WSCS POSTER ABSTRACTS – 2014 – SAN ANTONIO

Submission ID: 54726
Submission Title: Simple production method of umbilical cord derived mesenchymal stem cell using xeno-free materials

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Topic
Basic Research, Medicine, and Health

Problem
Production of safe stem cells is very important in regenerative medicine.

Background
Umbilical cord (UC) is a very promising source of mesenchymal stem cells (MSCs) for allogeneic use, as it can be collected easily without side effects to the donor. Moreover, UC-MSCs can be expanded more compared to bone marrow or adipose tissue derived MSCs, and for allogeneic use they are more readily available. Therefore, in this study, we developed a simple production method to get UC-MSCs using xeno-free material to provide safe MSCs for regenerative medicine.

Hypothesis
UC MSCs can be isolated and expanded easily in 10% PRP containing alpha MEM.

Research
We used multiple harvest explant method to isolate the MSCs. The medium for isolation and propagation was 10% platelet rich plasma (PRP) containing alpha MEM. Confluent cultures were harvested using TrypLE Select, combined and counted. Part of the results of primary cultures were expanded in T25 flasks (seeding around 5000/cm2), and the rest was cryopreserved. The results of passage-1 cultures were combined, and counted. Part of passage-1 cultures were checked for their

differentiation capacity and the rest was cryopreserved. Further, part of the passage 1 culture was cultured into passage-2, and part of passage-2 cultures were checked for their differentiation capacity, and the rest was cryopreserved.

Observations

From 5 cm of umbilical cord, we did explant culture in four 24 well plates. The number of harvest per well ranges from 0-5 times i.e. no harvest= 6, once= 20, twice= 34, three times= 27, four times=8, and 5 times= 1 well(s), respectively. Therefore, we harvested a total of 206 times from the 96 wells, and got a total of 3,595,600 cells from the primary culture. For passage-1, a total of 1,450,560 cells were expanded in twelve T25 flasks, and we got a total of 19,287,600 cells. For passage 2, a total of 871,280 cells were expanded in seven T25 flasks, and we got a total of 18,636,000 cells. If we use all of the cells from the primary culture result for expansion, we will get a total of 47,809,462 cells in passage-1. Further, if we use all of the putative result of passage-1 for expansion, we will get a total of 1,022,607,122 cells in passage-2. Induction of passage-2 cells showed differentiation capacity into osteogenic, chondrogenic and adipogenic lineage. However, differentiation into adipogenic cells yielded preadipocytes that contain smaller lipid droplets compared to those developed in bone marrow derived MSCs. Conclusion: UC-MSCs can be isolated and expanded easily in 10% PRP containing alpha MEM, and is suitable to fill the demand of safe allogeneic MSCs for patient use.

Submission ID: 55450
Submission Title: Modeling the diseased blood-brain barrier using patient-derived iPSCs: the case of Allan-Herndon-Dudley Syndrome

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Topic
Basic Research, Medicine, and Health

Problem
Allan-Herndon-Dudley Syndrome (AHDS) is a X-linked mental retardation characterized by an impaired thyroid hormone uptake at the blood-brain barrier (BBB). Current studies suggest an association between mutation in the monocarboxylate transporter 8 (MCT8) and impaired thyroid hormone uptake at the BBB. Yet, current in vivo and in vitro models fail to reproduce the pathophysiology observed in AHDS patients.

Background
The blood-brain barrier (BBB) plays an important function in the maintenance of the brain homeostasis. It allows the entrance of nutrients and hormones, whereas it limits the diffusion of small lipophilic compounds, including drug targeting the central nervous system. The dysfunction of the BBB in neurological diseases occurs in several neurological diseases, yet the contribution of genetic disorders at the BBB in neurodevelopmental diseases remains unknown.

Hypothesis
Induced pluripotent stem cells (iPSCs) have provided an promising tool for modelling neurological diseases by providing a patient-specific approach. We recently published a differentiation protocol capable to differentiate BMECs from established iPSCs. Therefore, we hypothesize that such protocol could be transposed in patient-derived iPSCs to model the BBB in neurological diseases.

Research
Using our recent differentiation protocol, we conducted a pilot study to differentiate BMECs from iPSCs derived from healthy and AHDS patients. We measured BMECs yield and barrier function,

expression and activity of key drug transporters. Finally we assessed thyroid hormone uptake in our monolayers.

Observations
Based on our established protocol, we were able to obtain BMECs from both healthy and AHDS patients. Notably, no major differences were observed between healthy and AHDS patients in terms of BMECs differentiation, yield or functional barrier outcome. No differences were observed in terms of drug transporters activity. However, we noted a significant decrease in thyroid hormone uptake in AHDS BMECs compared to healthy BMECs.

Submission ID: 55539
Submission Title: autologus Skeletal Muscle Graft Augmented with Autologous adult Type Bone Marrow Stromal Stem Cell used in Completely Transected Traumatic Chronic Spinal Cord Injury: Pilot study and new technique.

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Topic
Basic Research, Medicine, and Health

Problem
To determine the safety and feasibility of using autologous fresh harvested Skeletal muscle graft augmented with injection of adult type bone marrow stromal stem cell intrpositioned into complete transected chronic spinal cord injury using new technique. As spinal cord injury considered as difficult area to find treatment and many trailes was done to solve this problem. So used a new technique to try to solve this problem

Background
Traumatic insults to spinal cord induce both immediate mechanical damage and subsequent tissue degeneration leading to a substantial physiological, biochemical, and functional reorganization of the spinal cord. The spinal cord and skeletal muscle grafts were found to exert reciprocal trophic effects on each other, evidenced as a larger muscle mass in skeletal muscle grafts allowed to develop in the presence of spinal cord tissue. Concepts from developmental neurobiology would suggest that fiber growth and target identification would follow guidance cues and target recognition signals; some of these molecular cues may persist in the adult CNS or reappear after injury.

Hypothesis

Extracellular matrix can be a promising tool for treating chronic spinal cord injury and as skeletal muscle is the only longitudinal structure the looks like the longitudinal anatomy of the neural tissue and also its rich in elastin fibronectine and other moties which was investigated before as agood stimulating media for growing the out sproutting axons in vitro cultur. So we tried to use skeletal muscle as a scafold augmented with stem cell as a clinical trial for the management of completely transected chronic spinal cord injury.

Research

Six chronic (more than 6 months post trauma), traumatic spinal cord injury (SCI) patients with complete paraplegic motor power grade zero in both lower limbs with sensory level corresponding to the level of injury associated with loss of sphinctric control for both urine and stool. They had neurological level dorsolumber fractured. Participants were assessed at baseline and at 6 monthly intervals. Investigations was done including MRI Tractography to assesses the transected white matter ascending and descending tracts, Neurophysiology by testing for the peripheral nerve conduction of both tibial and peroneal nerves as motor and sural nerve as sensory and somatosensory evoked potential. Safety and tolerability were evaluated through monitoring for any adverse events and psychological evaluation was done. Operation and New Technique Operation summarized in identification of the injured area with removal of the intradural adhesion and intervening retro placed bone fragments. That was followed by removal of the gliotic and scare tissues followed by reconnecting both distal and proximal spinal cord ends using freshly harvested paraspinal skeletal muscle autologous graft which was immersed into the pre-prepared harvested adult autologous mesenchymal stem cell which was prepared by stem cell lab two days before surgery. The graft was tailored to be the same size as the defect between both ends and sutured in place using fine 8-0 proline threads. Closure was done in layers without drains to avoid CSF leak.

Observations

Results: Surgery was tolerated well by all American Spinal Injury Association (ASIA) Impairment Scale (AIS) A participants. There was smooth recovery with no early complications in the form of wound infection, CSF leak or post-operative bleeding or more new neurological deficits. But three patient complained of post-operative headache that improved within two week. MRI evaluation after six month revealed no developing syrinx or worsening in the parameters of Tractography but the muscle graft appeared as myelomalacia area with the same MRI findings. There was significant improvement in electrophysiological tests in three patients regarding improvement in the neuroexitability of both tibial and peroneal nerves with one patient showed improvement in the somatosensory evoked potential that revealed partial injury in the spinal cord. Also significant improvement was seen in functional scores as evaluated by Spinal Cord Independence Measure, as trunk balance, self-sitting, walking with crutches or walker and mild motor activity in the illiopsoas and lower abdominal muscle. Three patients out of six abled to change from indwelling urinary catheter to self intermittent catheter every 4 hours with early sensation with micturition. Conclusion: Autologous Skeletal muscle could be a simple good source of autologous scaffold for the 3D implantation of stem cell in the future management of traumatic completely transected spinal cord

injury.

Submission ID: 56799
Submission Title: Senescence, but not in vivo ageing, is associated with delayed differentiation, increased DNA damage and elevates TGF-β release in human primary myoblasts

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Topic
Basic Research, Medicine, and Health

Problem
Replicative senescence of cells in culture has been used as a model to study the ageing process in human muscle (Bigot et al., 2008). However, the relevance of this approach to in vivo ageing remains unclear. The aim of this study was to investigate if in vivo muscle ageing differs from in vitro muscle ageing by studying primary human muscle cells obtained from young and elderly people, before and after they reached replicative senescence.

Background
The serial passaging of normal somatic cells ultimately results in a state of permanent growth arrest called replicative senescence. This was initially shown for fibroblasts by Hayflick and Moorhead (1961) but also more recently, for human satellite cells (Decary et al., 1997). The senescing myoblasts have been used as a model to study muscle ageing. For example, senescent myoblasts obtained from an infant donor showed a delayed differentiation response compared to proliferating myoblasts from the same donor (Bigot et al., 2008). Serially passage C2C12 cells in vitro exhibited a reduced in MyoD and myogenin expression which are associated with decreased IGF expression (Sharples et al., 2011). However, there is uncertainty as to how in vitro ageing of cells reflects the physiological process of ageing of similar cells in vivo, e.g. when proliferating cells obtained from young and elderly subjects are compared. Satellite cell (muscle stem cell) activation and proliferation have been shown to be impaired in older animals in response to injury and represents one of the key age-specific defects in muscle repair (Conboy et al., 2003, Scime et al., 2009). There is also some

evidence that myoblasts obtained from muscles of elderly people do not differentiate as efficiently in vitro when compared with those from younger subjects (Beccafico et al., 2007, Beccafico et al., 2010, Jacquemin et al., 2004, Lorenzon et al., 2004, Pietrangelo et al., 2009). Critically, these conclusions are drawn from comparison of very limited numbers of subjects, often only one from each age group (Jacquemin et al., 2004, Lorenzon et al., 2004), and from populations that often have a low proportion of myoblasts (Pietrangelo et al., 2009) or have not been characterised in terms of desmin content (Beccafico et al., 2010).

