.4 fg of mRNA was required to infect a cell, representing at least 5000X smaller than that required by conventional transfection. We conclude that not only is our robotic approach a much more efficient approach but can also lead to more consistent outcome.

F-1504

N6-METHYL-ADENOSINE RNA MODIFICATIONS OF REPROGRAMMING GENES IN MOUSE AND HUMAN ES AND IPS CELLS

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N6-methyladenosine (m^{6}A) RNA modification is a recently emerging topic in the control of cell fate transition in mammalian embryonic stem cells. m^{6}A is one of the most prevalent modification on both

mRNAs and noncoding RNAs in eukaryotes with 12,000 m6A sites in over 7000 genes found. m6A methyltransferases affect p53 signaling pathways and apoptosis. Obesity risk gene FTO encoding the first identified m^6A demethylase mutations have been associated with increased risk for obesity and type 2 diabetes. Recently impairment in m6A regulation demonstrated prolonged expression of Nanog and dysregulation of ES cells to exit from self-renewal toward differentiation into several linages in stem cells. m^6A RNA immunoprecipitation (meRIP) is a method to monitor the status of m^6A and can help map the location of m^6A modifications. We have optimized the method by using a monoclonal antibody specifically targeted to m^6A modifications. The streamlined protocol is easy to process and can produce results with high SN ratio. With this robust protocol, we analyzed the m^6A RNA levels of the different reprograming genes in mouse and human ES cells and iPS cells.

F-1505

TRANSCRIPTIONAL ANALYSIS DURING HUMAN FETAL DEVELOPMENT UNCOVERS A TISSUE-SPECIFIC BARCODE AND HELPS ASSESS STEM CELL DIFFERENTIATION

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Gene expression is highly dynamic during fetal development and determines tissue specification and function. In humans, the transcriptional profile of different organs during development has not been systematically studied. However, this information would be extremely valuable to assess how effective differentiation of human (pluripotent) stem cells is. Moreover, this would generate information on the cascade of transcription factors that takes place during development in vivo for optimizing differentiation protocols in vitro. We have performed next generation sequencing (DeepSAGE) on 21 different human fetal tissues (plus the maternal endometrium) between 9 and 22 weeks of gestation. The results reveal a tissue specific signature independent of the developmental age. Interestingly the tissue signature remains into adulthood. We next developed a predictive tool, Keygenes, to access first the identity of fetal and adult organs and tissues from both next generation sequencing and microarray and applied that to predict the differentiation status of differentiated human stem cells to tissues of the three lineages (endoderm, ectoderm, mesoderm). Understanding how a particular tissue acquires its tissue identity will give insight into the development and maturation of tissues from a developmental biology perspective, but it will also represent a valuable tool to be used for regenerative medicine purposes.

F-1506

COMBINATION OF INTRACELLULAR FLOW CYTOMETRY WITH CELL SURFACE MARKER SCREENING IDENTIFIES A NOVEL CELL SURFACE MARKER SIGNATURE FOR HUMAN EMBRYONIC STEM CELL DERIVED NEURONS

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One of the major challenges in stem cell biology is identifying cell types of interest in heterogeneous cell cultures due to a lack of adequate cell surface markers to define cell types of interest. Intracellular markers are often known for many stem cell lineages however assaying for these markers necessitates cell fixation and permeabilization, whereas cell surface signatures that correlate to intracellular signatures remain few. Identifying cell surface signatures that correlate to intracellular expression of transcription factors of stem cell lineages would enable the enrichment of viable cell cultures for a particular cell type. To address this, we aimed to expand on methods of screening highly heterogeneous cultures by the use of multi-parameter intracellular flow cytometry to screen for novel cell surface signatures from neurons derived from human embryonic stem cells (hESC). Specifically, we first utilized intracellular flow cytometry and analyzed for the expression of neural stem cell markers Pax6, Sox1 and Sox2 and the neuronal marker doublecortin (DCX) to identify relevant neural cell populations. We then combined unbiased cell surface marker screening with intracellular flow cytometric analysis and validated potential cell surface marker signatures for neurons. The use of this multi-parameter screening strategy identified $CD200^{++}HLA\text{-}ABC^{dim/-}$ as a cell surface marker signature that allowed for the successful sorting of neurons from hESCs differentiation cultures. This new signature was compared with currently published methods that enable the sorting of neurons from cultures of neural stem cells that have been differentiated to neurons. The use of $CD200^{++}HLA\text{-}ABC^{dim/-}$ was more widely applicable for the enrichment of neurons in comparison to previous cell surface signatures. This multiplexed screening strategy can facilitate the discovery of new cell surface markers to specific cell populations where intracellular markers delineating cell lineages are already known.

F-1507

A MULTIPLEXED SINGLE CELL ASSAY TO TRACK PROLIFERATIVE HISTORY IN DIFFERENTIATING CELL SYSTEMS

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The variety of cell types comprising multicellular organisms were all derived from a single cell through progressive and coordinated stages of proliferation, death, and differentiation. Still, the linkage of cell division with lineage-specific differentiation, especially in complex human cell systems remains elusive. We believe that linking the fine details of proliferative status and history to cell fate committement will lead to important applications in human cell specification and regenerative medicine. A powerful technique for simultaneous, high-throughput proteomic monitoring of single-cell phenotypes, mass cytometry (CyTOF), has recently enabled deep analysis of differentiating cellular systems in primary human tissues, overcoming challenges related to stochasticity, heterogeneity, and asynchrony of these systems. However, a mass cytometry-based method for tracking the number of cell divisions (proliferation history) is not currently available. Here, we have adapted a well-established fluorescent dye dilution assay for tracking cell proliferation history, to mass cytometry. Specifically, we have identified a monoclonal antibody that can be tagged with a monoisotopic mass reporter and used to quantify carboxyfluorescein succinimidyl ester (CFSE) dilution in our 30+ parameter single cell assays. Control experiment results were quantitatively similar to CFSE dilution measured directly by flow cytometry, where ≥ 5 cell divisions can be distinguished.

As a proof of concept, mass cytometry CFSE dilution assays were then applied to primary human T lymphocytes activated in a rapid expansion protocol, currently applied in the clinic to expand T cells for adoptive transfer cell therapies. Using a mathematical model to extract proliferative history, we successfully profiled the temporal expression of a multitude of activating and inhibitory receptors across T cell divisions, and linked their maturation to the number of cell divisions from the naïve resting state. We further applied this assay to a cell line and to human pluripotent stem cell cultures. We believe that the suite of mass cytometry methods presented here will be generally applicable across various investigations involved in tracking cellular proliferation and differentiation across diverse stem cell systems going forward.

F-1508

A NOVEL, MULTIFACTORIAL APPROACH FOR HUMAN IPS CELL DIFFERENTIATION AND REPROGRAMMING USING AN AUTOMATED CELL CULTURE SYSTEM

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In vitro studies using pluripotent stem cells provide an essential tool for understanding hard-to-study in vivo developmental processes. Human iPSCs also holds great potential for disease modeling and cell-based therapies. A major challenge in the stem cell field, however, is to define the optimal condition for cell expansion, differentiation, and reprogramming. Since multiple intra- and extra-cellular signaling pathways are involved in each cellular process, a combinatorial approach to screen multiple factors is highly desirable. To facilitate the exploratory processes, we have developed an automated cell culture system for cell manipulation with environmental control. The system consists of an integrated fluidic circuit (IFC), an electro-pneumatic 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. Live cells or cell lysate can also be harvested from each culture chamber individually. Using this system, we have developed a novel non-integrating method for direct conversion of human BJ fibroblasts to neurons at high efficiency and cell viability using microRNA mimics and synthetic mRNAs. The system allows 3-day hands-off operation and long term cell culture. We demonstrated that human iPSCs can differentiate to neural progenitor cells or nociceptor neurons on the IFC after treatment with small molecules. Furthermore, using combinations of small molecules and signaling proteins in chemically defined media, we demonstrated directing human iPSCs into primitive lineages of all three germ layers on one IFC within four days. Finally, we have also developed protocol for RNA transfection that allows efficient delivery of exogenous mRNA such as nGFP into human iPSCs as well as successful siRNA knockdowns of either endogenous or exogenous genes. In summary, the automated microfluidic platform employs precise control of microenvironment of cells, facilitates studies of multifactorial combinations, and enables development of robust, reproducible, and chemically defined cell culture and manipulation.

F-1509

HIGH-FREQUENCY GENERATION OF TRANSGENIC MICE USING IMPRINTED GENES ENGINEERED ANDROGENETIC HAPLOID EMBRYONIC STEM CELLS

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Androgenetic haploid embryonic stem cells (AG-haESCs) could be established from mouse androgenetic blastocysts. By injecting AG-haESCs into mature oocytes, we can obtain live and fertile mice, which indicates that AG-haESC can substitute sperm cells, at least partially. It also provides a new approach for producing genetically modified mice rapidly. However, the efficiency is still very low and most of the pups generated by Intracytoplasmic AG-haESCs Injection (ICAI) were growth retarded and died shortly after birth, which most likely due to instable paternal imprinting status in the cells. Here, by using the CRISPR/Cas9 assisted gene targeting technology, we modified two critical imprinted genes in AG-haESCs. These cells, although at late passages, maintained paternal imprints, expressed classical ESC pluripotency markers, and contributed to various tissues. Strikingly, we got healthy fertile transgenic mice from these AG-haESCs by ICAI with high frequency, and the genetic modifications could be transmitted to offspring. Our results demonstrated that the imprinted genes-targeted AG-haESCs can serve as a reliable and efficient tool to produce gene-modified mouse models rapidly.

F-1510

IDENTIFICATION OF A NOVEL CELL SURFACE MARKER FOR THE ENRICHMENT OF TRANSPLANTABLE DOPAMINERGIC PRECURSOR CELLS FROM DIFFERENTIATED HUMAN PLURIPOTENT STEM CELLS

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Parkinson's Disease (PD) is the second most frequent neurodegenerative disorder, mainly caused by death of dopaminergic (DA) neurons in the ventral mesencephalon (VM). It has been shown that cell replacement therapies with fetal VM DA neurons can be beneficial for PD patients. Since the availability of fetal tissue is very restricted, human embryonic stem cells (hESC) or human induced pluripotent stem cells (hiPSC) are considered as an alternative source for the derivation of DA neurons for future cell therapy of PD. It has been shown that mesencephalic DA (mesDA) neurons arise from the midbrain floor plate in which FOXA2 acts as a key transcription factor. We successfully generated FOXA2+ cells in vitro by differentiation of hiPSC using a combined embryoid-body / dual SMAD inhibition protocol. In order to find sortable cell surface antigens, which correlate with the mesDA phenotype, we performed a flow cytometry based marker screening. The co-expression of FOXA2 was used to detect the target cells. Hereby, we identified a number of cell surface markers, of which one was

specifically co-expressed with FOXA2 and can be used to isolate mesDA progenitors utilizing MACS Technology. The sorted iPSCs were viable, enriched for midbrain specific markers such as LMX1A, lacked expression of pluripotency markers, and differentiated into mature dopaminergic neurons in vitro. Strikingly, immunomagnetic isolation of differentiated iPSCs prior to intrastriatal transplantation in a rat model of PD revealed that sorting did not affect graft survival, and that grafts of sorted mesDA cells were less proliferative and gave rise to dopaminergic neurons with a more mature phenotype as compared to those of unsorted cells. In conclusion, we find that our novel cell surface marker may be used as a tool to purify mesDA progenitor cells for regenerative therapy in PD patients.