Hypothesis
Myoblasts aged in vivo (obtained from old subjects) display a similar cellular phenotype to senescent myoblasts (aged in vitro) and these are distinct from that of myoblasts obtained from young subjects.

Research
Muscle biopsy samples were taken under local anaesthesia from the vastus lateralis muscles of 5 young (aged, 23-25) and 4 healthy elderly (aged, 67-82) subjects. Cells were cultured in a skeletal muscle cell growth medium until they reached senescence. The proportion of muscle cells (positive for desmin expression), differentiating cells and cells with double strand DNA breaks (positive for γ-H2AX) was assessed by immunocytochemistry, whereas TGF-β production was assessed in conditioned medium using a Luminex based assay. Cells were plated in growth medium in 96 well dishes at densities of 2000 (desmin, γ-H2AX) and 7000 (myogenin, MHC, TGF-β) cells/well. Cells were fixed 24 hours after plating for assessment of desmin and γ-H2AX or had their medium replaced with a serum free medium to induce differentiation. Several parameters associated with ageing process were compared: ability to undergo differentiation; presence of DNA damage; and production of TGF-β.

Observations
Marked heterogeneity between the different myoblast cultures was observed during multiple passaging. Several populations of cells maintained their initial desmin content over time (50-94%) and underwent 1-8 mean population doublings. However, others lost their desmin positive cells over time in culture (50-95% starting, 0% at senescence) and underwent 15-22 MPDs. Populations that did not maintain desmin expression were discarded from further analysis after the initial characterisation. The main finding of the study is that there is no difference in any of the parameters measured between the cells obtained from old and young people that had not undergone replicative senescence. However, the senescent cells had a decrease in the expression levels of myogenin (50 ± 3% young, 49 ± 3% old and 6 ± 1% senescent, P

Submission ID: 57752
Submission Title: Differential expression of adhesion-related proteins and MAPK pathways leads to suitable osteoblast differentiation of human mesenchymal stem cell (hMSC) subpopulations

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Topic
Basic Research, Medicine, and Health

Problem
In the past decade, there have been many significant advances in bone tissue engineering; however, proper cellular attachment to scaffolds remains a problem for bone graft construction. For instance, in studies involving engineering, several strategies have been developed to facilitate the adhesion and proliferation of osteoprogenitors in order to support their subsequent differentiation and matrix deposition. In this study, we addressed this challenge using an in vitro and in vivo approach. Based on the expression profile of the adhesion-related proteins and MAPK pathways, we appointed a subpopulation also a specific stage for the successful application of bone graft assembly.

Background

The successful engineering of functional tissues in vitro relies on the arrangement of synthetic scaffolds, viable cells and physiologically relevant signals, as well as cell adhesion. Cell adhesion to the extracellular matrix (ECM) is a fundamental process that connects integrins on cell surface to actin cytoskeleton through focal adhesions (FAs). These FAs are heterogeneous structures that vary in size, distribution and dynamics and, to certain extent, in their molecular constituents, which are also influenced by cell type and differentiation status. Moreover, cell contact with ECM proteins plays a critical role in regulating osteogenesis from hMSCs. We previously isolated two subpopulations of hMSCs based on the co-expression of the surface markers CD44, CD73 and CD105. Both subpopulations were able to initiate osteoblast differentiation. However, differences in the time required to reach osteoblastic maturity and in the efficiency of mineralization during induction with dexamethasone were evidenced.

Hypothesis

Because cell adhesion and osteoblast differentiation of human mesenchymal stem cells (hMSCs) are closely related, we hypothesized that it is possible to propose a strategy for the assembling of a successful bone graft; based on the expression of adhesion-related proteins and MAPK pathways by two hMSCs subpopulations which have been induced to the osteoblast lineage.

Research

Fluorescence-activated cell sorting (FACS) was used to isolated two subpopulations: CD44+/CD73+/CD105+ (CD105+), and CD44+/CD73+/CD105- (CD105-). Immunophenotypes, such as mesenchymal and hematopoietic markers, were analyzed by FACS. To understand the events related to cell adhesion in a functional framework, in this research, we identify by cellular and molecular markers, a time line for the osteoblastic differentiation process induced by dexamethasone. The expression of β1-integrin, talin, paxillin, vinculin and α-actinin was evaluated by western blot analysis. Changes in the FAK phosphorylation pattern and the involvement of the MAPK pathway were also studied during the differentiation stages using immunoprecipitation and Luminex technology. With the aim of explore connection between β1-integrin and the actin cytoskeleton at specific differentiation stage, their co-localization was assessed by confocal microscopy. In order to demonstrate the reparative ability of bone graft, we performed transplantation and analysis using a canine in vivo model. Before implantation, CD105+ and CD105- cells were cultured with osteogenic media as a monolayer and 1 more week on a bovine bone matrix Nukbone® (NKB®) as a three-dimensional structure. These assembled bioactive scaffolds were implanted in a critical-size defects created bilaterally in the femur of 6 Beagle dogs. The right defects were grafted with NKB® as control, and defects on the left with CD105+ graft or CD105- graft. After 7 weeks, animals were sacrificed and samples were subjected to histological sectioning. Bone-implant contact and bone area were evaluated by stained with H&E also Masson's trichrome stain and analyzed under light microscopy.

Observations

Immunophenotyping revealed the expression of the CD49a, CD49d, CD29, integrin α9β1, CD44, CD73, and CD105 antigens in both subpopulations. In contrast, CD90, CD45, CD34, CD14, and HLA-DR expression was not detected. The ability of both subpopulations to differentiate into

osteoblasts, adipocytes, and chondrocytes was evidenced using Alizarin red, Oil-Red, and Alcian blue staining, respectively. Interestingly, we observed a dissimilar osteoblastic differentiation potential between the subpopulations. CD105- cells showed stronger expression of secreted protein acidic and rich in cysteine (SPARC) and osteonectin, which was associated with more effective calcium deposition, than CD105+ cells. Further, based on the expression of α-actinin, the presence of FAKy397 and ERK1/2 activation during osteoblast differentiation, we define the maturation stage as critical for assembling of the bone graft (cellular component with NKB®). According to the adhesion efficiency evidenced by the in vitro assays also by the co-localization of the activated form of β1-integrin with the actin cytoskeleton, CD105- subpopulation showed a superior ability compare with CD105+ cells. Regarding in vivo trial, histological examination revealed extracellular matrix formation and active osteoblastic cell rimming in grafts cellularized with CD105-. At 7 weeks, the gap was occupied with a dense extracellular matrix and a trabecular meshwork was observed. A single osteocytic cell embedded with dense calcified tissues can be seen in the cross-section. In contrast, grafts cellularized with CD105+- showed a deficient endochondral reparative process with abundant fibrocartilaginous tissue. Taken together in vitro and in vivo assays, the integration of a NKB® scaffold and CD105- subpopulation at maturation stage represents a very attractive strategy for clinical use in orthopedic bioengineering.

Submission ID: 59895
Submission Title: Estimate the effects of mesenchymal stem cells (MSC) transplantation with or without hydrogel in chronic ischemic myocardial injury analyzed using stem cell-specific expression array

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Topic
Basic Research, Medicine, and Health

Problem
Mesenchymal stem cell therapy is shown to enhance the angiogenesis in chronic ischemic myocardium. But the cell survival proliferation after transplantation is a major obstacle to sustain the positive effects.

Background
Mesenchymal stem cell therapy has been shown to enhance the angiogenesis in chronic ischemic myocardium. However, the cell survival, migration and differentiation after transplantation are still the major obstacle to sustain the positive effects. As reports, hyaluronic acid based-hydrogel improves the cells survival and migration in vitro and in vivo. To increase the duration of MSC impact, we co-injected the MSCs and HA-serum hydrogels prior to the chronic ischemic myocardium and estimate the effects using a customized pig stem cell PCR array.

Hypothesis
Co-injection of autologous MSC with fresh serum mixed hydrogel (Hy-MSC) can enhance and prolong the effects of MSC in chronic ischemic myocardium.

Research
In a porcine model of chronic myocardial ischemia created by ameroid constriction of the circumflex artery, 6 x 107 MSCs were mixed with 1.2ml autologous serum based-Hydrogel (hyMSCs) and injected into the ischemic zone (ISZ). For comparison of MSCs without hydrogel were injected in a separate area within the ISZ as control. Cohorts of 2 animals were sacrificed at 2, 4, 6 and 12weeks (wk) post-injection. The samples from each animal were collected from 4 areas: the non-ISZ (N), the non-injected ISZ (ISCH), the hyMSCs- and MSCs- injected zone for pathological analysis (HE, IMC), and gene expression panel search and functional annotations by using a customized specific porcine stem cell qPCR array (96 genes/array), which contains key markers of cell-cell communication, cell growth and death, differentiation and stemness.

Observations
Compared to ISCH group, the proportion of significantly modulated genes in hyMSCs at 2, 4, 6 and 12wk were 19%, 33%, 55% and 11%, respectively. The up- or down-regulating panel of MSCs and hyMSCs at the 4 time points were similar. However, the upregulated genes in MSCs group were much less than that in hyMSCs at 6wk (33 vs 59 genes). Notably, the genes at 2wk for both the MSCs and HyMSCs treated areas were mainly linked to cell cycle and angiogenesis; the genes in the 4 and 6wk injected samples were more diverse and related to stem cell survival and differentiation markers, such as ASCL2, BMP2, CD44, COL1A1, GDF2, GATA4, IGF, KLF4, MSX1, MYC and OCT4. Compared to MSCs, hyMSC injected areas had statistically more differentially expressed genes. Our pathological analysis also found the hyMSCs' clusts were significantly more and greater than that of the MSCs only treatment samples at 4-, 6- and 12 wk post-injection time points. The pathological observation illustrated the cells co-transplantation with the hydrogel have better survival rate, suggesting the hydrogel enhances the MSCs survival and may prolong the MSCs role in the injected ischemic wounded area. These results indicate that HA-serum hydrogels can extend and increase the influence of MSCs.

Submission ID: 59994
Submission Title: The whole genome gene expression changes in cultured

human embryonic stem cells in response to low, clinical diagnostic relevant, and high doses of ionizing radiation exposures

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Topic
Basic Research, Medicine, and Health

Problem
The underlying molecular mechanisms and pathways triggered by ionizing radiation (IR) exposures in humans are far from being comprehensively understood, partly due to the lack of suitable model systems. It is not certain how low (less than 0.1 Gy) doses of IR (LDR) affect human cells, and which signaling networks and pathways govern cellular responses to such IR exposures.