F-1511

HEME OXYGENASE 1 ENHANCE PROLIFERATION OF PORCINE ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELL THROUGH NOX4-P53/P21 PATHWAY

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Unlike embryonic stem cells that can be self-renewal, adipose tissue derived mesenchymal stem cells (AdMSCs) undergo senescence after several sub-passages. Therefore, maintaining high proliferation ability is important to use AdMSCs in therapeutic purposes which require large number of cells. Heme oxygenase 1 (HO1) is an enzyme degrades heme into carbon monoxide, free iron, and biliverdin. These end products have anti-oxidant and anti-apoptotic effects thus HO1 expression is regarded as cell supportive and protective processes. In this study, we hypothesized that HO1 expression enhances proliferation of AdMSCs by the anti-oxidant and anti-apoptotic effects of HO1. We produced transgenic miniature pigs expressing HO1 gene and isolated AdMSCs from the transgenic pigs. Interestingly, we found that proliferation of AdMSCs from the transgenic pigs was evidently faster during in vitro culture compared to the AdMSCs isolated from wildtype miniature pigs. To investigate underlying mechanism of this enhanced proliferation in HO1 AdMSCs, we assessed expression of proliferation related genes in HO1 and wildtype AdMSCs. Quantitative PCR results confirmed that high expression of transgenic HO1 gene in HO1 AdMSCs while endogenous expressions of HO1 were similar between HO1 and wildtype AdMSCs. In HO1 AdMSCs, expression of p53 and p21 were significantly downregulated. Pro-apoptotic Bax gene was also downregulated and anti-apoptotic Bcl2 gene was upregulated in HO1 AdMSCs compared to wildtype. We also analyzed expression of Nox4, a NADPH oxidase previously reported as regulating cell proliferation and apoptosis by inhibiting p53 and p21. As expected, expression of Nox4 was significantly up-regulated in HO1 AdMSCs compare to wildtype. In conclusion, AdMSCs overexpressing HO1 showed enhanced proliferation compared to wildtype AdMSCs, which seems to be related with signaling mediated by Nox4 and p53/p21. *This study was supported by Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ009802), Rural Development Administration, Republic of Korea.*

F-1512

SCREENING KEY PROCES CONDITIONS FOR ANCHORAGE-DEPENDENT STEM CELL CULTIVATION USING SCALE-DOWN MODEL OF VERTICAL-WHEEL BIOREACTORS

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Processes development for allogeneic cell therapies currently utilize a 2D approach that is difficult to scale up for commercial manufacturing. Thus a new, scalable manufacturing platform is needed to meet projected large-scale demands for cell therapies such as stem cells. One promising 3D option is using suspended microcarriers in a single-use bioreactor; however, improvements are still needed in order to efficiently advance cellular therapy processes from laboratories to clinical trials to commercialization. The high cost of stem cell media and requirement of numerous experiments make process development difficult, even in benchtop-size bioreactors. Therefore, a scale-down, small-volume model that is representative of culture environments in larger volumes would be ideal for early stage process development. A family of single-use, Vertical-Wheel bioreactors with low shear was recently introduced and shows promise as a scalable 3D manufacturing platform. In particular, the scale-down model bioreactors can be used for more efficient and economical screening of process parameters. With 100 mL and 500 mL working volumes, these bioreactors are designed to work inside CO_2 incubators that will regulate temperature, pH, and dissolved oxygen levels, while agitation rate is independently controlled. Crucially, these bioreactors are able to represent the low-shear environment of larger size units while still suspending microcarriers uniformly with minimal power input. A screening and ranging study of key process parameters for stem cell cultivation was performed in these scale-down bioreactors, including microcarrier concentration, seeding conditions, media formulation, media exchange regime and frequency, and in-reactor cell harvest methods. A final stem cell density exceeding 2×10^6 cells/mL was achieved in a less than 500 mL working volume and without the use of any anti-foaming agents or shear protectants. Correlations were also found between cell growth, microcarrier clumping, and Kolmogorov scales, which can be applied to predict appropriate agitation speeds that will avoid shear damage during process scale up. This study will present the experimental data and show how small-scale, Vertical-Wheel bioreactors can improve the 3D suspension culture process for stem cell production.

F-1513

SINGLE CELL CULTURE OF HUMAN PLURIPOTENT STEM CELLS

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Human induced pluripotent stem cells (hiPSCs) of a lineage often show characteristic variations, including proliferation rate, differentiation capability and disease-triggering mutation, among individual cells. Understanding such variations is important but

challenging. Recently, single cell sorting and analysis have been achieved and more attention has been paid to the culture of hiPSCs at single cell levels. In this work, we propose a simple but yet robust platform for single cell culture of hiPSCs. This platform allowed us to obtain hiPSCs clones from single hPSCs and to ensure their expansion over 30 passages under defined, cytokine- and xeno-free conditions. Accordingly, the pluripotency of those single hiPSC originated clones have been evaluated by RT-PCR, immunocytochemistry, flow-cytometry, embryoid and teratoma formation. Our results show that those clones can effectively keep their pluripotency as well as their capability of self-renewal. However, the gene expression analyses have shown that some genes, such as MAGE family specifically expressed in germ or tumor cells, could have different expression levels among different clones, indicating that the individual cells may have different characters even in a same colony. More generally, our single cell culture platform consists of an easy approach to purify the hiPSCs colonies which can be used for both fundamental research and clinical applications. More investigations are needed for understanding how individual hiPSCs would have different self-renewal or differentiation properties.

F-1514

IDENTIFICATION OF A TIGHTLY-CONTROLLED, HIGHLY-ACTIVATABLE, DOXYCYCLINE-INDUCIBLE GENOMIC LOCUS

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Stem cell research requires strong, reliable and tightly regulatable transgene expression. In the mouse, the Doxycycline (Dox)-inducible tetO promoter frequently shows mosaic expression when introduced in the most commonly used genomic loci: ROSA26 and ColA1. We hypothesized that there are other genomic loci offering better properties for transgene expression. Therefore, we designed a screen to identify genomic sites allowing silent but Dox-activatable high (s/a sites) and overall transgene expression. Using a "detector cassette" constituted by a tetO promoter driving a beta-Gal reporter, and an integrated rtTA expression cassette, we screened a large number of random insertions in mouse ES cells. After random integration of the detector cassette, 200 independent clones were analyzed by X-Gal staining. Of them, 38 showed no expression in the lack of Dox, and strong and overall expression in the presence of Dox. Southern blot analysis with an internal probe identified 12 clones with single site, single copy integration of the detector cassette. The Dox inducibility of these 12 clones was further examined in in-vitro differentiated embryoid bodies. The best of these 12 clones was chosen to generate chimeric animals and establish a mouse line. In the absence of Dox, no beta-Gal expression was detected in the transgenic animals. Upon Dox administration, on the other hand, we observed strong beta-Gal expression in the heart, liver, lung, kidney, muscle, eye, pancreas, skin, intestine, and synovial fibroblasts of the joints. A detailed analysis of the expression properties is progress. We also identified the exact genomic location of the transgene insertion which information was necessary for utilizing this novel s/a candidate locus for future targeting with other vector designs.

F-1516

NANOFIBRILLAR CELLULOSE HYDROGEL SUPPORTS THREE-DIMENSIONAL CULTURE OF HUMAN PLURIPOTENT STEM CELLS

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Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) are pluripotent and capable of self-renewal. They can differentiate to all the cell types in human adult, and therefore they have great potential in research and therapies. To provide high quality cells for various applications, we have developed a three-dimensional (3D) culture system using a novel plant-derived nanofibrillar cellulose (NFC) hydrogel. Human ESCs and iPSCs cultured in the NFC hydrogel formed 3D spheroids with strong OCT4 expression. To propagate the cells, a cellulose enzyme, cellulase, was introduced to degrade NFC and to recover stem cell spheroids. The recovered stem cells were able to attach to Matrigel, vitronectin, laminin-511 and laminin-521-coated wells. The recovered stem cells cultured on two-dimensional (2D) surfaces exhibit typical stem cell morphology and express the pluripotency markers OCT4 and SSEA-4 but not differentiation markers muscle actin, β-tubulin type III or HNF3B. The cells cultured in the NFC hydrogel can be differentiated into the cells of three germ layers via in vitro embryoid body formation and in vivo teratoma formation, indicating that the cells retain pluripotency in 3D culture. This culture system is xeno-free, synthetic, and easy for shifting culture between 3D and 2D. It will be useful in stem cell-based drug research, cell therapy, and tissue engineering.

F-1517

SINGLE CELL EXPANSION OF HUMAN PLURIPOTENT STEM CELLS IN A XENO-FREE AND DEFINED CULTURE MEDIUM, STEMFIT®_AK

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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. It was also reported that cells maintained in StemFit®_AK could be successfully differentiated into somatic and intermediate precursor cells. Here, we further address characteristics of StemFit®_AK under various culture conditions. In comparison to a commercially available defined medium, StemFit®_AK yields higher cell numbers when dispersed human induced pluripotent cells (hiPSCs) are cultured on several cell culture matrices. This medium also supports efficient growth and colony formation, even at very low seeding densities that is required for cloning and single-cell analysis. hiPSCs maintained with StemFit®_AK further show the potential to be induced into more specialized progenies of all three germ layers when differentiated with widely-used induction protocols. Altogether, these results suggest that StemFit®_AK is an easy-to-use medium for stable culture and efficient expansion of hPSCs. We propose that StemFit®_AK is a reliable platform

for clinical applications of hPSCs and a versatile tool for stem cell research, including genome editing and studies for cell clonality and heterogeneity.

F-1518

ROLE OF WNT SIGNALING IN PROLIFERATION AND DIFFERENTIATION OF CARDIOSPHERE DERIVED CELLS UNDER HYPOXIC STRESS IN-VITRO

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Myocardial infarction associated with ischemia/reperfusion is a major cause of cardiovascular diseases resulting in major loss of cardiomyocytes, scar formation and subsequent deterioration of heart function. Resident cardiac stem cells (CSCs) are ideal candidates to treat CVDs because of allogenic, non-immunogenic nature. However, oxidative stress, inflammatory response and development of pro-apoptotic factors are the major aspects which hinder the CSCs in repairing the infarcted myocardium. Hypoxia Inducible Factor (HIF) regulates the pluripotency of stem cells in hypoxic niche by direct interaction with Wnt signalling cascade. The role of Wnt signalling in mediating differentiation of CSCs under hypoxic conditions is yet to be elucidated. Hence our aim is to investigate the mechanistic pathway of cardiosphere derived cells (CDCs) in proliferation and differentiation during hypoxia. We also aim to study the role of CDC in hypoxic conditions to prevent apoptosis and thereby enhance cardioprotection. Cardiac stem cells were isolated by explant method from adult rat hearts with intermediate cardiosphere (CS) formation step. CS were expanded as cardiosphere derived cells (CDCs) which were used for further studies. CSCs were characterized for stem cell marker, c-kit by confocal microscopy. Morphological characterization of CSCs and CDCs was performed by phase contrast microscopy and Hematoxylin & Eosin staining techniques. Gene expression of CDCs were analyzed by RT-PCR using markers such as C-kit, GATA-4, Nkx2.5, CD90 and vimentin. Immunocytochemistry of CDCs was performed to analyze nkx2.5 and α-sarcomeric actin markers. CDCs were treated with 10μM 5-azacytidine and allowed to differentiate to cardiomyocytes. Differentiation of 5-azacytidine treated CDCs was observed and was analyzed with nkx2.5 and α-sarcomeric actin markers using confocal microscopy and RT-PCR with the markers C-kit, GATA-4, Nkx2.5, CD90 and vimentin. CDCs were subjected to hypoxic conditions and the differentiation ability of CDCs was analyzed and the role of Wnt signaling pathway in mediating differentiation was being investigated. The results of this study will give a better understanding on efficiency of CDCs for therapeutic purposes.

F-1519

HIGH CONTENT SCREENING FOR MODULATORS OF DIFFERENTIATION IN HUMAN PLURIPOTENT STEM CELLS

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Central to the success of stem cell applications is the ability to modulate their behaviour in a controlled, scalable and clinically suitable manner. Currently, it is challenging to differentiate human pluripotent stem cells to the majority of cell types in vitro. Moreover, the resulting cells are often produced using undefined, suboptimal conditions lacking sufficient control, maturation and functional status. Due to strong evolutionary conservation of cellular signalling networks, it is reasonable to anticipate that a carefully crafted set of small molecules combined with phenotypic screening may identify suitable phenotypic and physiological outcomes. Therefore, screening each developmental stage with a select number of 'nodal' signalling regulators or their synergistic combinations might sufficiently identify modulators with high potential in therapeutic applications. Following this hypothesis, we identified a set of 96 potent, selective and pharmacologically diverse "drug-like" small molecules that target key signalling systems in the cell ranging from epigenetic and proteasome regulation, Hh/Smo, Notch, RTK, NHR, Wnt signalling and autophagy. In order to test compounds, we developed a quantitative high-content screening immunofluorescence assay to monitor their ability to modulate SOX2, OCT4 and SSEA1 expression. These markers indicate in a combinatorial way the cell's pluripotency or early differentiation status. Briefly, two human pluripotent cell lines were exposed to small molecules at various concentrations. Cell morphology, number and marker expression was analysed over 96 hours. Interestingly, treatment with several compounds resulted in the cells retaining pluripotency while many conditions were identified that resulted in loss of OCT4 or SOX2 expression or upregulation of SSEA1, indicating promotion of differentiation to the various germ layer lineages. As a result of this study, we confirmed that a combination of carefully selected small molecules and powerful screening platforms is sufficient to identify potent and efficient modulators of stem cell pluripotency and differentiation. Furthermore, differential pharmacology and drug/lead like character of these agents improves their chance to rapidly progress through optimization stages into the clinic.

F-1520

BIOREACTOR BASED STEM CELL EXPANSION IS SUPPORTED BY ADVANCED ANALYTICS

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As more cell therapeutics approach the commercialization phase, a gap in analytical equipment within traditional labs has been identified. Measuring glucose and lactate levels during bioreactor expansion can be used as an orthogonal method for assessing cell growth and optimizing feed protocols. Use of the Nova BioProfile FLEX® analyzer is standard for measuring nutrients and metabolites in traditional biopharma manufacturing settings. For potential application to cell therapy manufacturing, this study compared the standard nutrient and metabolite analytics to measurements with handheld glucose and lactate single-test analyzers and a compact blood gas and chemistry analyzer. Multiple assessments of control solutions were performed in order to characterize the variability of the standard method and the alternative equipment. After establishing the working range, we used the equipment to measure glucose and lactate levels in samples taken from bioreactors. We found the Nova equipment can be a portable, low-cost solution that provides a high level of comparability to the standard instrumentation. Additionally, the blood gas-chemistry analyzer can be used to measure process attributes including pH and dissolved oxygen, making it particularly useful in both the setup and calibration phase as well as the expansion phase.

F-1521

HUMAN PLURIPOTENT STEM CELL GROWTH ON LAMININ 521 COATED, MICROCARRIER CULTURES

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Human pluripotent stem cells (hPSC) are self-renewing cells having the potential of differentiation into the three lineages of somatic cells and thus can be medically used in diverse cellular therapies. One of the requirements for achieving these clinical applications is development of completely defined xeno-free systems for large-scale cell expansion and differentiation. Previously we demonstrated that microcarriers (MC) coated with mouse laminin-111 (LN111) and positive charged poly-l-lysine (PLL) critically enables the formation and evolution of cells/MC aggregates with high cell yields obtained under agitated conditions. In this paper we further improved the MC system into a defined xeno-free MC one in which the MCs are coated with recombinant human laminin 521 (LN521) alone without additional positive charge. The high binding affinity of the LN521 to cell integrins, enables efficient initial HES-3 cell attachment (87%) and spreading (85%) which leads to generation of cells/MC aggregates (400 µm in size) and high cell yields (2.4-3.5 x 10^6 cells/ml) within 7 days in agitated plate and scalable spinner cultures. The universality of the system was demonstrated by propagation of an induced pluripotent cells line in this defined MC system. Long-term pluripotent (>90% expression Tra-1-60) cell expansion and maintenance of normal karyotype was demonstrated after 10 cell passages. Moreover, tri-lineage differentiation as well as directed differentiation into cardiomyocytes was achieved. The new LN521 based MC system offers a defined, xeno-free, GMP compatible, scalable bioprocessing platform for the production of hPSC with the quantity and quality compliant for clinical applications. Use of LN521 on MCs enabled a 34% savings in matrix and media costs over monolayer cultures to produce 10^8 cells.

F-1522

INTER-ALPHA-INHIBITOR PROMOTES ATTACHMENT, SURVIVAL AND PROLIFERATION OF BOTH HUMAN AND MOUSE PLURIPOTENT STEM CELLS ON STANDARD PLASTIC IN SERUM-FREE MEDIA

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Pluripotent stem cells (PSCs) such as embryonic stem cells (ESCs) and induced pluripotent stem (iPSCs) have traditionally been grown on feeders or complex protein coatings in media containing serum or serum derivatives. However, these methods exhibit high variability and are therefore incompatible with applications requiring good reproducibility. Recently, several chemically defined media formulations have been described, but still require a coating agent. Several functionalized surfaces, the majority derived from integrin binding proteins, have been shown to support PSC attachment and growth, however they all require a costly and time-consuming coating step. Inter-α-inhibitor (IαI), a major component of human serum, is well known as an extracellular matrix remodeling protein and protease inhibitor, but recent studies indicate that IαI also plays a role in cell migration, proliferation and differentiation. We show, for the first time, that purified human IαI supplemented serum-free media is a xeno-free culture method that supports human and mouse PSC attachment, survival and long-term propagation on standard plastic for at least 30 passages maintaining pluripotency, differentiation capacity and genetic stability. In contrast to the standard E8 protocol in which human PSCs are cultured on vitronectin peptide-coated plates, E8 medium supplemented with IαI at the seeding step (herein named E8:IαI), is a xeno-free method that supports single cell passaging of human PSCs even in the absence of Rho-associated kinase inhibitor (ROCK) inhibitor. So far, eight different human PSC lines have been successfully adapted to the new E8:IαI protocol. Moreover, adaptation to E8:IαI from feeder cell culture proved to be highly efficient, roughly 400% improvement over vitronectin. The NCL-1 hESC line, which has previously proved intractable to feeder-free culture, was also successfully adapted to E8:IαI. IαI can be produced in large amounts at low cost, as our starting material is a side-fraction from human factor IX commercial production. In conclusion we present an improved and simplified culture method that is chemically defined, xeno-free and does not require any coating step. We anticipate that it will facilitate research use as well as clinical applications of PSCs and their derivatives.

F-1523

AN AUTOMATED IPSC-BASED TEST PLATFORM (DROP-TECH®) FOR TOXICITY ASSESSMENT OF SMALL MOLECULE COMPOUNDS IN EARLY STAGE DRUG DISCOVERY

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Safety failures due to toxicity of small molecule compounds are one of the major causes for high attrition rates in phase II and III clinical studies. Optimizing preclinical toxicity studies in physiologically more relevant models with a special focus on organ specific toxicity, in particular for cardiac, neuronal and hepatocellular tissues is of key importance. Induced pluripotent stem cells (iPSC) generated from somatic cells and differentiated into target tissue are considered as powerful tools with clear advantages over animal models and compensate for the lack of primary cells of human origin. We generated an automated embryonic stem cell test (EST) based on iPSCs as a model to assess toxicity in vitro. It models fundamental mechanisms in embryotoxicity, such as cytotoxicity and differentiation. Three endpoints are assessed to predict the toxic potential of a test substance, namely the inhibition of differentiation into beating cardiomyocytes, the cytotoxic effects on iPS cells and the cytotoxic effects on fibroblasts. We used quantifiable morphological assessment of contracting cardiomyocytes as an endpoint for differentiation in addition to highly predictive protein and expression markers specific for developing heart tissue. With this platform, the toxic potential of a compound can be assessed in vitro within 2-3 weeks.

F-1524

EFFICIENT ESTABLISHMENT AND LONG-TERM MAINTENANCE OF 3-DIMENSIONAL MOUSE INTESTINAL ORGANOIDS USING A NOVEL DEFINED AND SERUM-FREE MEDIUM

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The intestinal epithelium is rapidly renewed by Lgr5+ stem cells located at the crypt base. Recent advances in stem cell culture techniques have given rise to the organoid model that recapitulates the mammalian intestine in vitro. Isolated intestinal crypts are cultured in a 3D, semi-solid environment consisting of Matrigel® and a culture medium that is designed to mimic the intestinal stem cell niche. We have developed a serum-free medium, IntestiCult™ Organoid Growth Medium (OGM) that supports efficient establishment and long-term maintenance of mouse intestinal organoids. To address the importance of growth medium in this cell culture system, we performed an analysis of key experimental procedures required by users of the organoid system. Crypts were isolated from mouse upper intestines and re-suspended in a 50:50 mix of Matrigel® and IntestiCult OGM. Droplets of the suspension were placed in individual wells of pre-warmed culture plates to create domes containing ~300 crypts each and flooded with IntestiCult OGM. Crypts were cultured at 37°C with 3 medium changes per week. New crypt-like structures typically budded from organoids within 3 days of plating. After 7 days, organoids were dissociated using non-enzymatic medium and mechanical disruption into smaller aggregates. The resultant suspension was split 1:6 to establish secondary and subsequent cultures. Organoids were scored for morphology and characterized by immunocytochemistry (ICC) for specific markers at each passage. Efficiency of organoid formation from primary crypts was 64 ± 8% (mean ± SD; n=6) by day 5, yielding ~190 organoids per well. Organoids generally consist of an inner lumen surrounded by a complex arrangement of multiple crypt-like buds. Organoid formation efficiency increased to 86 ± 4% (n=4) after the first passage and remained consistently >85% over at least 12 passages (90 ± 3%, n=3). Markers for intestinal stem cells (Lgr5), polarized enterocytes (Villin), goblet cells (Muc2), enteroendocrine cells (Chg A), and Paneth cells (Lysozyme) were detected by ICC at each passage. In summary, IntestiCult™ OGM is a serum-free and defined medium for efficient formation and expansion of primary mouse intestinal organoids that provides a cost effective, valuable tool for studying intestinal function or stem cell biology ex vivo.

F-1525

CAS9/RNA-MEDIATED GENE EDITING IN MOUSE EMBRYONIC STEM CELLS

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Mouse pluripotent stem cells, either mouse embryonic stem cells (mESCs) or induced pluripotent stem cells (miPSCs), represent an excellent platform to study processes of developmental biology, model disease in vitro and in vivo or perform drug discoveries. In the last few years, the emergence of gene editing techniques based on engineered nucleases such as zinc-finger nucleases (ZFN) or transcription activator-like effector nucleases (TALENs), opened a wide range of gene targeting possibilities. However, the use of this technology has been limited by the complexity in the design, production and validation of these nucleases. Recently, the development of precise genome engineering tools based on the RNA-guided Cas9 nuclease from the type II prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR) adaptive immune system, enable efficient and easy to engineer targeted genome modifications in mammalian cells. Using this technology, we have generated a highly controlled doxycycline-inducible Cas9 mESC line, which harbor one single copy of Cas9 located in the type I collagen gene. Lentiviral delivery of small guide RNAs (sgRNAs) in doxycycline-treated mESCs demonstrated to be a very efficient manner to generate specific knockout mESCs lines. We have optimized the pipeline to obtain single or double knockout mESC lines in less than 5 weeks. These mESC lines could be used for in vitro or in vivo assays. Furthermore, we also generated a mESC library of knockout cells to perform forward genetic screenings and results will be presented. Finally, we will discuss our results regarding the generation of a doxycycline-inducible Cas9 mouse model for in vivo gene targeting.

F-1526

INTEGRATING BIOPROCESS OPTIMIZATION AND OMICS TOOLS TOWARDS THE DESIGN OF NOVEL CARDIAC STEM CELL THERAPIES

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The challenges of developing cell therapies are manifold including the lack of expertise in product characterization and specialized cell manufacturing. Our work has been focused on production and characterization of challenging SC-based products for application in both autologous and allogeneic cell therapies for cardiac repair: cardiomyocytes (CM) derived from induced pluripotent SC, human mesenchymal (hMSC) and cardiac SC (hCSC). Herein, a systematic approach was developed using robust methodologies for up- and down-stream bioprocessing. We have also applied proteomic-based protocols, transcriptomic and metabolomic tools to allow a more comprehensive characterization of cell quality and to support bioprocess optimization towards high cell productivities. Our strategy for iPSC-derived CM production consisted in designing an integrated bioprocess by combining expansion, differentiation and cell lineage selection steps in environmentally controlled bioreactors operating in perfusion. By optimizing different bioprocessing parameters we were able to improve by 1000-fold the cardiomyocyte differentiation yields and obtain a pure population of functional CM. Metabolome and fluxome analysis is being applied along the differentiation process. This quantitative study will improve our understanding on the contribution of metabolic pathways to the regulation of hPSC self-renewal and the metabolic requirements associated with CM differentiation. We developed an efficient protocol for scalable expansion of hMSC and hCSC using microcarrier-based stirred culture systems. For downstream bioprocessing, novel filtration-based methodologies were applied for harvesting, concentration and washing of cells after expansion in bioreactor systems, ensuring high recovery yields (>80%) of cells with high viability, quality and

potency. Mass spectrometry tools (nanoLC-MS) have been applied to obtain a comprehensive characterization of hCSC secretome and receptome. Receptome analysis rendered the identification of more than 2000 proteins, including plasma membrane proteins, cell surface markers (e.g. myoferlin) and more than 100 plasma membrane receptors (e.g. EGF receptor, frizzled family receptor 6).Secretome data are still under evaluation. Up to now about 500 proteins were identified.

F-1527

3D RECONSTITUTED VASCULAR-NSC NICHE AND THE HOMEOSTATIC FUNCTION OF VASCULAR MICROENVIRONMENT IN NSC MAINTENANCE

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Neural stem cell niche is a specialized microenvironment that maintains the capacity of both self-renewal and multipotency of neural stem cells (NSCs) through various microenvironmental cues including growth factors, and interactions of cell-cell and cell-ECM. The most key component in NSC niche is the vasculature. NSCs closely locate to and interact with blood vessels in order to maintain their homeostasis. To increase our knowledge of the mechanisms governing the behaviors of NSCs, it should be required to comprehensively understand their instructive microenvironment. Here, to investigate the role of vascular microenvironment(VE) in NSCs' behavior and homeostasis, we reconstituted 3D vascular-NSC niche in a microflulidic system. Microfluidic system is composed of PDMS containing the 150µm height of micropatterend channels. The channels comprise 3 units; two side channels for vascular formation and one central channel for 3D NSC culture in ECM. To reconstitute 3D vascular-NSC niche, NSCs isolated from embryonic brains (E13) were mixed with collagen solution (pH 7.4, 2 mg/ml) and injected into the central channels. After gelling collagen containing NSCs by incubating at 37°C in 5% CO^2 for 30 minutes, mouse brain endothelial cells (bECs) were seeded into two side channels. With the reconstituted 3D vascular-NSC niche, we evaluated the effects of VE on NSC behavior under different circumstance including basal, proliferation (PRO) and differentiation (DIFF)-inducing conditions. As a result, the VE enhanced NSC self-renewal and regulated its differentiation fate under the basal condition. In comparison with NSCs in the absence of VE, those in the presence of VE maintained relatively high self-renewal capability, and mostly differentiated into gila cells, not neurons. We also observed that NSCs, closely located to VE (within 160 µm), tended to differentiate into astrocytes and extend their feet toward bECs. VE differently responded and consequentially effected in NSCs' fate under DIFF-and PRO inducing condition. Under the DIFF-inducing condition, VE alleviated NSC differentiation and promoted proliferation to maintain NSC homeostasis, but, under the PRO-inducing condition, VE affected on only NSC proliferation by decreasing its self-renewal with GFs consumption for NSC homeostasis.