Background
The apparent controversy of such data is in a marked contrast to societal needs to predict health risks from diagnostic radiation in the clinic, natural background radiation, and environmental radiological accident exposures.

Hypothesis
We established a novel, human embryonic stem cell (hESC) culture-based model to examine radiobiological effects in human cells. To gain deeper understanding of LDR responses, in the present study, our goal is to elucidate the whole genome gene expression changes in distinct hESC lines following both low (5 rad), and, as a reference, high (100 rad) IR exposures.

Research
We utilized system biology approaches, such as DNA microarrays, to interrogate whole genome gene expression alterations, and we examined the dynamics of transcriptional changes in hESCs profiling “early” (2 h post-IR) and “late” (16 h post-IR) responses. The whole genome gene chip technique is capable of detecting statistically significant changes in gene expression in both H9 and H14 hESCs even after IR exposures as low as 5 rad.

Observations
“Early” (2 h) gene expression signature after 5 rad of IR involves the induction of only 11 genes in

H9 and 17 genes in H14 line of hESCs. Gene expression changes appear to be modest at best (only 3 genes at ≈ 2.0–2.5 folds in H9, and less than 2.0 folds in H14) and strongly implicate p53 signaling and DNA damage response (DDR). The lack of “late” (16 h) response gene expression changes compared to mock IR exposure may suggest a transient alterations following LDR (5 rad) in both H9 and H14 hESCs. High dose (HDR, 100 rad) responses in both H9 and H14 hESCs are distinct from those observed after 5 rad LDR. However, only 2 and 6 genes were found to be unique to LDR in H9 and H14. The patterns of repressed, down-regulated genes are almost exclusively unique to distinct IR exposure scenarios for H9 hESCs. Very good correlation is found between independent DNA microarray and TaqMan qRT-PCR datasets for established DDR genes confirming the validity and soundness of the scientific approach used. We aim to include more hESC lines in our screening assay to identify common themes and pathways affected by LDR and HDR IR exposures, and to examine the degree of variability in gene expression among distinct hESC lines, to generalize our findings.

Submission ID: 60570
Submission Title: Photobiomodulation increases proliferation and mineralization response of dental pulp stem cells encapsulated in BMP-4 loaded biomaterial

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Topic
Basic Research, Medicine, and Health

Problem
The use of biomaterials, stem cells and growth factors have shown promising results in Regenerative Dentistry. However, new therapies, such as laser phototherapy, have been emerging as a new candidate to accelerate and improve the quality of the new dental tissue formation. Here we evaluated the use of photobiomodulation in the proliferation and differentiation response of dental pulp stem cells (DPSCs) when seeded onto an injectable and thermoresponsible hydrogel (Pluronic F127) loaded with bone morphogenetic protein 4 (BMP4).

Background
Laser phototherapy (LPT) has recently shown to be a promising candidate for dental tissue de novo regeneration by activating latent TGF-B via production of reactive oxygen species, thus promoting tertiary dentin formation. Despite the limited remodeling potential of dental pulp tissue, especially in mature teeth, this tissue does in fact demonstrate a reparative capacity. Little is known about the LPT effects on the pulp tissue; this therapy could significantly accelerate and increase the likelihood of functional dental pulp and dentin tissue neoformation.

Hypothesis
Our hypothesis is that photobiomodulation can improve DPSCs response towards proliferation and odonto/osteo differentiation.

Research
Normal human-impacted third molars (n=5) were collected consenting patients in the School of Dentistry at the Universidade de São Paulo (CAE 09731212.2.0000.0075). DPSCs were previously characterized and cell pellets were ressuspended in Pluronic F127/BMP-4 (1:1 v/v) in 48-well microplates. Cells were then irradiated every other day, up to 7 days, with two different energy densities using a continuous-wave diode laser [GaAlAs, 660 nm, 0.028 cm2, 20 mW, 0.71 W/cm2, 3 J/cm2 (4s) or 5 J/cm2 (7s)]. PKH26 and MTT assays were used to assess cell proliferation, while Alizarin Red S staining and PCR analysis were used to evaluate odonto/osteo differentiation.

Observations
Under oxidative stress, cells seeded onto hydrogels without BMP4 and irradiated with 5 J/cm2 exhibited increased cell proliferation rate. In the presence of BMP4, irradiated groups with both 3 and 5 J/cm2 have shown early mineral deposition. After 14 days of odonto/osteogenesis induction, cells onto BMP-4 loaded hydrogels and irradiated with 5 J/cm2 produced massive nodule formation compared to the control groups. Mainly at 5 J/cm2, DPSCs significantly expressed mRNAs related to odonto/osteoblast differentiation, such as collagen I, osteocalcin, dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP-1) and heat shock protein 27 (HSP27). Altogether, these data demonstrate that BMP4-loaded thermoresponsible hydrogels allow stem cell proliferation and differentiation and LPT notably improves this response.

Submission ID: 61284
Submission Title: Landscape of a novel RNA binding protein L1TD1 protein-protein interactome in human Pluripotency

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Topic
Basic Research, Medicine, and Health

Problem
Identifying the a novel RNA binding protein l1TD1 protein-protein interaction network in human pluripotency

Background
Pluripotent human embryonic stem cells (hESCs) can differentiate into all somatic cells of the human body and hold tremendous potential for developmental biology, drug screening and regenerative medicine. The importance of the core pluripotency transcription factors, OCT4, NANOG and SOX2, in the maintenance and induction of pluripotency has been well documented. However, a variety of post-transcriptional processes control and alter the original message after transcription. In addition, protein degradation by proteasomes has been implicated to have vital role in the pluripotency maintenance. The role of post-transcriptional regulators and protein networks in the maintenance of pluripotency are still largely unknown, especially in human cells. L1TD1 is highly and specifically expressed in pluripotent cells under the control of OCT4, NANOG and SOX2. We have previously reported that L1TD1 is essential for the maintenance of pluripotent state in human cells . L1TD1 is a RNA binding protein (RBP) similarly to LIN28, which is one of the well characterized post-translational regulators of pluripotency. RBPs have a fundamental role in a wide variety of cellular processes, including RNA transcription, splicing, processing, localization, stability and translation. Moreover, each RBP has unique and specific roles. Due to its highly specific expression and vitality in human pluripotent cells, we expect L1TD1 to regulate the maintenance of pluripotent state

Hypothesis
The interactome for Oct4, Sox2 and Nanog, along with other individual transcription factors, has been reported in mouse ES cells (mESCs). However, the functional protein-protein interactions in hESCs have remained unexplored. Notably, the protein networks distinct from transcriptional control in pluripotent cells remain unknown. We hypothesis that identifying novel RNA binidng protein L1TD1 protein-protein networks in human pluripotency significantly expands the current knowledge RBPs and provides important resource of protein interactions in pluripotent human cells.

Research
To elucidate interacting and functional components involved in the regulation of human pluripotency, we have carried out, for the first time, the characterization of proteins associated with L1TD1 using immunoprecipitation followed by Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Furthermore, we validated selected interactions in hESCs and human induced pluripotent stem cells (hiPSCs).

Observations
Our data reveals that L1TD1 has an important role in RNA splicing, translation, protein traffic and degradation. L1TD1 interacts with multiple stem cell specific proteins, many of which are still

uncharacterized in the context of development. Further, we show that L1TD1 is a part of the pluripotency interactome network of OCT4, SOX2 and NANOG bridging nuclear and cytoplasmic regulation and highlighting the importance of RNA biology in pluripotency.

Submission ID: 62577
Submission Title: 1α,25-dihydroxyvitamin D3 synchronizes circadian genes in adipose-derived stem cells

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Topic
Basic Research, Medicine, and Health

Problem
A large number of molecules have been proposed to synchronize the expression of circadian genes in cultured cells. Among these molecules, the cell differentiation inductors forskolin and dexamethasone are used as standard methods in the synchronization of circadian genes in vitro. 1α,25-dihydroxyvitamin D3 (1α,25-(OH)2D3), the biologically active form of vitamin D, and its analogues have been demonstrated to induce differentiation of numerous types of benign and malignant cells, e.g. 1α,25-(OH)2D3 has been found to enhance the osteogenic phenotype in embryonic and adult mesenchymal stem cells, but little is known about its role in circadian synchronization. The aim of this study was to determine if the biologically active form of vitamin D alters the expression of circadian genes in adipose-derived stem cells (ADSCs).

Background
The role of 1α,25-(OH)2D3 in the integrity of bone and calcium homeostasis has been well established. In addition, 1α,25-(OH)2D3 has been associated with many important functions, such as metabolism control, cell growth, differentiation, anti-proliferation, apoptosis, and adaptive/innate immune responses. The circadian rhythm regulates a variety of biological processes, in addition to its role in regulating the sleep-wake cycle. Many of the circadian regulated processes are related to the functions associated to 1α,25-(OH)2D3. Furthermore, a genetic pathway has been identified recently underlying the observation that vitamin D deficiency alters the expression of the circadian genes Npas2 and Per2 during osseointegration. The work presented here has been published as: Gutierrez-Monreal M, et al. “A Role for 1α,25-Dihydroxyvitamin D3 in the Expression of Circadian Genes”. Journal of Biological Rhythms, 2014.

Hypothesis
The hypothesis of the work presented here is that 1α,25-(OH)2D3 is able to synchronizes the expression of the core circadian genes Bmal1 and Per2 in adipose-derived stem cells.

Research
To determine if 1α,25-(OH)2D3 affects the expression of circadian genes, we measured the expression by qPCR of two core clock genes, Bmal1 and Per2, and two clock output genes, Dec1 and Bglap, which are involved in cell differentiation and bone homeostasis, respectively. ADSCs were serum shocked for two hours with 50% horse serum, serum shocked for two hours with 50% horse serum and supplemented with 1α,25-(OH)2D3, supplemented with only 1α,25-(OH)2D3, spiked for two hours with 1α,25-(OH)2D3, and under the presence of free-serum medium as a control condition. ADSCs were harvested every four hours over a sixty hour period for the five mentioned experimental conditions.

Observations
The most important result was that 1α,25-(OH)2D3 was able to synchronize circadian gene expression in ADSCs, as observed through the oscillation of the Bmal1 and Per2 genes. The rhythmic expression of Bmal1 and Per2 showed different amplitudes for each condition, while the control has a more stable profile through the 60 hour period. Furthermore, the expression profiles of Bmal1 and Per2 were in opposite phases, as expected. The genes Dec1 and Bglap showed no circadian fluctuation in any of the experimental conditions. The expression profiles were similar to the basal expression found in the control condition. These results showed that the expression of Dec1 and Bglap genes through time was not affected by the treatments in ADSCs. These results suggest an important role of this hormone in the maintenance of circadian rhythms at the cellular level. Our findings demonstrate that adult mesenchymal stem cells may be entrained with either serum shock or 1α,25-(OH)2D3, suggesting relationships between circadian oscillations and stem cell properties such as pluripotency and proliferation.