F-1528

NON-INVASIVE FOCUSED ULTRASOUND IMPROVES TARGETING AND THERAPEUTIC EFFICACY OF NEURONAL-PRIMED MESENCHYMAL STEM CELLS IN A RAT MODEL OF PARKINSON'S DISEASE

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Stem cells are an attractive candidate to treat neurotraumatic and neurodegenerative diseases; however their therapeutic efficacy is often limited by the insufficient delivery of systemically administered cells or undesirable side effects at sites. For the clinical use, the therapeutic risk associated with delivery and efficiency needs to be reduced. Focused ultrasound may suggest as an innovative approach, which is non-invasive intervention to promote drug delivery to the brain. Here, we induced primed-neurospheres (pNS) derived from microRNA (miR)-treated bone marrow mesenchymal stem cells (MSCs), and evaluated pathology as well as behavioral recovery. We selected three miRs, which are involved in the proliferation and differentiation by regulation of hypoxia inducible factor (HIF)-1α. MiR complex induced neural differentiation from MSCs, expressing early neuronal proteins. Furthermore, these cells generated nestin-positive neurospheres and dopaminergic (DA) neurons. The efficiency of MSC and pNS delivery was validated in 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson's disease (PD). We have used low intensity focused ultrasound (LIFUS) to open transiently the blood-brain barrier (BBB) to improve the stem cell transplantation into 6-OHDA lesion of the brain. LIFUS was delivered with 0.2~1.0 KPa to selectively stimulate with 3.5 W/cm2 of acoustic intensity. We transiently confirmed BBB opening stained with Evans blue by using microbubbles. Transplantation of MSC and pNS revealed cell survival of 7 days compared to cell-transplanted group without LIFUS treatment in the striatum and substantia nigra. In this region, tyrosine hydroxylase positive cells were detected with Laminin between the grafted cells and host cells. We also explored greater amelioration of behavioral impairments response to amphetamine and apomorphine for 4 weeks; however there was little behavioral change in control group. These data provide evidence that LIFUS can offer effective delivery, and promote therapeutic efficacy for non-invasive stem cell therapy to clinical PD. *This work was supported by research fund of Catholic Kwandong University International St. Mary's Hospital (CKURF-201407050001).*

F-1529

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

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The need for predictive, in-vitro cardiac safety or neurotoxicity screening drives further development of automated, high-throughput compatible drug evaluation systems. Recently, pluripotent stem cells are evaluated as a new, more predictive model for cardiovascular risk assessment or neurotoxicity screening pertaining to in-vitro assays. We have developed cell handling protocols for the use of induced pluripotent stem cell derived (iPSC) cardiomyocytes and neurons on planar patch clamp systems. We also screened iPSC cardiomyocytes by using a new hybrid screening method that combines impedance (cell contractility) with MEA-like extracellular field potential (EFP). These electrophysiological measurements provide a non-invasive, label-free, high temporal resolution approach for iPSC screening. We present chip-based approaches, which allow parallel patch clamp recordings without compromising neither data quality nor sophistication regarding technical features. Development of a miniaturized, modular system, and its integration in fully automated robotic platforms enables highly efficient, parallel ion channel screening with the chip-based approach in the industry standard of the microtiter plate format. While cell usage is a crucial constraint with cells of limited availability, such as primary 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. In summary, 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.

F-1530

CONSTRUCTING PERIPHERAL NERVE-INNERVATING CELL CULTURE MODEL DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS USING MICROFABRICATION TECHNIQUE

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Peripheral nervous system governs activities of inner organs and various body movements. Due to its limited proliferative and regenerative potentials, loss of peripheral neurons caused by injuries or neurodegenerative diseases is responsible for fatal cognitive and motor disabilities. Thus, generation of peripheral neurons in vitro and establishment of co-culture system of peripheral neurons and target organ tissues are useful for clarifying the progressive mechanism of peripheral nervous-related diseases. In this study, we attempt to develop a co-culture method of human induced pluripotent stem cell-derived peripheral neurons and another tissue cells (e.g. central neurons and cardiac myocytes), in order to construct peripheral nerve-innervating cell culture models. Peripheral and central neurons and cardiac myocytes were differentiated and generated from 201B7 human induced pluripotent stem cells with appropriate small molecules treatments. Then, by using a microfabricated poly(dimethylsiloxane) device having two culture compartments and connecting microchannels, we compartmentalized human induced pluripotent stem cell-derived peripheral neurons and another tissue cells, and connect them via the microchannels. The microfabrication based peripheral nerve-innervating cell culture model would provide useful experimental platform for peripheral nervous-related diseases.

F-1531

SCALABLE MONOLAYER PSC CULTURE ON LN521 WITH ROBUST SINGLE CELL PASSAGE AND FREE WEEKENDS UNDER XENO-FREE AND DEFINED CONDITIONS

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The lack of defined, xeno-free, easy and robust methods for efficient expansion of human pluripotent stem cells (hPSC) has hindered both the advancement of basic research, due to high experimental variation and poor quality cells with phenotypic and genetic changes, and human cell therapy requiring absolute safe methods and large numbers of low passage cells. By using a human recombinant protein naturally expressed by hPSCs, LN-521, we can culture hPSCs for over 80 single cell passages without any abnormal genetic aberrations and with maintained expression of pluripotency markers. Cells cultured on LN-521 grow twice as fast compared to other matrices and can be split 1:20 or up to 1:30 as single cells without the addition of artificial ROCK inhibitor. The simplicity and reliability of the procedure, speed of cell amplification and the genetic stability of the cells make LN-521 suitable as reagent in clinical trials for PSC-based therapy. Furthermore, true clonal growth, important for cell fate tracking, gene function analyses and editing, without inhibitors of anoikis, is possible by using LN-521 and E-cadherin. The same authors also demonstrated chemically defined and xeno-free derivation of new clinical hESC lines from single blastomers. In essence, circumventing the ethical dilemma of destroying the surplus embryos donated by couples going through fertility treatments. LN-521 provides a biorelevant niche for hPSCs and we are now able to show that hPSCs can be cultured without the need of daily feeding. Data from 10 consecutive passages on three different lines reveal no significant differences in cell morphology, proliferation or the expression of pluripotency markers such as Oct4 and Nanog. The results show that the expression levels are similar to both the daily fed group and starting samples. Chromosome analysis after 10 passages indicated normal karyotype for all groups. In conclusion, we show that LN-521 is an optimal matrix for hPSC culture due to its biological relevance allowing derivation, clonal cultivation and robust long-term pluripotent cell growth. 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.

F-1532

APPLICATION OF THE CRISPR/CAS9 SYSTEM FOR EFFICIENT GENE TARGETING IN COMMON MARMOSET ESCS

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Gene targeting technology in non-human primate embryonic stem cells is an important tool for preclinical study and for human hereditary disease research. However, gene targeting in non-human primate ESCs has not been well-developed. CRISPR-Cas9 system is a recently developed genome editing tool using directed DNA double strand break (DSB). In this study, we established the CRISPR/Cas9 system in ESCs of common marmoset, a small non-human primate, and evaluated the effect on gene targeting efficiency. For this purpose, we constructed Cas9/gRNA expression vectors and targeting vector for the marmoset ACTB locus. The targeting vector was introduced into common marmoset ESCs with or without Cas9/gRNA expression vector. After drug selection, the numbers of colonies were counted and their genotypes were examined. Further, the effect of homology arm length of targeting vector in homologous recombination efficiency was examined. As a result, the application of CRISPR/Cas9 system increased the efficiency of homologous recombination by gene targeting in common marmoset ESCs. The targeting vector with shortened homology arm could also generate homologous recombinants efficiently. Next, by using this technology, we demonstrated gene targeting in Proteolipid protein 1 (PLP1) gene, a responsible gene of Pelizaeus-Merzbacher disease, known as a hereditary leukodystrophy. A targeting vector and Cas9/gRNA expression vector for the PLP1 locus were introduced into common marmoset ESCs and homologous recombinants were successfully obtained. Taken together, this gene targeting strategy is useful for disease modeling in common marmoset ESCs.

F-1533

A THREE-DIMENSIONAL BONE MARROW STROMAL CELL DERIVED EXTRACELLULAR MATRIX INCREASES MESENCHYMAL STEM CELL PROLIFERATION AND INTENSIFIES THEIR ANTI INFLAMMATORY PROPERTIES

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Mesenchymal stem cell (MSC) applications for regenerative medicine and treatment of diseases associated with a robust inflammatory response have shown great potential due in part to MSC trophic factor production. The critical barriers associated with such applications include obtaining high numbers and high quality, multipotent MSCs. We have developed a three-dimensional, bone marrow stromal cell-derived extracellular matrix (ECM) that increases the yield and potency of MSCs when compared to tissue culture plastic. Mass spectrometry and scanning electron microscopy analyses indicate that the ECM is a complex protein network composed primarily of extracellular matrix proteins organized as a three-dimensional structure. Culturing of adipose-, bone marrow-, and cord blood-derived MSCs on bone marrow stromal cell-derived ECM improved the cell yield by a minimum of 2-fold when compared to tissue culture plastic. Specific for bone marrow MSCs, cells expanded on ECM had increased expression of stage specific embryonic antigen (SSEA)-4, a multipotent MSC marker (72±11% for ECM and 30±6 % for tissue culture plastic). Concomitantly, gene expression analysis demonstrated that bone marrow MSCs cultured on ECM had amplified expression of anti-inflammatory factors interleukin-10 and transforming growth factor beta-3 (12 fold and 1.8 fold when compared to tissue culture plastic, respectively). These results indicate that bone marrow stromal cell-derived ECM efficiently expands and improves the quality of MSCs versus culture on tissue culture treated plastic.

F-1534

CELL LINE AUTHENTICATION USING SNP TRACE™: A HIGH-THROUGHPUT, COST-EFFECTIVE PLATFORM FOR FINGERPRINTING, IDENTIFYING CONTAMINATION, AND DETERMINING CELL LINE ORIGIN

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Induced pluripotent cell research generates an abundance of derivative cell lines. Curating thousands of cell lines is challenging and essential for effective annotation and quality maintenance. Misidentified and cross-contaminated cell lines are ongoing problems. It is estimated that 15%-35% or more of human cell lines are misidentified, resulting in a waste of resources and publication of false or misleading data. The current ANSI standard for cell line authentication is short tandem repeat (STR) profiling, a methodology with several acknowledged disadvantages causing misclassification. Here we introduce and evaluate a high-throughput panel of 96 single-nucleotide polymorphism (SNP) assays on Fluidigm® microfluidics technology for authentication of human cell lines and tissues. The Fluidigm SNP Trace Panel was tested on 907 human cell lines previously characterized by 8- or 16-locus STR profiling. Pairwise comparison of the 907 cell lines indicated SNP Trace discriminated between unrelated samples with a high degree of confidence. We also report gender typing of cell lines and show SNP Trace was able to identify cell lines from a single origin and detect intrahuman cross-contamination as low as 5%, which is equal to or better than detection by STR. In conclusion, SNP Trace rapidly and accurately identified the fingerprints of human samples in a cost-effective and time-efficient manner. This study has generated a database of 907 SNP fingerprints for future comparison and provides a reliable, fast, and economical alternative to STR profiling.

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

F-1537

INVESTIGATING THE MOST EFFECTIVE FORM OF CONVEYING LECTURE MATERIAL: IS IT TIME TO FLIP BACK THE FLIPPED CLASSROOM?

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We assessed the efficacy of traditional lectures versus online videos with respect to student learning in an undergraduate introductory biochemistry course (SCRB 25 - Biochemistry and Human Metabolism) via a prospective randomized study. Students were initially randomized to one of two groups - attendance of in-class lectures only versus use of pre-recorded online videos only - for the first half of the term and then crossed over to the other group for the second half of the term. Pre-course, mid-course, and post-course assessment tests/surveys that covered the course material allowed us to independently assess the effects of traditional lectures and online videos on student learning for the two halves of the course. While active learning has been established to improve student performance in science, technology, engineering, and mathematics (STEM) courses, our study examines whether pairing active learning sessions with traditional lectures may lead to superior performance compared to pairing with online videos, i.e., the flipped classroom model. These findings may have important implications in today's educational landscape.