Submission ID: 69074
Submission Title: The lymph node as a new site for kidney organogenesis

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Topic
Basic Research, Medicine, and Health

Problem

There are nearly 400,000 end-stage renal disease (ESRD) patients in the United States and approximately 2,000,000 worldwide, increasing 4-5% annually. All ESRD patients will need dialysis or transplantation to stay alive and a severe organ shortage is driving research for alternative therapies. Despite promise, cell-based regenerative medicine still remains a challenge, due to an inability to completely recover structurally complex organ functions.

Background

A potential approach to replace organ functions is through organogenesis. Renal capsule grafting is a well-established method of growing rudimentary organs in vivo for extended periods. However, whereas ectopic transplantation under the kidney capsule is suitable in rodents, it is clinically unrealistic as the human kidney capsule and parenchyma cannot be easily separated to permit cell transplantation.

Hypothesis

Given our recent finding that the lymph node (LN) can serve as an in vivo factory to generate or sustain complex structures like liver, pancreas, and thymus, we investigated whether it could also support kidney organogenesis from mouse renal embryonic tissue. The specific hypothesis behind the proposed research is that LNs are highly vascularized, prerequisite for glomerular filtration and hormonal function.

Research

To investigate whether mid-embryonic mouse kidneys could integrate into a host mouse LN and undergo morphological maturation, renal tissues were harvested from C57BL/6 GFP+ embryos and injected directly into jejunal LNs of adult wt C57BL/6 mice. Mice were euthanized for analysis at pre-defined time points, and repopulated LNs were examined at the cellular and molecular level. To investigate whether host bone marrow cells contributed to kidney organogenesis within the LN, GFP BM chimeras were generated. To assess whether the ectopic kidney could proliferate in response to growth stimuli, host mice underwent left nephrectomy.

Observations

When embryonic kidney fragments were transplanted into the LN, host blood vessels integrated into the developing glomeruli suggesting access to the bloodstream, a critical challenge to achieve filtration. Importantly, not only did the LN furnish the developing tissue with host cells, but also provided it with homeostatic signals, since nephrectomy augmented ectopic kidney maturation. Finally, although functional organogenesis from embryonic kidney single-cell suspension is challenging within the LN due to kidney complexity, we demonstrate that this innovative platform can be exploited to study the behavior of multiple cell populations with stem/progenitor features.

Submission ID: 69166
Submission Title: Characterization of peritoneal mesothelial cells as a potential source of stem cells

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Topic
Basic Research, Medicine, and Health

Problem
Recently, regenerative therapy using stem cells began a revolutionary trend. However, the source of stem cells is limited for cell therapy.

Background
Stem cells have the distinct ability to self-renew and to differentiate into various cell types. Mesothelial cells line the body's serous cavities including peritoneum and are able to be isolated from spent peritoneal dialysis fluids (PDF).

Hypothesis
This study investigated whether the peritoneal mesothelial cells from PDF can be used as a potential source of stem cells for cell therapy.

Research
The pertoneal mesothelial cells were isolated from spent peritoneal dialysis fluids and cultured in stem cell medium and characterized by the expression of markers using RT-PCR and FACS. The differentiation of peritoneal mesothelial cells into osteoblast, chondrocytes and hepatocytes was induced by differentiation media and determined by Alizarin red, Alcian blue and Indocyanine green staining, respectively.

Observations
The growth of peritoneal mesothelial cells isolated from PDF was continued to passage 7th without any growth retardation. RT-PCR analysis revealed that peritoneal mesothelial cells expressed CK8, CK18, UPA, and Desmin. The peritoneal mesothelial cells expressed CD73 and CD90, and also showed the sphere formation. The differentiation potential of peritoneal mesothelial cells in PDF into osteoblasts, chondrocytes and hepatocytes was demonstrated using Alizarin red, Alcian blue and Indocyanine green staining, respectively. Especially the expression of AFP and ALB was detected

from hepatic induced peritoneal mesothelial cells. Therefore, this study revealed that peritoneal mesothelial cells from PDF have the property of adult stem cell regarding to growth and differentiation potential, which makes the use of peritoneal mesothelial cells for cell therapy be possible

Submission ID: 69612
Submission Title: Impact of Space Flight on Mouse Bone Marrow Cell Development

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Topic
Basic Research, Medicine, and Health

Problem
This study addresses the impact of space flight on mouse bone marrow cell development.

Background
Bone marrow is a complex microenvironment that is necessary for the generation of red and white blood cells (hematopoiesis) in adult animals. This organ can be disrupted by numerous factors including stress, bone changes, alterations in circadian rhythm and irradiation; all of which occur during space flight.

Hypothesis
We tested the hypothesis that space flight will impact the developing cell populations.

Research
We had access to normal mouse bone marrow from mice that were part of the 13-day Commercial Biomedical Test Module-2 (CBTM-2) experiment that was flown on a space shuttle flight, Space Transportation System (STS)-118. We used flow cytometry to assess the expression of molecules that define the maturation/activation state of cells from the humeri of C57BL/6J mice. We also differentiated mouse bone marrow cells in the presence of recombinant macrophage colony stimulating (rM-CSF) factor for 14 days during the flight of space shuttle STS-126. We used flow cytometry to assess cell surface molecule expression and RNA was also preserved during the flight

to perform a microarray for additional information. This work was funded by NASA grants NAG2-1274 and NNX08BA91G and NIH grant RR16475.

Observations
In the in vivo studies, there were no composite phenotypic differences between total bone marrow cells isolated from flight- and ground-control mice. However, there were subpopulation differences in Ly6C, CD11b, CD31, Ly6G, F4/80, CD44 high, and C-Fos. In the in vitro studies, there were significant differences in the numbers of macrophages that developed in space compared to controls maintained on Earth. There were significant changes in the distribution of cells that expressed CD11b, CD31, F4/80, Mac2, Ly6C and C-Fos. We also found a pattern of transcript levels that would be consistent with an outcome of relatively normal differentiation but increased proliferation by the bone marrow macrophages that were assayed after 14 days of space flight.

Submission ID: 70000
Submission Title: Experimental Approaches to Derive CD34+ Progenitors from Human and Nonhuman Primate Embryonic Stem Cells

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Topic
Basic Research, Medicine, and Health

Problem
Current clinical use of CD34+ cells are either autologous or allogeneic transplantation from adult tissues, which might not be practical in future application. With the advancement of embryonic stem cells, the controlled/directed differentiation of pluripotent stem cells has become an alternative source for obtaining large CD34+ populations of clinical relevance.

Background
CD34-based stem cell therapy has existed for more than a decade. Injection of CD34+ hematopoietic stem cells selected by monoclonal antibodies reconstituted bone marrow hematopoietic capacity after lethally irradiated in baboons. Years later, the discovery of circulating endothelial progenitor cells positive for CD34 has deeply impacted the growth of this field, extending beyond hematopoietic reconstitution to show that the progenitors in circulation could repair the vascular damage. To date, the clinical trials using CD34+ cells cover various diseases including acute and chronic ischemic heart failure, spinal cord injury, liver cirrhosis, and peripheral vascular diseases. A recent PubMed search using the keyword “CD34 cell therapy” resulted in 6,948 articles about this topic. Because of the therapeutic potential, we need to identify easily accessible and

reliable sources, which is the main focus for future translational application.

Hypothesis
With the advancement of embryonic stem cells, the controlled/directed differentiation of pluripotent stem cells has become an alternative source for obtaining large CD34+ populations of clinical relevance.

Research
Differentiation of the embryonic stem cells will give rise to inexhaustible CD34+ cells, which becomes an exciting approach for biomedical research and for regenerative medicine. Here, we review the main methods that have been published for the derivation of CD34+ cells using the human and nonhuman primate stem cells. Therefore, it enables us to oversee the current status of this field, to compare the methods used for this purpose, and to consider the issues in translating the bench science to bedside therapy.

Observations
Although a plethora of CD34+ cell generation methods have been reported, the rationale of the differentiation approach remains unexplained under most circumstances. Similarly, few protocols have not been validated yet. However, successful ESC-based therapies depend developing a simple and efficient protocol for the differentiation of ESC into functional CD34+ progenitors. This is certainly the focus of the translational aspect of stem cell biology. It is believed that the integration of understanding the early events of embryogenesis in culture and establishing scientifically sound test system for identification of useful cell type(s) will accelerate the CD34+ cell therapy for treating the patients.

Submission ID: 70100
Submission Title: Weekend-free culture of pluripotent stem cells

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Topic
Basic Research, Medicine, and Health

Problem
The conventional methods for culturing human embryonic stem cells (hESCs) have required a daily-based medium change. Apart from being tedious and private life disturbing because of weekend feeding, it is also very costly due to the large amount of medium consumed during the whole culture

period. In this study we describe a system that allows for cultivation of hES/iPS cells without feeding them during weekends.

Background
Recently Rodin and colleagues (Rodin et al, Nature Communications 2014; Rodin et al, Nature Protocols 2014) have shown that LN-521™, a human recombinant laminin protein and naturally expressed in the inner cell mass of the early embryo, allows robust long-term self-renewal and single-cell clonal cultivation of human pluripotent stem cells. LN-521™ provides a biorelevant niche for hPSCs and we are now able to show that hESCs can be cultured more efficiently and economically even without the need of daily feeding.

Hypothesis
By successfully recreating the special stem cell niche with Laminin-521, receptors and signalling pathways of critical importance for pluripotent stem cells are correctly activated. This leads to less dependence on a very complex cell culture medium as the matrix itself has growth factor like properties stabilising the cells and that the requirement of daily feeding is not needed.

Research
In this study, three different feeding patterns, daily feeding, one feeding and no feeding, were applied to maintain hESC line HS181 on LN-521™ for 10 consecutive passages.