F-1538

DATA SHARING IN STEM CELL TRANSLATIONAL SCIENCE: A POLICY STATEMENT BY THE INTERNATIONAL STEM CELL FORUM ETHICS WORKING PARTY

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Last years the stem cell field has thrived, particularly due to the convergence of stem cell science with genomic technologies enabling intense genetic characterization of stem cell lines. Today, the sharing of genomic and health-related data for biomedical research to achieve statistical significance and to foster translational medicine in the field of stem cell science is of the utmost importance. Indeed, international data sharing stimulates scientific progress and is more efficient and economical. Yet, in the absence of proper governance and security, the risk of privacy infringements of research participants and their family members may increase. Data and sample sharing constitute a scientific and ethical imperative but need to be conducted in a responsible manner in order to protect individual interests as well as maintain public trust. In 2014, the Global Alliance for Genomics and Health (GA4GH) adopted a common *Framework for Responsible Sharing of Genomic and Health-Related Data* (hereinafter the *"Framework"*). The goal is to develop harmonized approaches both to enable effective and responsible sharing of genomic and clinical data and to catalyze data sharing projects that drive and demonstrate its value. A prerequisite for applying this *Framework* in practice is the use of data in compliance with national and international laws, general ethical principles, and best practice standards that respect restrictions on downstream uses. The GA4GH *Framework* is applicable to data sharing in the stem cell field, however, adaptation and interpretation is required so as to provide guidance for this specific context. In this presentation, we apply the principles and core elements of the GA4GH *Framework* to the context of stem cell science. Although all the (interconnected) principles *of the Framework* and *Stem Cell Charter* apply, the International Stem Cell Forum (ISCF) Ethics Working Party here limits itself to further discussion of those principles that are specific to the aims of translational stem cell research, including (1) engagement, (2) data quality and safety, (3) privacy, security and confidentiality, (4) risk-benefit analysis and (5) sustainability.

F-1539

A NEW MORAL STATUS FOR PARTHENOGENETIC DERIVED HUMAN "EMBRYOS" AND ITS IMPLIFICATION FOR STEM CELL RESEARCH, PATENTABILITY, AND THERAPY IN EUROPE

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In the aftermath of the European Court of Justice's (CJEU) decision in the case of Brüstle v Greenpeace of 2011 that patent claims encompassing embryonic stem cells (hESCs) extracted from of human embryos were patent-ineligible in the European Union on morality grounds a rash of stories has predicted the destruction of hESC research in Europe. However, stem cell research has continued since then and brought a new legal question to the CJEU. The new case concerned the patentability of parthenogenetic activation of oocytes (parthenotes) for the production of hESCs. Such activated ova can develop into the blastocyst phase but are unable to develop further into a complete human being. In December 2014 the CJEU ruled that the principle of the Brüstle case must be taken to mean that in order to be classified as a 'human embryo', an unfertilised human ovum whose division and further development have been stimulated by parthenogenesis must necessarily have the 'inherent capacity of developing into a human being'. The Court stressed that the emphasis is on whether the parthenote can develop into a human being, rather than whether it can commence this process. First, this ruling is a turning away from the Brüstle ruling, since parthenotes were considered to be human embryos in Brüstle. Second, the ruling applies to all techniques which produce human non-totipotent entities since the ruling abstracts to all human entities which do not have an inherent capacity of developing into a human being. However, the Court left open what "capacity of developing into a human being" means, it did not determine a minimum developmental stage. Therefore, it is still unclear what a human being or a human embryo is - at least - in patent law. For this reason, this project brings forward the argument that - legally - it is necessary to define a human embryo not (only) by a potential developmental process (i. e. totipotency), but by a certain developmental stage (e. g. blastocyst, neural groove, or other justifiable stage) which has to be at least theoretical reachable (reference point) for the concrete entity. Additionally; it must be pointed out, that the word "embryo", even if it is defined by a developmental stage, is just a linguistic operator and different from the subsequent constitutional question on how to treat legally prenatal (developing) human entities.

F-1540

SECURING INFORMED CONSENT AT STEM CELL DRIVES: AN IMPLEMENTATION OF WORLD MARROW DONOR ASSOCIATION GUIDELINES

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Securing informed consent at time of donor registration to a stem cell donor-database is an important moral, ethical, and legal obligation. Moreover, two studies by Switzer et al. (2003, 2004) found that donors who felt less informed at various points in the donor recruitment, evaluation, and workup process were more ambivalent about donation and less likely to proceed with the donation process if asked. In 2003, the World Marrow Donor Association (WMDA) published a set of suggested procedures for securing informed consent of potential stem cell donors. However, implementation

of these guidelines at stem cell drives remains unclear. We have previously proposed that stem cell drives should include five components: prescreening, informed consent, registration, swabbing, and reconciliation. Here, we propose an approach to implement WMDA's informed consent guidelines at stem cell drives. We have adapted the WMDA guidelines to apply to the five station stem cell drive design. At the prescreening station, potential donors learn about the principles of stem cell donation, and are informed about donor eligibility requirements including health restrictions. At the informed consent station, registrants are handed an information brochure, shown diagrams illustrating donation procedures, and educated about potential risks of donation, donor/patient anonymity, donor nonremuneration, and the donor's right to withdraw. At the registration station, registrants complete and sign a consent form, and learn about the process for data collection, storage, usage, and confidentiality. At the swabbing station, the registrant is asked several probing questions to confirm they understand the material covered in the informed consent station. Finally, at reconciliation, registrants are asked if they have any additional questions, and they are instructed of their responsibility to update their health/contact information as applicable. By presenting this novel approach to securing informed consent, we aim to contribute to the discussion regarding the optimal strategies for stem cell donor education at time of recruitment to a donor-database. Our proposed implementation of WMDA's informed consent guidelines could be easily adopted by any individuals or groups that organize stem cell drives.

F-1541

DILEMMAS OF TREATING PARKINSONS DISEASE WITH STEM CELLS

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Should we treat patients with Parkinson's disease(PD) using stem cells? Are fetal, cord tissue adult derived or even induced pluripotent stem cells (iPSC) the ethical solution to the moral issues regarding embryonic stem cells? How should we design and devise protocols to conduct pilot studies using stem cells in PD to assess their safety and efficacy? Over the last several years there has been a revolution in our team`s ability to make stem cells from different sources and use them for therapeutic gain in disorders of the brain especially PD. These experiences have helped us to determine the best strategies to be adopted and the potential chances for success in developing new cell therapies to clinical application in different stages of PD. We have steadfastly adhered to scientific, technical, ethical, regulatory and logistic issues in effectively translating laboratory cell-based protocols to patients in clinical trials i.e.translating from bench to bedside. We are quite optimistic about the likelihood of success in developing radical new approaches to a range of devastating, and currently difficult to treat or even untreatable, neurodegenerative conditions, however we also caution that the problems are and will be far more, complex and the solutions are likely to be slow paced and very costly to achieve, in order to overcome significant ethical and regulatory as well as scientific challenges.

F-1542

RECOGNITION AND FACT IN CLINICAL TRIALS OF STEM CELL TRANSPLANTATION FOR SPINAL CORD INJURY

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Numerous clinical trials of stem cell transplantation (SCT) had been performed to patients with spinal cord injury (SCI) since almost a decade before, however SCT is not approved as one of treatment tools for SCI in the clinical setting until now. The aim of this study is to delineate the recognition of clinicians and SCI patients about SCT including stem cell-received patients in Korea, and find a way to move forward to the clinical field for stem cell therapy. Total 188 patients with chronic SCI including 20 SCT-received patients and 51 clinicians were conducted a survey; SCT-performed and non SCT-performed clinicians, and SCT-received and non SCT-received SCI patients. The survey constituted common and group-specific questions related to recognition about stem cell therapy, national policy and supports for SCT. For SCT-received patients, physical examination including neurological and functional assessments were also performed and compared with physical status before SCT in their previous medical recording. SCT-received patients had a lot of expectation of moderate-to-complete functional improvement following SCT (70%) compare to non SCT-received patients groups (27.4% for patients and 43.5% for clinicians). All participants worried about complications following SCT, and agreed with necessity of active rehabilitation program even after SCT. Functional improvement following SCT was not obvious in SCT-received patients. About 15% of them felt some improvements following SCT, however physical examination revealed that neurological level of injury was not changed (60%) in most cases, and 20% cases showed even worsening. The proportion of bladder dysfunctions including frequency and incontinence was not different between non SCT-received and SCT-received patients. Some of SCT-received patients suffered from side effects following SCT, and neuropathic pain was more obvious than non SCT-received patients (95% versus 63.1%). We found that all clinicians and SCI patients had the same opinion and recognition to stem cell therapy, and stem cell received patients had a lot of expectation in stem cell therapy. However we could not conclude that stem cell therapy enhance any neurological nor functional improvements in SCI patients, and neuropathic pain was increased more following stem cell transplantation.

F-1543

DESIGNING GMP FACILITY FOR CLINICAL-GRADE PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) hold a wide variety of potentials for regenerative medicine, such as applications in disease modeling, drug discovery, and cellular therapy. Many of these applications require expansion of hPSCs under settings compliant to good clinical practices. As a core infrastructure of regenerative medicine using hPSCs for starting material of cellular therapy and tissue engineering, we have been constructing National Center

for Stem Cell and Regenerative Medicine which contains three major facilities: good manufacturing practice (GMP) facility for the production of clinical-grade hPSCs, laboratories for the quality control and banking of the research-grade hPSCs. Designing of GMP facility for the hPSCs is challenging since the standard processes for the production of clinical-grade hPSCs have not been completely established yet. Additionally, unlike to the chemical drugs, production of hPSCs in the GMP facility needs to consider efficient expansion and contamination issues at all of the work flow. Here we present our preliminary design draw for GMP facility and process flows.

CANCER CELLS

F-1545

ESTABLISHING A NOVEL IN VITRO MODEL OF PROSTATE CANCER STARTING FROM PROSTATE STEM/PROGENITOR CELLS

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Cell lines representing the progression of prostate cancer are scarce. Previously, we have established and characterized a new prostate luminal epithelial cell line (PLum), with *Pten/TP53* deletions, derived from a prostate epithelial stem/progenitor-enriched cell population. The deprivation of androgens from established PLum-orthotopic tumors resulted in tumor regression and eventually castration-resistant growth. Cells derived from orthotopic tumors have been isolated to develop androgen-dependent versus androgen-independent model. In this study, several experiments were conducted to establish and investigate the functional differences of the newly isolated androgen-dependent (PLum-AD) and androgen-independent (PLum-AI) prostate cancer cell lines. Unlike PLum-AD cells that grew in serum-free medium, PLum-AI cells grew better in 5% FBS-containing medium. Both cell lines remained faithful in morphology to their in vivo source, where PLum-AD showed a typical epithelial morphology (in vivo source: adenocarcinoma) and PLum-AI showed an epithelial-to-mesenchymal morphology (in vivo source: sarcomatoid carcinoma). Furthermore, upon immunofluorescent analysis, PLum-AD cell expressed mostly prostate epithelial markers while PLum-AI cells expressed mesenchymal cell markers. In addition, QRT-PCR and Western blot analysis confirmed the epithelial and mesenchymal morphology of PLum-AD and PLum-AI respectively. To assess for the presence of stem/progenitor cell population, the cells were subjected to sphere-formation assay. Both cell lines had the capacity to form spheres, where PLum-AD cells formed regular-shaped spheres and PLum-AI cells formed mostly large stellate shaped spheres consistent with their mesenchymal-like nature. In addition, both cell lines are tumorigenic where PLum-AD resulted in an adenocarcinoma while PLum-AI resulted in a sarcomatoid carcinoma when transplanted subcutaneously in NOD-SCID mice. Altogether, these data suggest that the newly isolated cell lines represent a new in vitro model of prostate cancer progression. More work has to be done to decipher the molecular mechanisms involved in this progression that would eventually lead us to new therapeutic targets.

F-1546

TUMOUR-INITIATING CELL FUNCTION AND SENSITIVITY TO GENOTOXIC STRESS IS CONTROLLED BY PROTEIN TRANSLATION RATES

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Although elevated protein synthesis is key physiological tasks for cancer cells, the role of mRNA translation regulation in tumour-initiating cells is not yet understood. Recent studies have revealed that tissue stem cells produce less protein than their progeny. However whether protein translation is also low in tumour-initiating cells and whether protein translation rate is cause or consequence of the tumour-initiating cell state remains unclear. Here, we show that tumour-initiating cells from squamous tumours synthesize less protein than their immediate progenitors in vivo. Mechanistically we show that inhibition of post-transcriptional cytosine-5 methylation of transfer RNAs alter their processing, which in turn activate stress responses. Our analysis reveal that activation of stress response pathways drives both a global reduction of protein synthesis and altered translation of specific mRNAs that together promote stem cell functions and tumourigenesis. We conclude that tumour-initiating cells must revoke translation inhibition pathways and module their translational programme to regenerate a tumour.

F-1547

IN VIVO SCREENING OF TRADITIONAL CHINESE HERBAL MEDICINE FOR ESTROGENIC ACTIVITIES AND THEIR EFFECTS ON BREAST CANCER STEM CELLS

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Hormone replacement therapy (HRT) is commonly applied to relieve menopausal symptoms, but increased exposure to hormones exacerbates the risk of cancers, particularly breast cancer. Phytoestrogens are plant-derived compounds found in a wide range of traditional Chinese herbal medicine (TCM), which show much lower affinities to estrogen receptors (ERs) than to estradiol (E2). Moreover, phytoestrogens may act as selective estrogen receptor modulators (SERMs). In this study, TCM and their active ingredients were screened using an in vivo system; the effects of these substances on breast cancer stem cells were investigated in vitro. We used transgenic medaka for estrogenic activity screening, in which green fluorescent protein (GFP) is expressed in the liver upon exposure to estrogenic substances. Among 37 herbs and 38 active ingredients, one herb and one of its 3 active ingredients showed estrogenic activities using this in vivo screening system. To further confirm the inhibitory effects of this active ingredient on breast cancer cells, we found that the active ingredient reduced the percentage of $CD44^{+}/CD24^{-/low}$ subpopulation and the mammosphere formation in adherent MCF-7 cells. We then sorted $CD44^{+}/CD24^{-/low}$ cells from MCF-7 cells and determined the direct effects of the active ingredients on this subpopulation. Our results showed that it inhibited mammosphere formation in

CD44+/CD24−/low cells with decreased size and number and loose morphological characteristic. The ingredient also induced S phase arrest of CD44+/CD24−/low cells at low concentrations and G0G1 phase arrest of these cells at high concentrations; this cell cycle arrest was accompanied by increased levels of cell cycle regulators, including Chk2 and p21. Moreover, this active ingredient induced apoptosis and downregulated mitochondrial membrane potential of CD44+/CD24−/low cells. The apoptosis-induction effects was further supported by the upregulated expression of apoptotic regulators, including Bcl-2/adenovirus E1B 19kDa interacting protein 3 (BNIP3), death-associated protein kinase 2 (DAPK2), Cleaved Caspase 7, and PARP.

F-1548

GERMLINE DELETION OF PK-M2 IN THE MOUSE IS COMPATIBLE WITH EMBRYONIC AND POSTNATAL DEVELOPMENT BUT LEADS TO SPONTANEOUS HEPATOCELLULAR CARCINOMA

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The pyruvate kinase gene, Pkm, encodes the PK-M1 and PK-M2 isoforms, which are the result of alternative splicing of mutually exclusive exons. While PK-M1 is considered the adult pyruvate kinase isoform, PK-M2 has been closely linked to embryogenesis, tissue regeneration, and cancer. PK-M2 is therefore thought to play an important mechanistic role in meeting the specific energetic and biosynthetic requirements of highly proliferative cells. We show that, contrary to expectations, expression of PK-M2 is widespread in adult tissues and is not restricted to proliferating cell types. To interrogate the requirement for PK-M2 in the adult mouse, we generated a germline loss of function mutation of PK-M2 (Pkm2-/-). Importantly, this Pkm2-/- allele does not affect the function of the other isoform, PK-M1. Surprisingly, complete loss of PK-M2 is compatible with normal development and Pkm2-/- mice express PK-M1 throughout embryogenesis and into adulthood. Phenotypic characterization of young adult mice shows that PK-M2 mutant mice are fertile and grossly normal. Upon aging, however, a clear metabolic phenotype becomes apparent. By 62 weeks of age, 67% of Pkm2-/- male mice develop large hepatocellular carcinomas with significant stromal infiltration. Tumor development in these older mice is preceded by increased weight gain and increased fasting blood glucose. Our results demonstrate a novel role for PK-M2 in maintaining systemic energy homeostasis and thereby preventing spontaneous hepatocellular carcinoma.

F-1549

MICRORNA-600 REGULATES BREAST CANCER STEM CELLS VIA WNT/B-CATENIN SIGNALING

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Recent advances in breast cancer research highlighted the role of breast cancer stem cells (bCSCs) in tumor progression and metastasis. Better understanding of bCSCs biology such as self-renewal and differentiation programs is crucial to derive anti-bCSC therapy and improve breast cancer care. Recently, it has been proposed that microRNAs play a key role in CSC regulatory circuits. To decipher bCSCs regulatory mechanisms, we conducted two functional pan-genomic screens of oligonucleotides libraries, one containing miR-Mimic for miRNA overexpression and the other containing miRNA antagonist sequences (Locked Nucleic Acid, LNA) for miRNA knock-down. We identified the microRNA-600 as a bCSCs regulator able to switch bCSCs from a self-renewing state to a differentiating state. Using an algorithm (miRonTop) specifically designed to predict miRNA's targets, we identified stearoyl coA-desaturase 1 (SCD1) as a target gene of miR-600. SCD1 is responsible for Wnt proteins modifications by lipids. These modifications are crucial for Wnt secretion and activation of Wnt/b-catenin pathway. We validated the role of miR-600 in the control of bCSC fate through Wnt/b-catenin regulation. In vivo ectopic overexpression of miR-600 in patient derived xenografts (PDX) induces a decrease in the bCSCs frequency. Moreover, miR-600 was markedly downregulated in primary tumors samples from patient associated with a poor overall survival. Our study suggests that miR-600 is a key player in the Wnt/B catenin signaling and that further efforts toward development of miR-600-based therapeutics are fully warranted.

F-1550

IDENTIFYING AND TARGETING DRUG RESISTANT STEM CELLS IN ADENOMA

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The conventional approach has largely been unsuccessful in curing cancers, and this could be not targeting cancer stem cells (CSCs). We have hypothesized that tissue stem cells, cancer stem cells (CSCs) may have adopted dual active-quiescent mechanisms. We tested this hypothesis in the mouse model of APC^{Min}, which carries a dominant mutation ($Apc^{\Delta 850}$) that predisposes to multiple intestinal neoplasia in the mouse. 1. We first established the tumorigenic kinetics in APC^{Min} model, thus to be able to start the treatment when the tumor had

been established. We tested a novel approach of targeting cycling cancer cells with chemotherapy and quiescent drug-resistant CSCs with Cox2 inhibitor. We demonstrated that only this combined treatment with chemo and Cox2 inhibitor could reduce tumor number significantly. 2. We compared the stem cell division rates among different groups, we found that the number of tumor stem cells and their rates of division were reduced following combination treatment. This correlated well with a significant reduction of Brdu incorporation in tumors. We identified quiescent chemo-resistant cells by combining CldU (for slow cycling cells) and IdU (for active cycling cells) labeling methods. 3. We further bred APC^{min} with $Bmi1^{Cre-ER}$ and $Rosa26^{YFP}$ mice. This APC^{min} $Bmi1Cre^{-ER}Rosa26^{YFP}$ model provided evidence that quiescent drug-resistant cells enrich with Bmi1+ cells. We examined lineage tracing at different time points and found that multiple single $Bmi1^{+}$-YFP cells representing multiple clones were able to divide, further giving rise to the tumor. We are in the process of testing whether combination treatment can reduce $Bmi1^{+}$ initiated tumor growth. In summary, we have established an efficient treatment method based on understanding the cellular and molecular mechanisms underlying drug-resistance.

F-1551

CULTURED HUMAN CIRCULATING TUMOR CELLS FROM COLON CANCER WITH SELF-RENEWAL AND MULTIPOTENT POTENTIAL

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Circulating tumor cells (CTC) have attracted a broad interest in the cancer research and clinical communities but their characterization remains minimal, partly because of their small numbers and of the limited approaches to purify them. Here we designed conditions aiming to enrich and maintain in culture CTCs that display self-renewing abilities. We generated three CTC cell lines from the blood of patients with stage IV colorectal cancer (CRC). These cell lines express multiple Cancer Stem Cell (CSC) markers, dialay a high colonosphere-forming frequency in vitro, are highly tumorigenic after injection in immuno-compromised animals and displays multi-lineage differentiation ability in vitro and in vivo. We also found that these CTC lines expressed differential patterns of genes involved in drug resistance and accordingly that they were poorly sensitive to 5-FU-based cytotoxic treatment. In contrast these cells displayed, to various degrees, enhanced sensitivity to regorafenib compared to a panel of cells isolated from human colorectal tumour samples in our laboratory. Genomic analysis of one of these cell lines allowed us to detect the presence of genetic alterations in multiples genes including those encoding BRAF, APC, PI3K, P53, SMAD4 and ATR, and sub-cloning experiments led to the detection of a differentially expressed Androgen Receptor variant in some but not all cells of this cell line, suggestive of some degree of genetic heterogeneity. Our results demonstrate for the first time that isolation of CTCs using their self-renewal properties can offer critical insight into the contribution of Cancer Stem Cells towards metastasis development, and suggest that CTCs grown using this approach could be used to anticipate treatment response in patients with CRC.

F-1552

ACTIVATION OF AURORA A KINASE THROUGH FGF1 FGFR SIGNALING AXIS SUSTAIN THE NEURAL STEM CELL CHARACTERISTICS OF GLIOBLASTOMA CELLS

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Fibroblast growth factor 1 (FGF1) binds, activates FGF receptors and regulates cell proliferation and neurogenesis. Human FGF1 gene 1B promoter (-540 to +31)-driven SV40 T antigen has been shown to lead to tumorigenesis in transgenic mice. FGF-1B promoter (-540 to +31)-driven green fluorescence (F1BGFP) has further been shown to isolate neural stem/progenitor cells (NSPCs) with self-renewal and multipotency from developing and adult mouse brains. In this study, we provide several lines of evidence to demonstrate that FGF-1B promoter activity is crucial for AurA expression and kinase domain activation (Thr288 phosphorylation) in the maintenance of glioblastoma (GBM) cells and NSPCs: (i) Treatment of FGF1 increases AurA expression in human glioblastoma. (ii) Using fluorescence-activated cell sorting, F1BGFP reporter facilitates the isolation of F1BGFP(+) GBM cells with higher activation levels of FGFR and AurA. (iii) FGFR inhibitor, SU5402, and AurA inhibitor, VX680, could both down-regulate F1BGFP-dependent AurA activity. (iv) 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. (v) F1BGFP reporter could identify NSPC-like cells with higher AurA activation, self-renewal capacity and multipotency from human GBM tissue. (vi) Flow cytometric analyses demonstrated that F1BGFP(+) GBM cells possessed different NSPC markers, including CD133, EGFR, SSEA1 or SSEA4. (vii) Inhibition of AurA by VX680 reduced the neurosphere formation of human GBM cells, mouse ESCs, mouse ESC-derived NSPCs, primary mouse brain NSPCs. Taken together, our results suggest that activation of AurA kinase through FGF1/FGFR signaling axis sustains NSPC characteristics of GBM cells, thus is an important mechanism for the malignancy of GBM.

F-1553

HIGH INTERSTITIAL FLUID PRESSURE REGULATES TUMOR GROWTH AND DRUG UPTAKE IN HUMAN GLIOBLASTOMA STEM CELLS

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High interstitial fluid pressure (IFP) represents a barrier for drug uptake in human GBM, the most common malignant primary brain tumor in adults. Although studies have clarified the role of tumor vasculature, it is unclear if elevated IFP and swelling of cancer cells regulate tumor growth and drug uptake. Inhibition of NKCC1 activity with bumetanide augments temozolomide-mediated apoptosis in

vitro and blocks invasion in vivo by regulating cell volume. However, concerns about side effects following bumetanide treatment warrants development of new approaches. Induction of antisecretory factor (AF) is safe in patients and lowers IFP in subcutaneous tumors. A specially produced cereal (SPC) diet and delivery of AF peptide produce elevated endogenous and exogenous AF levels, respectively. The goals were to 1) identify the relationship between IFP levels, tumor growth and drug uptake in GBM xenografts, and 2) to test if AF-induction represents a novel IFP-reducing strategy. Mechanical compression increased proliferation of GBM stem cells grown in a 3D-matrix. We developed methodology to correlate IFP levels and tumor growth in human GBM xenografts. Incubation of 3D tumorspheres with AF blocked compression-induced proliferation in vitro. SPC-induced AF expression in tumor cells lowered IFP levels, inhibited proliferation, induced apoptosis, and increased survival in mice xenografted with human GBM stem cells. SPC diet and intranasal injection of AF increased uptake of doxorubicin in GBM xenografts. Similar to bumetanide, an inhibitor of NKCC1 activity, AF reduced restoration of cell volume under hyperosmotic conditions. In vivo measurements demonstrated that AF-induction also reduced cell volume in vivo. Our studies suggest that elevated IFP promotes tumor growth and reduces drug uptake in human GBM. AF-induction represents an attractive strategy to inhibit tumor growth, increase drug uptake, and ultimately improve the survival of GBM patients.

F-1554

ENDOGENOUS MOZ IS CRITICAL FOR MOZ AND MLL FUSION LEUKEMIA THROUGH REGULATION OF HOXA9 AND MEIS1 EXPRESSIONS

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Monocytic leukemia Zinc finger protein (MOZ), a Myst-type histone acetyltransferase, is involved in chromosome translocations associated with FAB M4 or M5 subtype of acute myeloid leukemia (AML). We have shown that MOZ was critical for self-renewal of hematopoietic stem cells (HSCs) and for expressions of HoxA9 and Meis1 that were major target of MOZ/MLL fusion genes. To clarify the roles of MOZ in AML, we introduced leukemia-associated fusion genes, such as MOZ-TIF2, MLL-AF10 and PML-RARA, into fetal liver hematopoietic stem/progenitor cells (HSPCs) prepared from MOZ+/- or -/- fetus by retrovirus system. These cells were analyzed for colony-forming ability in vitro and leukemia-inducing activity in vivo. MOZ-TIF2 or PML-RARA immortalized MOZ-/- cells, whereas MLL-AF10 did not immortalize MOZ-/- cells. However, AML was not induced in mice transplanted with MOZ-deficient cells that were transduced with either MOZ-TIF2 or MLL-AF10. Expression analysis showed that HoxA9 expression was induced by MOZ-TIF2, but not induced by MLL-AF10 in MOZ-/- cells. On the other hand, Meis1 expression was induced by neither MOZ-TIF2 nor MLL-AF10 in MOZ-/- cells. Chromatin immune-precipitation analysis indicated that MOZ-TIF2 was accumulated at HoxA9 locus but not at Meis1 locus in MOZ-/- cells. Active histone modifications were decreased and repressive histone modifications were elevated at Meis1 locus in MOZ-/- cells. These results indicate that immortalization and leukemia-inducing ability are consistent with expression of HoxA9 and Meis1, respectively. When Meis1 was ectopically introduced in MOZ-/- cells, AML development induced by MOZ-TIF2 was recovered. In contrast, when Meis1 was conditionally deleted, AML development was significantly delayed. Taken together, these results suggested that endogenous MOZ played crucial roles in MOZ/MLL fusion leukemia through maintenance of active chromatin status at HoxA9/Meis1 locus and that endogenous MOZ was candidate of therapeutic target of MOZ/MLL leukemia.