Observations
Data show no significant differences observed between the three feeding patterns based on the microscopic observation of cell morphology and proliferation. After only 4 days the single-cell plated cultures were ≥80% confluent and cell viability was at all times ≥90% for all three groups. The pluripotency of cells after 10 passages was evaluated with immunocytochemistry (ICC) and the results show >90% Nanog expression, and >80% Oct-4 expression. RT-PCR showed consistent result with ICC staining in terms of transcription levels of Nanog and Oct-4. Moreover, no differentiation marker (Pax6, Brachyury, and sox17) was detected. Chromosome analysis after 10 passages indicated normal female karyotype for all groups. Our preliminary conclusion is that by culturing hESCs on the biologically relevant niche-protein LN-521™ reduced feeding frequency or no feeding at all is possible in an easier and more predictable manner than previously shown. Ultimately this benefit most researchers in terms of both labor and material cost savings and also allows for more standardized culture protocols.

Submission ID: 70552
Submission Title: Prostate cancer exosomes can differentiate bone-marrow derived mesenchymal stem cells into myofibroblast-like cells

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Topic
Basic Research, Medicine, and Health

Problem
There is a growing evidence that mesenchymal stem cells (MSCs) recruit to the tumour microenvironment of prostate cancer. Once at the tumour site, MSCs promote tumorigenesis, but very little is understood about the mechanism behind this.

Background
Changes in the fibroblastic stroma often accompany carcinogenesis, and are characterised by accumulating myofibroblast population. TGFβ, secreted by cancer cells is thought to be the principle factor responsible for forming cancer associated myofibroblasts, which promote tumour growth partly through pro-angiogenic activities. Such changes contribute towards disease progression, and correlate with poor clinical outcome. Mesenchymal stem cells of bone marrow origin have been proposed to be a possible source of cancer associated myofibroblasts. The factors secreted by cancer cells that impact MSC fate however are poorly understood.

Hypothesis
Here, we hypothesise that cancer derived exosomes, which are nanovesicles expressing sTGF-β, can differentiate bone-marrow derived mesenchymal stem cells (BM-MSC) into disease promoting myofibroblast.

Research
Exosomes from prostate cancer cell line (DU145) were isolated by ultracentrifugation on a sucrose/D2O cushion and characterised by nanoparticle tracking analysis, western blot and TGFβ (ELISA). BM-MSC were defined by cell surface phenotype and their capacity to undergo adipogenic differentiation. Soluble TGF-β1 (sTGF-β1), DU145 exosomes or DU145 conditioned media rendered exosome deficient (by ultracentrifugation or Rab27a knock down) were used as stimuli for MSC differentiation. The ALK-5 inhibitor SB431542 or neutralising antibody was used to block exosomal

TGFβ. Myofibroblast differentiation of MSC was assessed by immunohistochemistry for αSMA expression, as well as vascular endothelial growth factor-A (VEGF-A) and hepatocyte growth factor (HGF) evaluation using ELISA. The effect of exosomally-differentiated BM-MSC in modulating migration and proliferation of both endothelial (HUVEC) and tumour cells was examined using the Via Count dye and a scratch assay, respectively. Tubule-formation assay was carried out in vitro through co-culture of exosomally-differentiated BM-MSC with endothelial cells to evaluate cell organisation.

Observations
BM-MSC underwent adipogenic differentiation staining positive for Oil Red O. Purified prostate cancer exosomes strongly inhibited adipogenic differentiation of MSC, differentiating instead into α-smooth muscle actin positive myofibroblast-like cells, with elevated VEGF-A and HGF secretion. Rab27a knockdown decreased secretion of exosomes into culture media, which in turn failed to drive myofibroblastic differentiation of MSC. Similar results were generated by ultracentrifugation depletion of exosomes. Exosomally-differentiated MSC were found to support endothelial cell proliferation, migration and tubule-formation consistent with a pro-angiogenic phenotype. Furthermore, exosomally-differentiated MSC strongly supported prostate cancer cell proliferation and motility. Therefore, we conclude that exosomally-differentiated MSCs exhibit tumour promoting features, akin to myofibroblasts at tumour sites.

Submission ID: 70619
Submission Title: Immune-modulatory properties of human Adipose Derived Stem Cells (hADSCs) isolated by a mechanical approach.

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Topic
Basic Research, Medicine, and Health

Problem
Autoimmune diseases are currently treated using immunosuppressive agents or small molecule inhibitors, but these approaches have side effects in the long term including increased risk of infections. Searching for new techniques to avoid these problems and to obtain a more resolute

treatment for autoimmune diseases, many groups of researchers have focused on the study of cellular therapies. Cell populations of immune and non-immune origin show a potential to control the homeostasis of the immune system, including regulatory T cells, natural killer T cells, myelo-monocytes and adult mesenchymal stem cells (MSC). The demonstrated ability of adult MSC to differentiate into various cell lineages combined with their immunosuppressive properties could facilitate tissue repair, making them a candidate source for autoimmune diseases treatment. Bone marrow derived MSC are considered the gold standard for such applications; however, several limitations exist, including low MSC frequency, the painful isolation procedure and the decline in cell numbers with donor age. Thus, there is increasing interest in identifying alternative MSC sources. One alternative is subcutaneous white adipose tissue that provides an advantage over other MSC sources due to the ease with which tissue can be accessed (under local anesthesia and with minimum patient discomfort) and the ease of isolating stem cells from the harvested tissue. In our study we analyzed the immunomodulatoty capabilities of human adipose derived stem cells (hADSC) obtaining by a mechanical method, in order to avoid the potential drawbacks of traditional enzyme-based methods including exposure of cells to bacterial enzymes with potentially pathogenic contamination, manipulation of the cells and the cost associated with the procedure.

Background

In addition to the application of hADSC as precursors of differentiated cells for cell replacement, the unique immunobiology and secretome of hADSC are increasingly appreciated for their therapeutic potential. Like MSC from other sources, hADSC are immunoprivileged due to lack of expression of class II major histocompatibility complex and co-stimulatory molecules on the cell surface. This potentially allows for allogeneic transplantation of hADSC into immune-competent recipients with minimal immune reactions in the host. In addition, hADSC have immunomodulatory properties and can promote tissue repair through immunosuppressive effects exerted via direct cell-to-cell interaction or secreted factors such as prostaglandin E2, leukemia inhibitory factor and kynurenine. The Lipogems® device is a patented instrument (PCT/IB2011/052204), which allows processing of lipoaspirated adipose tissue, through mechanical, non-enzymatic, micro-fragmentation. Processing consists of a progressive reduction in size of the adipose tissue fragments, while eliminating oil residues and blood components from the final product, through 3 different steps: (1) fragmentation of lipoaspirated adipose tissue by a 4mm2 mesh; (2) emulsification and removal of oil and blood residues by a saline flow through the device; (3) fragmentation of emulsified lipoaspirated adipose tissue by a 1mm2 mesh. We can obtain hADSC populations from Lipogems® product for expansion in culture by various methods, including enzymatic digestion of the tissue fragments, a single centrifugation or directly placing tissue fragments in culture. Each of those hADSC have been systematically characterized for growth features, phenotype and multipotent differentiation potential, and they fulfill the definition of mesenchymal stem cells provided by the International Society of Cell Therapy: plastic-adherent on standard cell culture vessels; multipotent differentiation potential (adipogenic, chondrogenic and osteogenic); positive for CD73, CD90 and CD105, and negative for CD11b or CD14, CD19 or CD79α, CD45 and HLA-DR in their cell surface immunophenotype.

Hypothesis

Starting from the knowledge that without enzymatic adipose tissue digestion we were able to obtain a MSC population, we demonstrated that the mechanical dissociation allows for the isolation of a higher percentage of the pericytes subpopulation compared with the traditional enzyme-based method. We hypothesize that the mechanical dissociation method to isolate hADSC, avoiding enzymatic digestion of adipose tissue will have greater immunosuppressive effects compared to hADSC obtained by enzymatic digestion.

Research

hADSC were obtained from subcutaneous adipose tissue of donors subjected to elective liposuction procedure under local anesthesia. Each tissue sample was processed by the following methods: 1. Enzymatic digestion with collagenase I and the resulting cell populations were termed Processed Lipoaspirate cells (PLA cells); 2. Mechanical dissociation followed by enzymatic digestion with collagenase I, and the resulting cell population s were called Lipogems® - collagenase hADSCs; 3. Mechanical dissociation followed by a single centrifugation and the resulting cell populations were termed Lipogems® hADSCs; 4. Mechanical dissociation and directly seeding of the tissue product into culture, and that cell populations were named Lipogems® product hADSCs. The cells prepared by each method were placed in culture dishes and expanded for two passages. The various hADSC were compared in term of viability, yield of cells, expression of surface markers, differentiation capability and immunomodulatory properties. PLA cells and hADSC populations obtained by mechanical dissociation at passage zero (p0), and after culture at passage two (p2) were phenotypically characterized by flow cytometry. After detachment with Trypsin/EDTA, cells were washed and 1x105 cells were re-suspended in PBS without Ca2+ and Mg+ for staining with the following antibodies: anti-CD90 FITC, anti-CD73 Brilliant Violet 421, anti-CD105 PE, anti-CD146 Alexa Fluor 647, anti-CD31 biotin, anti-CD34 PE/Cy7, anti-CD44 Brilliant Violet 421, anti-CD45 Brilliant Violet 510, anti-CD19 PE, anti-CD14 APC, anti- HLA-DR PC7. When staining with biotin-conjugated antibodies was performed the Brilliant Violet 605 streptavidin was used as chromofore. Dead cells often give false positive results, as they tend to bind nonspecifically to many reagents. Therefore, removing dead cells from flow cytometry data is a critical step, so live and dead cells discrimination was performed. Samples were fixed with 1%paraformaldehyde and then analyzed by flow cytometry (BD LSRII flow cytometer, BD Biosciences) using BD FACSDIVATM software (BD Biosciences). The immune-related capabilities of hADSC were studied by co-culture at different ratios with isolated T lymphocytes in the presence of a CD3 stimulus. Briefly, CD3+ T-cells were obtained by a negative selection of peripheral blood mononuclear cells isolated from the buffy coat of a healthy donor after Ficoll-PaqueTM gradient centrifugation. After two culture passages, hADSC were plated in round bottom 96well plates at different concentration (from 2x104cells/ml to 2.5x106 cells/ml) in RPMI, supplemented with 10% FBS and βmercaptoethanol. T-cells (2x105) were plated and activated by Dynabeads® human T activator CD3/CD28 (1:1 ratio with T lymphocytes). Lymphocyte proliferation was assessed by flow cytometry using CellTraceTM Violet Cell Proliferation Kit labeling. After 3 days, T lymphocytes were collected and assessed for the expression of CD3, CD4, CD8, CD25, CD127 and Forkhead box P3 (Foxp3) by flow cytometry analysis.