F-1555

DISEQUILIBRIUM OF BMP2 LEVELS IN THE HUMAN BREAST STEM CELL NICHE LAUNCHES EPITHELIAL TRANSFORMATION BY OVER-AMPLIFYING BMPR1B CELL RESPONSE

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The microenvironment (niche) governs stem cells (SC) fate by balancing their self-renewal and differentiation. Accumulating evidence indicates that the tumor niche plays an active role in cancer but its definition and properties for tumor initiation, progression and resistance are not yet established. While Bone Morphogenetic Proteins (BMP) are key regulators of stem cells and their niche, BMP2 and BMP4 have also emerged as important regulators of cancer stem cells. Using primary tissue, we analyzed BMP molecules expression in the breast microenvironment and signaling in normal mammary cells and tumors. We identified that under physiological conditions, BMP2 controlled the maintenance and differentiation of early luminal progenitors, while BMP4 acted on stem cells/myoepithelial progenitors. BMP2 levels appeared to be specifically increased in luminal tumors microenvironment, provided by endothelial and stromal cells while tumor cells do not produce it. Using MCF10A cell line, a model of immature human mammary epithelial cells, we investigated the impact of deregulation of BMP levels on cell transformation. Chronic exposure of MCF10A cells to high BMP2 levels in the presence of IL6 initiated transformation towards a luminal tumor-like phenotype, mediated by the receptor BMPR1B as showed by anchorage independent growth and in vivo experiments. Very interestingly, in vivo BMP2-long term treated cells organized close to neovessels that colonized the co-injected matrigel. This was not observed when we xenografted cells expressing shBMPR1B confirming that BMPR1B was required for transformation and identifying it as the predominant receptor involved in luminal tumors from the earliest stages to established tumors. Our data also suggests that microenvironment-induced overexpression of BMP2 may result from carcinogenic-pollutant exposure. We have then revealed a major role of the BMPs to fuel cell transformation and expansion by over-amplifying a natural stem cells response. Altogether, our data provide insight into the etiology of breast cancer by revealing a new mechanism through which the stem cell microenvironment represents a driving force to promote transformation and dictate the ultimate breast tumor subtype.

F-1556

CRISPR TECHNOLOGY FOR THERAPEUTIC APPLICATIONS: INDUCING GENOMIC INSTABILITY IN HUMAN OSTEOSARCOMA CELLS WITH NUCLEASE-DEFICIENT CAS9

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Cancer is known to be one of the main death contributors worldwide, with an array of different behaviors that make them difficult to cure or contain. In order to further understand how this group of diseases behaves, we use the very efficient CRISPR technology to target tumorigenic cells. RNA transcripts have been observed to be prevalent in tumorigenic cells. Also, previous studies have found that the integrity of centromeric heterochromatin is dependent on these RNA transcripts being silenced. Following the idea that disrupting non-coding RNA suppressors leads to an increase in RNA production, that generates genomic instability, and as a result can cause tumorigenicity, we have developed a tool to explore the effects of endogenous satellite transcript over-expression through a drug-inducible lentiviral system. With the use of CRISPR technology we have designed pRARm2Cas9VP64, which is being used with human osteosarcoma cells to test if the intent to generate genomic instability is achievable. This dCas9 carries the property that allows it to generate structural instability in the centrosome of these cells. This is firstly achieved through the point mutations in two catalytic residues with which it maintains the ability to attach to target dsDNA, but the cleaving effect on the helix is neutralized. Once pRARm2Cas9VP64 reaches its target site, the potential transcription activator (the tetrameric VP16) will induce generation of non-coding RNA that in turn will produce genomic instability. We hope that this plasmid construct takes us one step closer to understanding the mechanisms that cancer has adopted, and subsequently opens the door to novel cancer therapies.

F-1557

AUTOPHAGY IS REQUIRED FOR INTESTINAL STEM CELL MAINTENANCE AND HYPERPLASIA CAUSED BY UVRAG DEFICIENCY IN DROSOPHILA

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Stem cells are critical for the maintenance of adult tissues and organs, including the intestinal tract of mammals and Drosophila. Here we show that intestinal stem cells in Drosophila depend on high levels of baseline autophagic degradation. Stem cells lacking core autophagy genes fail to maintain proliferative capacity and are progressively lost, leading to increased leakage of the gut and premature death of affected animals. In contrast, loss of the putative autophagy regulator UVRAG, a tumor suppressor potentially involved in colorectal cancer, causes abnormal proliferation of stem cells and accumulation of undifferentiated progeny. We find that hyperplasia induced by lack of UVRAG requires a functional autophagy pathway, which raises the possibility of applying autophagy inhibitors for treating UVRAG-deficient cancers. Our results establish the fundamental role of autophagy for gut functioning through long-term maintenance of intestinal stem cells, which is also important for longevity.

F-1558

THE EXAMINATION EFFECT OF TRABECTEDIN (ET-743) ON CD133HIGH / CD44HIGH HUMAN PROSTATE CANCER STEM CELLS IN TWO-DIMENSIONAL (2D) AND THREE DIMENSIONAL (3D) SYSTEM

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Trabectedin is an anti-cancer molecule that was originally isolated from Ecteinascidia turbinata. Trabectedin is active against a variety of tumor cell lines growing in culture at nanomolar concentrations. However, it is still waiting to discover that the effect of Trabectedin in cancer stem cells. The present study focused on the effect of Trabectedin in cell proliferation, cell cycle progression and apoptosis in prostate cancer stem cells. We therefore, isolated CD133 +high/CD44 +high human prostate cancer stem cell line. Cells were treated with Trabectedin in a dose and time dependent manner to determine the inhibitor effect of Trabectedin. Cell viability and proliferation were analyzed and efficiency of Trabectedin was assessed using sphere-forming assay. Annexin-V and immunofluorescence analysis were performed for evaluation of apoptotic pathways. According to results cytotoxic effects of Trabectedin were demonstrated in high dose treatment. This drug induced G2/M phase arrest. Our data demonstrate that Trabectedin inhibits cancer stem cell spheroid formation by increasing the cell death and apoptosis. Trabectedin decreased formation of E-cadherin-dependent multicellular spheroids in 3D system. Trabectedin-induced growth inhibition and apoptosis was associated with increased expression of caspase-3, caspase-8, caspase-9, p53 and decreased expression of bcl-2 in a dose-dependent manner. It was found that Trabectedin induced apoptosis through extrinsic and intrinsic pathways in DU-145 cells but only extrinsic pathway in PC-3 cells. In conclusion, our results indicated that Trabectedin has cytotoxic and apoptotic effects in prostate cancer stem cells. These data reveal that after completing in vivo and phase studies Trabectedin may be an effective chemotherapeutic molecule for prostate cancer treatment.

F-1559

EFFECT OF PRO-INFLAMMATORY ENVIRONMENT ON QUIESCENT/ACTIVATION PHENOTYPIC SWITCH OF MELANOMA CANCER STEM CELL

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Accumulating data suggest that both cancer development and recurrence depend on the ability of resistant tumor cells to adopt a quiescent or dormant phenotype following anti-cancer treatment.

Dormant cells persist in various tissues of the body without causing clinical manifestations until they are reactivated and lead to tumor recurrence. Mechanisms that control the activation of quiescent tumor cells remain poorly understood. However, tumor microenvironment, especially inflammatory factors, which increase incidence of cancer, appears to be critical. Our goal is to determine whether a pro-inflammatory environment contributes to the quiescence/activation phenotypic switch in melanoma. For this, we used a GFP inducible expression system coupled to histone2B, to identify quiescent cells (H2B-GFP+) in a 3D skin equivalent (SE) model and conditioned media obtained after stimulation of human lymphocytes with anti CD3/CD28 antibodies (SCM), to mimic the inflammatory environment observed in patients in vivo. Our results show that pro-inflammatory environment increased the number of H2B-GFP+ melanoma label retaining cells (LRC), the number of melanospheres and the pool of ABCB5+ and rhodamine 123 excluding melanoma cells, the traits corroborating melanoma stem cell phenotype. In the skin equivalents, the conditioned medium prevented pigmentation of the SE epidermis and increase the stem cell compartment, suggesting that pro-inflammatory factors increase the melanoma stem pool by inhibiting their differentiation fate. These results imply, that by regulating inflammation we may be able to control cellular quiescence linked to tumor dormancy and cancer recurrence.

F-1560

STEM-LIKE CELL BASED PATIENT DERIVED XENOGRAFT (PDX) MODELING OF COMMON AND RARE CANCERS IN HUMANIZED MOUSE MODELS

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There has been significant progress in understanding the biology of many human cancers, yet therapeutic advances have been limited. In vitro cell culture model systems and in vivo preclinical model systems that lack human compatibility remain the predominant vehicles for testing the efficacy of therapeutics. Tumor tissue heterogeneity and the associated compatibility of the tumor microenvironment, as well as signaling cross-talks and their regulatory feedback mechanisms are often underestimated in the above models of human cancer. It is possible that one or more of these factors may interfere with the capacity to successfully translate preclinical studies to clinical applications. Therefore, we have undertaken studies to identify the stem-like tumor-initiating cells (TICs) from both the common human malignancies (prostate, lung, breast, kidney, and pancreas) as well as the less-studied rare cancers (sarcomas, uveal melanomas, paragangliomas, and chordomas). Using TICs and/or primary tumor specimens from consenting patients, we investigated methods of generating tumors that approximate the heterogeneity of their original parent tumors. Although the TICs are log fold efficient in inducing tumorigenesis compared to bulk tumor cells, these cells represent only a very minor population within the gross tumor tissue. Therefore, obtaining sufficient numbers of TICs for functional investigations has been the major challenge. Here, we present our experience with the use of two feasible approaches to help overcome the aforementioned difficulties: (1) to propagate the limited amount of resected patient tumor specimen using PDX models, followed by isolation of TICs from those propagated xenograft tumors; and (2) to purify the TICs first when a sufficient amount of the patient's tumor specimen is available and subsequently transplant these TICs to recreate the tumor, validate its original parent tumor heterogeneity, and then purify the TICs for repeating the tumor regeneration and carrying out the other preclinical characterizations. We suggest humanized mice to provide a patient-specific tumor microenvironment and orthotopic xenotransplantation to provide tissue-specific signaling. We isolated TICs by sphere formation and/or by prospective purification using their novel cell surface markers.

F-1561

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN NEURAL STEM CELLS AND IN BRAIN TUMOR STEM CELLS

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Glioblastoma multiforme (GBM) is the most common primary brain tumour in adults. Patients with newly diagnosed glioblastoma have a median survival of approximately 14 months. Cancer stem cells are supposed to be the cells, which are resistant to conventional treatment and thus responsible for tumour relapse. As brain tumor stem cells (BTSCs) share many similarities with neural stem cells (NSCs) it is of significant importance that targeting BTSCs does not have a negative effect on the healthy NSCs. To analyse the differences between these two cell types, we induced brain tumours in mice by viral overexpression of PDGF and a constitutive active form of AKT. We purified BTSC from these tumours and from healthy mice and used ribosome profiling to investigate the differences in gene expression between these two cell types. Until recently, analyses of gene expression have focused on the abundance of mRNA species as measured either by microarray or by RNA sequencing. With the ribosome profiling technique only the small mRNA pieces, which are occupied by ribosomes are sequenced, thus providing information about the differentially translated genes. With this method we identified many differentially expressed genes in BTSCs compared to NSCs. Some of the differentially expressed genes were expected results, e.g. the upregulation of the PDGF pathway due to overepression of PDGF or the expression of already known BTSC markers. These results confirmed the validity of our method. Additionally many new targets were identified and their expression could be further confirmed by Western Blot analysis and by immunofluorescence stainings of mouse and human brain tumour slices. The targets we are currently working on could also be identified in a number of human glioma cell lines. In our future studies we will asses the importance of these genes for the growth and relapse of brain tumours in vivo by using animal models with the goal to identify new therapeutic targets for treating GBM. With our approach we might provide for the first time molecular targets, which are exclusive for brain cancer stem cells.

F-1562

ROLE OF THE PLURIPOTENCY MARKER SOX2 IN MELANOMA FORMATION AND MAINTENANCE

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Melanoma is a highly aggressive tumor, which in most cases requires by oncogenic mutations in the B-Raf/Ras/MAPK signaling pathway. This cancer develops from the malignant transformation of melanocytes, which originate from in the neural crest lineage. Sox2 is a transcription factor highly expressed in embryonic stem cells, in which it plays a crucial role in maintaining pluripotency. Its expression is absent from adult melanocytes in both human and murine skin. In contrast, recent studies have shown that Sox2 expression is upregulated upon melanoma formation in patients suggesting an oncogenic role for Sox2. Therefore, we will adress the functional role of Sox2 in vivo by means of a Tyr::NRasQ61K Ink4a-/- mouse model spontaneously developing metastatic melanoma after 4-6 month. Further, we try to elucidate possible molecular mechansims and genetic networks that could explain the role of Sox2 regarding tumorigenesis using human melanoma cells. To decipher the role of Sox2 in melanoma initiation, conditional tamoxifen-based Sox2 ablation using the Cre-LoxP system was performed before the mice developed any primary lesions. Interestingly, Sox2 ablation did not inhibit melanoma initiation compared to the control animals. X-Gal staining revealed high recombination efficiency confirming the conditional Sox2 knockout (cKO). Moreover, Sox2cKO lymph node and lung melanoma metastases could be detected. In summary, melanoma cells lacking Sox2 expression were still able to form primary melanomas and to invade to distant organs. Next, we performed a stable SOX2 knockdown (KD) in human melanoma cells. Although the cells did not show a significantly reduced or increased proliferation pattern compared to the control, invasion assays showed decreased migration capacity of these cells in matrigel. Consistent with these data, genes promoting melanoma cell invasion were strongly downregulated upon SOX2KD. Further, SOX2KD cells showed higher expression of melanocytic differentiation markers. These findings were confirmed in vivo using a xenograft model of stable SOX2KD melanoma cells injected in immunocompromized mice. Hence, our data suggest Sox2 is involved in melanoma cell invasion but might be compensated in vivo.

F-1563

EQUATING DIFFERENTIATION PATTERNS OF COLORECTAL TUMORS WITH CANCER STEM CELL CHARACTERISTICS TO UNRAVEL THE MYSTERY BEHIND TUMOR AGGRESSIVENESS

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Incidence and morbidity of Colorectal cancer (CRC) is perceptible worldwide. Recent evidence suggests that a population of cancer cells called cancer stem cells (CSC) is responsible for invasion, metastasis, heterogeneity, therapeutic resistance and recurrence of CRC. Targeting these CSC can be a promising modality of treatment for aggressive CRC. In this study we elucidate the link between CSC, differentiation grade and tumor aggressiveness. Paraffin embedded tissue samples of different histopathological grades of primary, untreated colorectal cancer were analyzed for the expression of four CSC markers CD44, CD326, CD24, and CD166 using immunohistochemistry. Marker based isolation of CSC and non-CSC-bulk-tumor cells from fresh colorectal tissue and HT29 & HCT116 CRC cell lines was done using FACS. Tumor sphere assay was performed with the sorted subsets. Microarray was done using Agilent whole genome 4x44K array slides to study transcriptomic changes between CSC and non-CSC-bulk-tumor cells for both high grade & low grade CRC. Data was analysed using Flow Jo, GeneSpringGX 13 and GeneGOMetaCore. Validation was done using Real time PCR. There was a statistically significant difference ($p<0.05$) in the expression of CD44, CD326 and CD166 between cases and controls. FACS showed higher prevalence of CSC in primary high grade CRC as compared to low grade CRC. Sorted and cultured CSCs formed tumor spheres. Gene expression analysis of sorted CSCs showed over expression of the classical stemness markers including Oct4, nanog, c-myc, klf4, MSH1 as well as EMT markers including MMPs, Snail, Twist and ZEB1. Our data also identified novel genes AHSA1, CFH, ACSS1 and NUPR1, specific to CSC. On the basis of our novel findings, we hypothesize that tumor aggressiveness of high grade CRC may be attributable not only to a higher number of CSC but also to a difference in expression profile of these CSC compared to the CSC of low grade CRC.

F-1564

ONCOGENICITY OF OCT4A IN PEDIATRIC BRAIN TUMOR

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Atypical teratoid rhabdoid tumor (AT/RT) is a highly aggressive and one of the most fatal pediatric tumors that occurs in very young infants. This malignant embryonal tumor of the central nervous system resembles teratomas due to the presence of embryonal, epithelial and mesenchymal cell constituents. OCT4A, encoded by a splicing variant of the *POU5F1* gene, is highly expressed in embryonic stem cells (ESC) and its aberrant expression was previously correlated with poor outcome in pediatric brain tumors. In this study, we found that stable overexpression of OCT4A, confirmed by qRT-PCR and western blot, in a patient-derived cell line of AT/RT significantly increased cell proliferation, clonogenic activity and generation of neurospheres in vitro. Overexpression of OCT4A also significantly enhanced tumor growth in a subcutaneous model of tumorigenesis. These findings indicate that aberrantly increased expression of OCT4A, an OCT4 isoform with well characterized function in ESC pluripotency and self-renewal, contribute to AT/RT development and aggressiveness.

F-1565

TARGETING THE KINOME TO INDUCE DIFFERENTIATION IN HUMAN BREAST CANCER STEM CELLS

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We have established a cell isolation and cultivation method enriching for mammary cancer stem cells (CSCs) from patients suffering from triple negative breast cancer (TNBC). We combine hypoxia, serum-free conditions and a 3D environment to establish cell lines that retain the potential to self-renew and differentiate both in vitro and in vivo - the two fundamental properties of stem cells. So far we have established nine steadily growing primary breast cancer cell lines which we are in the process of analyzing for differentiation potential, keratin marker expression, colony forming ability and drug sensitivity. The tumorigenicity of the cell lines was assessed by transplantation of cells into cleared fat pads of NOD/SCID mice - the "gold standard" to identify potential CSCs. Down to 10 cells were sufficient to establish tumor grafts that resembled the tumors of origin. Differentiation therapy, first mentioned in the 1950s, is having a comeback in recent years. This therapy approach is based on the use of agents that induce differentiation of CSCs, thereby directing them toward a comparatively benign, non-tumorigenic, non-CSC state. We identified several kinases whose knockdown drives human TNBC stem cells towards a luminal or myoepithelial fate. After lentiviral or siRNA kinase knockdown, the triple negative breast cancer cell line MDA-MB-468 showed loss of myoepithelial markers via immunofluorescence and microarray analysis. The knockdown also resulted in a slower proliferation rate as well as diminished tumorigenic potential (publication in preparation). As a next step, knockdown will be performed on the established primary breast cancer cell lines and drug sensitivity will be assessed after knockdown of each identified kinase. Together with an improved clinical characterization of breast cancer subtypes, using new CSC markers, this approach could be part of future cancer treatment in the clinic and would be a first step towards individual patient therapy.

F-1566

MSN-CISPLATIN-SIRNA IS POTENTIAL THERAPEUTIC APPROACH FOR MALIGNANT HCCS

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Hepatocellular carcinomas (HCCs) with expression of stem/progenitor cell markers including CD133 have been reported to have more aggressive biological behavior and poor prognosis, has been suggested to generate cancer stem cells. CD133+ liver cancer cells are characterized by resistance to chemotherapy, increased colony formation, and in vivo cancer initiation at limited dilution. Mesoporous silica nanoparticles (MSNs) have attracted much attention for their preeminent attributes including high surface area and easy functionalization. However, the multiple-application of MSNs in cancer stem cells (CSCs) remained mostly unclear. Combination with Cisplatin and siTGFb, we successfully demonstrated that MSNs could not only greatly enhance the toxicity of Cisplatin but also inhibit the expression of CD133. Consistently, we demonstrated that MSNs treatment negatively regulates tumor growth by directly suppressing Oct4 and Sox2 in HCC-CSCs, whereas it positively regulates hepatic maturation genes. We therefore evaluated the treatment effect of MSN-Cisplatin-siRNA on HCC-CSCs in vivo. MSN-Cisplatin-siRNA delivery in HCC-CSCs significantly inhibited their tumorigenic and CSC-like abilities, and facilitated their differentiation into CD133− non-CSCs. In addition, MSN-Cisplatin-siRNA delivery effectively suppressed their expressions of drug-resistance and anti-apoptotic genes, and dramatically increased the sensitivity to chemotherapeutic drugs. Lastly, MSN-Cisplatin-siRNA treatment led to significant inhibition of tumorgenesis in HCC-CSCs. Compare with Cisplatin alone, in vivo and in vitro evidence of MSN-Cisplatin-siRNA delivery significantly suppressed tumorigenesis, and synergistically improved survivals in orthotopic HCC-CSCs-transplanted immunocompromised mice. Therefore, MSN-Cisplatin-siRNA is potential therapeutic approach for malignant HCCs.

F-1567

TUMOR SUPPRESSOR ROLE OF HIF-1ALPHA IN LEUKEMIA-INITIATING CELLS IN MURINE FLT3-ITD-INDUCED MYELOPROLIFERATIVE NEOPLASM

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Hypoxia-induced signalling is a major regulator in normal and malignant hematopoiesis. The main proteins involved in this signalling are the hypoxia-inducible factors (HIFs), a family of dimeric transcription factors. The oxygen-regulated subunit HIF-1α plays a crucial role in the quiescence and self-renewal of hematopoietic stem cells as well as leukemia-initiating cells (LICs). Better understanding of the requirement of the molecular response to hypoxia in LICs could lead to new therapies targeting this pathway. Accordingly, we have investigated the effect of HIF-1α loss on the phenotype and biology of $FLT3^{ITD}$-induced myeloproliferative neoplasm (MPN). Using a combined transgenic mouse model (*Mx1*-Cre; $Hif\text{-}1\alpha^{fl/fl}$; $Flt3^{ITD/+}$) we show that deletion of HIF-1α leads to a more severe MPN phenotype reflected by a shorter survival of the mice, higher numbers of white blood cells and myeloid cells in peripheral blood, as well as more severe splenomegaly and hepatomegaly. Loss of long-term hematopoietic stem cells (Lin- Sca1+ cKit+ CD48- CD150+) and multipotent progenitors (Lin- Sca1+ cKit+ CD48- CD150-) in the bone marrow, and their increment in the spleen, were the most pronounced effects on a cellular level upon the loss of HIF-1α. The fact that $Hif\text{-}1\alpha^{\Delta/\Delta}$ $FLT3^{ITD}$ MPN is transplantable indicates that loss of HIF-1α did not imply a defect in LICs self-renewal, as described previously, and additionally, argues for a cell-intrinsic effect of HIF-1α on $FLT3^{ITD}$-induced MPN. Because HIF-1α status influences the energetic activity of the cell, we examined the metabolic condition of these neoplastic myeloid cells (mitochondrial activity, levels of reactive oxygen species, proliferation and apoptosis). Together these data indicate a metabolic adaptation of these malignant cells to their new niche (spleen) and an improvement of their tumorigenic capacities (less quiescence and more proliferation) when HIF-1α is lost in these cells. These findings are in contrary to what has been previously described for the role of HIF-1α in LICs and lead us to propose that HIF-1α could act as a tumour suppressor gene, inhibiting proliferation in myeloid

malignancies. Our results provide evidence that targeting HIF-1α can lead to disease progression of MPN so its potential use as a therapeutic target should be further evaluated.

F-1568

MUSASHI ENHANCED GS3K-B AND PROMOTED STEMNESS-RELATED CHEMORESISTANCE IN GLIOBLASTOMA

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Glioblastoma multiforme (GBM), one of the deadly malignant brain tumor, and is easily to recur and develop resistance to clinical chemotherapeutics. It has been demonstrated that cisplatin treatment select for resistance but also for a more oncogenic phenotype characterized by high self-renewal and oncogenic resistance. Musashi-1 (Msi-1), a neural stem cell marker, is found to be elevated in several cancer tissues and positively regulates cancer progression. In this report, we found that Msi-1 promoted tumourigenicity and enhances drug resistance of human GBM cells in response to cisplatin, a chemodrug. We further showed that Msi-1 could activate and interact with phosphorylated-GS3K-b, and lead to a protective effect against mitigating cisplatin-induced cell death. Knockdown of Msi-1 effectively suppressed the phosphorylation of GS3K-b and blocked b-catenin translocating into nucleus in cisplatin-resistant GBM cells, while overexpressing Msi-1 reversed these effects. Notably, Msi-1 could further upregulate b-catenin-driven stemness signature, and expression of Oct-4, Nanog, and ABCG2, thereby enhanced tumorigenicity and chemoresistance of GBM cells. Moreover, the result of in vivo animal study demonstrated that knockdown of Msi-1 effectively suppressed stem-like properties, synergistically enhanced the chemotherapeutic effect, and significantly improved survival rate in GBM-transplanted immunocompromised mice. Taken together, our findings indicated that Msi-1 may provide a positive contribution to oncogenic resistance, in partly, through interplaying with GS3K-b and modulating WNT/stemness mechanism.

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