Observations

Lipogems® Collagenase hADSCs and PLA cells obtained from fresh tissues were analyzed for

surface marker expression by flow cytometry immediately after isolation. Centrifugation after the mechanical dissociation without enzymatic treatment did not provide sufficient cells to proceed with the analysis. The percentage of hematopoietic cells (CD45+) collected was between 70 to 90% for both cellular preparation methods (n=5). Within that population there were no differences in the proportion of cells expressing CD14 (macrophages and neutrophils), CD19 (B lymphocytes), CD3 (T lymphocytes), HLA-DR (antigen presenting cells and activated T cells), CD44 (effector memory T cells) and CD31 (monocytes and some types of T cells). Non-hematopoietic cells (i.e. CD45-) were also compared between the isolation methods. PLA cells showed 6.5% of CD45- whereas Lipogems® Collagenase hADSC had 20.7%, showing a relative enrichment of these cells by the mechanical dissociation method. This population of cells was further characterized by the expression of mesenchymal markers (CD90, CD73, CD105 and CD44). PLA cells showed 10.0±3.7 % of CD90+, 8.9 ± 5.2% of CD73+ and 10.6±4.0% of CD105+, whereas Lipogems® Collagenase hADSC had 20.2±5.7% of CD90+, 17.2±4.6% of CD73+ and 27.60±15.0% of CD105+ cells. The increased expression of these markes in Lipogems® Collagenase hADSC compared to PLA cells suggest an increased recovery of MSC after the mechanical dissociation. We evaluated the presence of the pericyte population (CD146+ CD31- CD34-) and the supradventitial population (CD146- CD31- CD34+). They were 0.55±0.16 % and 5.04±2.38 % respectively for PLA cells and 0.24±0.22 % with 11.10±4.75 % for Lipogems® Collagenase hADSC. After two passages in culture, cell surface marker analysis by flow cytometry highlighted that the hematopoietic population (CD45+) were completely lost. The expanded cells obtained were comprised almost exclusively of MSC based on the expression of mesenchymal markers (e.g., CD90, CD73, CD105 and CD44) at high percentage (~100%.) However there was a different proportion of the pericytes subpopulation depending on the isolation procedure, such that the hADSC obtained from the direct tissue culture after mechanical dissociation showed higher levels (about 20%, p value ≤ 0,01, analyzed by two-way ANOVA with Bonferroni correction for multiple comparison). The immunomodulatory capabilities of all hADSC populations were studied by co-culture at different ratios of MSC to T lymphocytes in the presence of a CD3 stimulus. Data showed that hADSC obtained by mechanical dissociation with enzymatic digestion at a ratio of 1:10, (i.e., 1 hADSC for 10 T lymphocytes) suppressed 50% of CD4 and CD8 T lymphocytes proliferation, reaching the strongest suppressive effect equal to 80% at the ratio of 1:1. The suppression was weaker using hADSC isolated without enzyme (ratio 1:10 equal to 10% of suppression and ratio 1:1 equal to 70% of suppression). We also tested if the presence of PLA cells and Lipogems®- derived stem cells could increase the percentage of induced regulatory T (iTreg) cells, which are a suppressive cell population involved in immune tolerance. We found a similar trend of increased iTregs population after co-culture with PLA cells or Lipogems®- collagenase hADSC (e.g. 40% more at the ratio 1:10). There were no changes of iTregs with co-culture of hADSC isolated by mechanical dissociation without enzymatic treatment. These data showed that hADSC obtained using the Lipogems® methodology provides cell populations with immunosuppressive properties that are influenced by the isolation method utilized. Isolation without enzyme in proliferation assay brings a decrease in hADSC suppressive effects with no change in the number of iTregs. Further investigations, expanding case studies and analyzing in detail the interaction between cell populations, for example searching for different secreted factors, could reveal the role of the distinct cell subpopulations obtained without the use of enzymes.

Submission ID: 70873
Submission Title: Investigating endogenous LINE-1 retrotransposition in human induced pluripotent stem cells

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Topic
Basic Research, Medicine, and Health

Problem
The generation of iPSCs offers a new perspective on the use of stem cells in the regenerative medicine field. Patient-specific iPSCs could then be derived to correct genetic defects in potential cell therapy. However, the safety of these cells has not been thoroughly assessed, especially their genomic integrity. Here, we investigated whether endogenous LINE-1 retrotransposons are mobile in iPSCs, thereby resulting in somatic mutations.

Background
The long interspersed element-1 (LINE-1 or L1) is a retrotransposon which replicates by a 'copy-paste' mechanism. It is capble of inducing insertional mutations that can result in diseases when genes are disrupted. It was recently shown that the copy number of L1 and other retroelements is stable in induced pluripotent stem cells (iPSCs). However, by using an engineered reporter construct over-expressing L1, another study suggests that reprogramming activates L1 mobility in iPSCs. It is therefore not clear whether iPSCs harbor somatic mutations from endogenous L1 retrotransposition.

Hypothesis
We hypothesize that upregulation of L1 transcription in iPSCs results in novel L1 somatic insertion events, thereby potentially compromising genomic integrity of these cells.

Research
L1 expression was assessed in isolated iPSC clones as well as during the reprogramming process by quantitative real-time PCR. To detect potential somatic insertions in iPSCs , we used targted high

throughput sequencing of L1Hs, the youngest and most active L1 retrotransposon in the human genome. However, we used a novel sequencing strategy. As opposed to conventional sequencing direction, we sequenced from the 3' end of L1Hs to the genomic DNA, thus enabling the direct detection of the polyA tail signature of retrotransposition for verification of true insertions.

Observations
We observed that L1 transcription was up-regulated in iPSC clones and that trannscriptional up-regulation was in fact initiated during the reprogramming process. In addition, deep coverage sequencing allowed us to detect seven potential somatic insertions with low read counts from two iPSC clones. Negative PCR amplification in parental cells, presence of a polyA tail and absence from seven L1 germline insertion databases highly suggested true somatic insertions in iPSCs. Furthermore, these insertions could not be detected in iPSCs by PCR, likely due to low abundance. We conclude that L1Hs retrotransposes at low levels in iPSCs and therefore warrants careful analyses for genotoxic effects.

Submission ID: 71123
Submission Title: Belgian Stem Cell and Umbilical Cord Blood Donation and Banking: Towards a Sustainable Government Policy with SMART Measures

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Topic
Ethics, Law and Society

Problem
Belgium has a marrow donor program registry (MDPB-reg), 14 stem cell banks (SC), 5 umbilical cord blood (UCB) banks and 8 non-hematopoietic banks. These are all public organisations financed

with state aid. Which strategy should the Belgian federal government implemented to guarantee a sustainable SC policy and thus assure access to affordable cellular therapies and regenerative medicine for the patients in Belgium?

Background

Since 2008 the MDPB-reg receives financial support for the HLA typing of potential SC donors based on a contract with the public National Institute for Health and Disability insurance (NIHDI). In 2009 based on the national Cancer Plan, structural state aid for the SC and UCB banks changed drastically. These government measures resulted in two main financial resources for both the public SC and UCB banks: 1: A lump-sum (€3,426,000) form the Federal Public Service for public health (FPS-ph) 2: A volume of activity based reimbursement system (€6,679,881) of the NIHDI Resulting in the current total budget of €10,105,881 annually for this sate aid mechanism . There is no specific funding for public non-hematopoeitic cell banking activities. This government strategy is five years in place. What is the outcome? The minister of public health called for an assessment of this policy in the timeframe 2013-2014. A thematic working group (TWG) of Belgian experts lead by the WIV-ISP Cancer Centre was established to evaluate the government policy. Based on its conclusions the TWG formulated a strategy with recommendations in the format of SMART (specific, measurable, attainable, relevant & time limited) measures to guarantee a sustainable government SC policy in the future.

Hypothesis

During the assessment, the TWG studied 3 major topics. A first topic was the MDPB-reg. The central question was: “Bearing in mind that haematologists prefer young, healthy male donors and that the chance to find a matching donor is 1/15000, what should be the main objective of the MDBP-reg recruiting strategy for the next five years?” Secondly the TWG discussed the UCB donations. How can the donation process be ameliorated in order to guarantee high quality UCB units? How should maternities and UCB banks support the donating parents? A last topic was the state aid for SC and UCB banks. The current lump-sum financing lead to a proliferation of the number of SC banks. Is the current financing a disadvantage for the SC and UCB banks with a high volume of activity? Should the the lump-sum state aid totally be shifted towards the NIHDI volume of activity based reimbursement system? In addition is structural state aid for the non-hematopoietic banks desirable?

Research

In the time frame April 2013-September 2014 the TWG engaged in a fact finding multi- aspect qualitative research method. In the TWG, experts of the main stakeholders – the public SC and UCB banks, the MDPB-reg, patient organisations and public authorities – were represented. The TWG expert panel discussed the mentioned three main topics: the MDPB-reg, the UCB donation and the business model for the financing of public SC, UCB and non-hematopoietic banks. Each of these three sessions lasted approximately two hours. Prior to each meeting the moderator prepared a discussion note which was send to all participants at least ten days before the TWG meeting. The discussion notes presented (1) quick literature reviews regarding the issue, (2) a list of best practices, (3) questions to be answered during the meeting and (4) a list of preliminary indicators for

the SMART measures . To assess all the topics from their political, economic, social, environmental and technologic perspective and relevancy a PESTEL analyse was being used,. All the data, the discussion notes and the conclusions of the TWG expert panel were catalogued and analysed using software for qualitative data research (QSR Nvivo10). Finally, all the results were reported in the recommendations addressed to the minister of public health: –'Belgian Stem Cell and Umbilical Cord Blood Banking: Towards a Sustainable Government Policy with SMART Measures–.

Observations

In 2013 the MDPB-reg counts for 65,956 HLA typed potential donors or 0.55 of the total Belgian population. (EU average is circa 0.73%) In Germany 5.82% of the population is registered as a potential SC donor. The TWG concluded that specifically recruiting within ethnic minorities will results in higher genetic or HLA haplotype diversity and thus create more added value for the registry. To reach this objective a pilot study to specifically recruit donors in ethnic minorities will be implemented. Massive broad public campaigns, however, should be avoid to assure better control over the targeted allocating of the available HLA typing budget. A second main discussion of the TWG expert panel concerned the UCB banks. Where in 2012 the EU average of UCB units per capita in stock is only 4.41/10000 inhabitants, in Belgium this ratio is 15.3 per 10000 inhabitants. The 5 Belgian public UCB banks hold an accumulated stock of 16,952 UCB units. There is no reason to expand the available infrastructure of 5 UCB banks and the approximately 45 maternities with UCB donation capabilities. However, the TWG expert panel recommends to implement a the level of the banks: "UCB coaches"; i.e. professionals that assist the maternities in (1) educating donors, (2) better guaranteeing the quality of the donated UCB, and (3) reducing the administrative burden in the harvesting procedure. Third the funding system of the public SC, UCB and non-hematopoietic banks was assessed. Per capita, Belgium has a high density of public banks compared to its neighbour countries: 1 SC bank per 695690 inhabitants and 1 UCB bank per 2087670 inhabitants. In The Netherlands, France and Germany this respectively is 1/1,295,923 & 1/16,847,010 ; 1/919,890 & 1/5,937,477 and 1/1,505,664 & 1/1,355,0976. Specifically for Germany it should be mentioned that it has a significantly high number of private UCB banks compared to B, F and NL. To reduce the number of public SC banks in the long run, the TWG expert panel is of the opinion that the lump sum state aid for SC banks should stop and entirely shift into a NIHDI volume based reimbursement. This NIHDI reimbursement system also should better represent the production cost of the therapies (UCB unit handling, PBSC, CD34 selection, bone marrow harvesting ,etc.). This system would advantage the bigger SC banks since they benefit economics of scale. The lump-sum state aid for the public UCB banks however should remain. To be profitable UCB banks should have a sales turn-over of at least 0.8% of their stock whereas for the Belgian banks this is only 0.5% by average. The new business model would cost €10,401,730 (€716,200 lump-sum and €9,685,530 volume of activity funding). In addition, within the limits of the EU directives and the EU regulation regarding state aid, public funding for up to 7 non-hematopoietic public banks is desirable. These banks are all related to a major Belgian hospital. They are a driving factor in the national program for translational research for both cellular therapies (e.g. ATMP) and regenerative medicine.

Submission ID: 71126
Submission Title: Assessing Parkinson’s Disease Progression using iPS Cell Derived Disease Specific Cells

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Topic
Basic Research, Medicine, and Health

Problem
Leveraging the genetic diversity of a clinical population, ORIG3N is introducing novel approaches for evaluating disease states utilizing Induced Pluripotent Stem (iPS) Cells. By using a clinically diverse population of inherited and idiopathic Parkinson's Disease (PD) patients and non-symptomatic family members, we will address differences in disease progression in both patient populations.

Background
Parkinson's Disease (PD) is a progressive disorder resulting in the degeneration of motor abilities in the patient. Our objective is to develop a cell based Parkinson’s Disease Progression platform to facilitate the evaluation of rate of disease progression in idiopathic PD. The disease pathology of idiopathic PD in slow versus fast progressors has been difficult to characterize in a clinically relevant manner.

Hypothesis
We will ultimately identify an in vitro disease-modeling platform for profiling disease progression. To this end we will use differentiation of induced pluripotent stem cells (iPSCs) as a route to access target cell neuronal lineages with specific genetic backgrounds that include autosomal genetic PD and sporadic or idiopathic PD.

Research
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 forms of PD, but also in those from patients of idiopathic-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.

Observations
The objective is to provide genetically defined, disease related platform for assessing disease progression using PD-patient specific neurons in culture. By monitoring the rate of disease pathology through maturation of dopaminergic neurons via stem cell differentiation we will be able to correlate disease phenotype with the clinical disease progression distinguishing slow and fast progressors.

Submission ID: 71355
Submission Title: Cell Line Variability in the Differentiation of Human Pluripotent Stem Cells to an Otic Progenitor-like Fate

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Topic
Basic Research, Medicine, and Health

Problem
Reports of inner ear mechanaosensitive hair cells and sensory neurons from pluripotent stem cells for the treatment of sensorineural hearing loss have resulted in inefficient or incomplete phenotype conversion. Furthermore, the differentiation potential of each cell line varies and to our knowledge, no studies have compared the differentiation potential of human embryonic stem cells (ES) and induced pluripotent stem (iPS) cells to an otic progenitor cell-like fate.

Background
Approximately 280 million people worldwide suffer from hearing loss. Prolonged exposure to loud noise, ototoxic drugs and aging contributes to hearing loss. Damage to sensory hair cells (HCs) within the cochlea is the primary cause of hearing loss which is followed by sensory degeneration of the nerve. Mammalian inner ear cells do not regenerate, resulting in permanent hearing loss. At present, there are no biological treatment options available for sensorial hearing loss resulting in the use of hearing aids and/or cochlear implants, which do not restore normal hearing.

Hypothesis
Human pluripotent stem cells (hPSCs) have had a significant impact on translational medicine, as these cells have been used for cell replacement, disease modelling and pharmacological screening. These cells can have a number of applications for improving our understanding and ability to treat hearing loss. During development the auditory system develops alongside the central nervous system and is dependent for proper formation upon signaling from the developing adjacent neural ectoderm. In this study, we hypothesize that when directing hPSCs towards a neural fate, a sub-population of cells will become otic progenitor cells.

Research
We and other investigators have defined methods to generate otic progenitor cells from hPSCs. To facilitate translation of hPSC-based applications relevant to treating hearing loss, we differentiated induced pluripotent stem cells (iPSCs) to otic progenitor-like cells and also compared its differentiation potential to human embryonic stem cells (hESCs). Differentiating cells were tested for the presence of transcriptional factors after 7, 14, and 30 days of differentiation. Analysis was then performed using qPCR and immunohistochemistry for gene and protein markers of the pre-placodal region and otic placode.

Observations
Our results show gene transcript and protein expression profiles are consistent with that seen during early mammalian inner ear development in both hESCs and iPSCs. Modification of cell maintenance protocols to enable feeder layer free handling of hPSC will be necessary to allow for the generation of a robust populations of otic progenitor-like cells from hPSC for translational purposes. Ongoing studies aim to further optimize the culture paradigm using conditions for translational studies addressing hearing loss.

Submission ID: 71813
Submission Title: The Effect of Prolonged DMSO Exposure on Recovery and Growth of Adipose-derived Stem Cells

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Topic
Basic Research, Medicine, and Health

Problem
The current prevalent practice in the cryopreservation of cells is to cryopreserve them within 30 minutes of exposure to dimethyl sulfoxide (DMSO) to avoid toxicity. Manufacturing realities can make this constraint difficult to meet. Does prolonged exposure to DMSO affect the viability and potency of the cells?

Background
In a frozen state, DMSO replaces the water in a cell and prevents ice crystal formation. At room temperature, DMSO is considered to be toxic to cells. In most cell culture labs, it is commonly believed that cells need to be frozen as quickly as possible, or at least within 30 minutes, limiting their exposure to DMSO and thereby reducing toxicity.

Hypothesis
DMSO is not as toxic to cells as commonly believed. The less viable cells will die off during the initial exposure period but the more viable cells will withstand longer exposure and, given recovery time, will proliferate.

Research
A known number of human adipose-derived mesenchymal stem cells were plated and cultured in 24-well tissue culture plates and exposed to standard cryopreservation medium (10% DMSO, 90% serum) for 30 minutes, one hour, two, six or eight hours, then frozen at -80°C. Corresponding controls were left in standard cell culture medium (10% serum, 1% antibiotic) for the same time points, then transferred to standard cryopreservation medium and also frozen at -80°C. After three days, samples and controls were thawed and rinsed, fresh cell culture medium was added, and all samples and controls were incubated under standard conditions (37°C, 5% CO_2, 100% humidity) for up to two weeks. Pictures and cell counts were taken immediately post-exposure, and three days and one and two weeks post-thaw; live cells were counted using Presto Blue, a metabolic assay that relies on the reducing power of living cells to measure their proliferation.

Observations
We have shown that prolonged DMSO exposure is not toxic to cells as believed. Samples exposed to DMSO for longer than two hours had the lowest cell count but were the fastest to recover and proliferate. After one week cells grew to 100% confluence and cell counts were not statistically different from one another, independent of exposure time. In conclusion, cells can be exposed to DMSO for longer than currently thought possible, providing significant advantages in manufacturing settings.

Submission ID: 72154
Submission Title: Efficient differentiation of midbrain dopaminergic and forebrain GABAergic neurons from human pluripotent stem cells

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Topic
Basic Research, Medicine, and Health

Problem
Midbrain dopaminergic (DA) and forebrain GABAergic (GABA) neurons derived from human pluripotent stem cells (hPSCs) are excellent sources for various disease modeling and drug screening including Parkinson's diseases, schizophrenia, epilepsy and anxiety. So far, huge efforts have been focusing on the efficient induction and maturation of midbrain dopaminergic and forebrain GABAergic neuron from hPSCs. However, the protocols from different laboratories vary in the cell culture methods, total culture duration and final outcomes.

Background
Currently, no commercial product is available for the efficient differentiation of midbrain dopaminergic and forebrain GABAergic neurons from human pluripotent stem cells.

Hypothesis
To meet customer requirements, two culture medium systems have been developed for the efficient induction and maturation of midbrain DA neurons and forebrain GABA neurons from hPSCs.

Research
During the development of these two medium systems, high throughput screening was used to screen chemicals. Design excellence was used to optimize the medium formulation. Real-time PCR, immonocytochemical staining, and flow cytometry were used to evaluate experimental results.

Observations
The medium kit for DA neurons includes floor plate induction, floor plate cell expansion and DA maturation media. The floor plate cells induced from hPSCs expressed midbrain and floor plate markers such as LMX1A, FOXA2 and OTX2. The induced floor plate cells can be expanded in floor plate expansion medium with an enormous increase in cell number. The expanded floor plate cells maintained midbrain and floor plate marker expression and differentiated into tyrosine hydroxylase (TH) and beta III tubulin positive DA neurons in DA maturation medium. The medium kit for GABA neurons contains three media: forebrain neural stem cell (NSC) induction medium, ventralization medium and GABA maturation medium. The induced forebrain NSCs expressed neural marker

SOX1 and rostral marker OTX2 and PAX6. After the treatment with venrtaization medium, cells expressed ventral marker NKX2.1 and rostral marker OTX2. The differentiated cells from ventral forebrain precursor cells expressed GABA and beta III tubulin, indicating GABA neuronal phenotype. By comparison to the published protocols, these two differentiation kits have several advantages including the easiness for using, short culture duration and high efficiency

Submission ID: 72174
Submission Title: Development of a 3D multicellular culture model to promote self-organization, differentiation and vascular network formation for lung tissue engineering

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Topic
Basic Research, Medicine, and Health

Problem
Chronic respiratory diseases are leading causes of death and morbidity, and currently lack curative therapies. At their end-stage, chronic lung diseases require lung transplantation for therapy, but the supply of donor organs is extremely limited. Regenerative medicine provides long-term hope for addressing both the epidemic of chronic lung diseases, and the shortage of donor organs, but critical hurdles remain to be overcome (Abreu SC, et al. 2009; Kotton DN and Morrisey EE. 2014). While recent studies have made great progress delineating the mechanisms of lung development and developing methods to drive iPS cells toward mature lung lineages (Hogan BL, et al. 2014; Gomperts BN. 2014), relatively little progress has been made in designing strategies by which these advances might be translated into tissue repair, and ultimately advanced to human studies. One critical limitation is the lack of available methods to generate complex three-dimensional lung tissue in culture suitable for eventual translational applications.

Background
To generate highly organized 3D tissues that mimic organ structure and function, tissue engineers have attempted to mimic the in vivo organogenesis process by manipulating critical aspects of the ex vivo environment (Gjorevski N, et al. 2014). During embryonic development, nearly all the endodermal organs, including the lungs, derive from organ buds, which are induced by different cell types to coordinate their assembly and then undergo repeated rounds of outgrowth and branching to grow into a full mature organ (Zorn AM and Wells JM. 2009). These observations led us to propose

that three-dimensional lung-bud formation can be recapitulated in vitro by co-culturing lung epithelial cells with endothelial and mesenchymal lineage cells.

Hypothesis
We hypothesized lung organoids can be formed in vitro to support self-organization, differentiation and vascular network formation in a 3D multicellular system.

Research
To enable cells to self-organize into a 3D structure (rather than attaching to planar culture dish) (Rock JR, et al. 2009), we coated culture plates with 40% Corning Matrigel combined with PneumaCult- ALI Maintenance Medium. The former is an extracellular matrix preparation derived from a gelatinous protein mixture secreted by Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells. The latter is a media formulation optimized to induce primary human bronchial epithelial cells (HBECs) mucociliary differentiation at the air-liquid interface (ALI). After solidification of the thick Matrigel layer, a single cell suspension including human bronchial epithelial cells (NHBE), human microvascular lung endothelial cells (HMVEC-L) and human fetal lung fibroblast (IMR90) was combined with 5% Matrigel and PneumaCult- ALI Maintenance Medium, and was seeded on top of the Matrigel layer. The ratio of each type of cell was human NHBE: HMVEC-L: IMR90 = 10:7:2 (Takebe T, et al. 2014). The cells were fed with 5% Matrigel in PneumaCult- ALI Maintenance Medium every other day and observed by light microscopy. Samples were collected at varying time points up to 28 days and processed by frozen section. Immunofluorescence staining and confocal imaging were applied for analysis.

Observations
The cells rapidly migrated into the 40% Matrigel layer and formed a 3D spheroid within approximately 24 hours. We visualized early formation of an endothelial network that was homogenously distributed in the 3D spheroid, using Cell Tracker labeled cells. Continuous remodeling and cellular self-organization was visualized by immunofluorescence staining and confocal imaging. Epithelial structures expressing the luminal cell marker KRT8 were formed by the first week, and were clearly distinct from the basal cell population, marked by P63 expression, that was present at baseline upon cell seeding. The fibroblast (Vimentin+) network formed in a dense and highly organized fashion that surrounded the epithelial structures. Occasional vascular tubes were noted randomly distributed in the fibroblast network at approximately week one, though vascular network formation and persistence remains to be optimized. Between week one and week three the cells continued to differentiate and self-organize into additional luminal structures, some of which contained abundant secreted mucus (visualized by MUC5AC immunostaining) inside the lumen. These observations indicate that the lung organoid contains differentiating goblet cells, the cell type responsible for secreting mucus onto the luminal surface of the airways. Markers of ciliated cells, including FOXJ1 and alpha Tubulin, were also visualized by immunofluorescence staining and confocal imaging, indicating ciliated cell differentiation inside the organoid. The size of the organoid remained stable after 3 days in culture and relatively little proliferation was observed, as indicated by Ki67 expression, consistent with rapid differentiation being favored over proliferation. Finally, the lung organoids formed by these methods in vitro were mechanically stable and could be manipulated

physically by forceps or spatula, supportive of future studies aimed at organoid transplantation.

Submission ID: 72229
Submission Title: The effects of dimethyl sulfoxide (DMSO) on the neural differentiation of Induced Pluripotent Stem Cells from Non-Human Primate

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Topic
Basic Research, Medicine, and Health

Problem
The differentiation potential of pluripotent stem cells varies sub¬stantially across cell lines. Certain cell lines have a higher capacity to differentiate into derivatives of some germ layers (Osafune, Caron et al. 2008; Bock, Kiskinis et al. 2011). For example, HUES 8 is best for pancreatic differentiation and HUES 3 for cardiomyocyte generation (Osafune, Caron et al. 2008). These non-trivial differences in developmental potential among pluripotent stem cell lines point to the importance of optimizing the differentiation of pluripotent cells for cell therapy (Osafune, Caron et al. 2008).

Background
In 2000, Woodbury and colleagues found rat MSCs treated with DMSO can elicited neuronal morphologies with a time course and induced neuron marker expression (Dale Woodbury n.d.). The formation of embryoid bodies (EB) in response to exposure to dimethyl sulfoxide (DMSO) is the main protocol that has been used to induce the differentiation of P19 cells into cardiomyocytes (Edwards, Harris et al. 1983; Arreola, Spires et al. 1993; Skerjanc, Slack et al. 1994; Abilez, Benharash et al. 2006; Angello, Kaestner et al. 2006; Jasmin, Spray et al. 2010). Recently, it was described a method to help overcome restrictions on the differentiation propensities of human pluripotent stem cells by culturing them in DMSO (Chetty, Pagliuca et al. 2013).

Hypothesis
In this study, we used marmoset iPS cells-based in vitro models to show the effects of different concentrations of DMSO on neuron, based on gene expression.

Research
Culturing iPS cells in dimethyl sulfoxide (DMSO) increased expression of NKX2.2, NKX6.1, Scip, Olig2, Sox10, NCAD and Sox1 in three marmoset iPS cell lines in a differentiation protocol in which cells are exposed to a combination of SAG (sonic hedgehog agonist), dorsomorphin, SB431542, retinoic acid and FGF2. We performed a comparative analysis of the transcriptomes of the three marmoset iPS cells lines and their differentiated derivatives, using Affymetrix RNA microarrays. 865 genes were found to be upregulated; 993 genes were found to be downregulated. We focused on GDF3, Prune2, LAMA1, CRYAB, ITGA8 and CDH5 as examples of genes identified by microarray analysis as being upregulated in DMSO-treated differentiated cells, and CDH20, RORA, Stc1, Eno2, DSC1 and CCND2, which were scored as being downregulated in DMSO-treated cells. Overall, qPCR validation was in complete agreement with the changes observed by microarray analysis. Furthermore, immunocytochemical analysis showed increased levels of NKX2.2, NKX6.1, Sox10, and Olig2, confirming their differentiation into the neural lineage.

Observations
This approach should be generally valuable for directed neural differentiation of pluripotent stem cells for experimental cell therapy.

Submission ID: 72380
Submission Title: Engineering of Macaque CD4+ T Cells and CD34+ Hematopoietic Stem Cells resistant to in vitro SIV Infection using Zinc Finger Nucleases Technology

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Topic
Basic Research, Medicine, and Health

Problem
Antiretroviral therapy dramatically decreases in vivo viral replication to levels below clinical detection but does not eradicate infection for persistent low-level HIV-1 replication and latent provirus in resting CD4+ T cells. Immune activation therapy is being used as an alternative approach to increase the turnover rate of latent virus reservoir through activation of infected cells that comprise

this reservoir, to promote cell death and accelerate virus clearance. However, on-going studies have yet to demonstrate complete virus eradication.

Background

CCR5 is the major HIV co-receptor, and individuals homozygous for a 32-bp deletion in Ccr5 gene are resistant to infection by CCR5-tropic HIV-1. Therefore, the CCR5 co-receptor provides a unique opportunity to exploit gene knockout technologies for anti-HIV therapy. The Berlin patient highlights the potential therapeutic benefit of CCR5 disruption in treatment and possible eradication of HIV infection. Various gene therapy approaches to block CCR5 expression are currently being evaluated, including the expression in hematopoietic cells of CCR5-specific intrabodies, ribozymes and siRNAs. The targeted cell populations include both mature peripheral T cells and Hematopoietic Stem Cells (HSC). The loss of CCR5 in HSC appears to have no adverse effects on hematopoiesis.

Hypothesis

Recently, autologous hematopoietic stem cell transplantation have been shown to be a safe, feasible, and reasonable approach for AIDS-related lymphoma in patients who meet criteria for transplantation. Assuming that hematopoietic stem cells could be collected before the patient becomes HIV-infected, the ultimate HIV therapy post-infection would be: 1. Early ART post HIV-infection to keep viral replication below detection, limiting infection to latent provirus in resting CD4+ T lymphocytes; 2. Establishment and transplantation of CCR5-modified autologous hematopoietic stem cells collected before HIV infection, using current technologies, under the control of immunosuppressive drug and during ART; and, 3. Interruption of ART after repopulate with the HIV-resistant stem cells. We would hypothesize that this semi-myeloablative-conditioning regimen could lead to the killing of all cells that, in theory, harbor the virus. The transplantation of the autologous HIV-resistant hematopoietic stem cells would allow the complete recovery. Since human hematopoietic stem cell transplantations before HIV-infection are currently unlikely to be available, the hypothesis could be tested on a suitable animal model such as the nonhuman primate mode for AIDS. A successful “proof-of-concept” experiment would demonstrate the feasibility of using molecular-based strategy to prevent viral infection.

Research

we are pursuing the use of engineered Zinc Finger Nucleases (ZFNs) to permanently disrupt the CCR5 open-reading frame. We are targeting the second extracellular loop of CCR5 to obtain cells with mutations which mimick a CCR5delta32 mutation. We used a reporter plasmid to check the activity of the ZFNS. CCR5-targeted ZFNs are currently being evaluated in vitro in our laboratory, targeting mature CD4+ T cells and hematopoietic stem cells isolated from naïve-uninfected macaque blood, bone marrow and umbilical cord samples. We are also adopting newly established technologies Transcription Activator-like nucleases (Talens) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) to disrupt CCR5 gene in the cells. We engineered SIV-resistant macaque CD4+ T cells using CCR5-ZFNs.

Observations

After nucleofection of mRNAs encoding for ZFNs into CD4+ cells isolated from macaques, we show

that these cells were resistant to in vitro SIVmac239, SIVmac251, and SIVagm infections as shown by the absence of p27 expression. We then focused on the modification of HSC isolated from macaque femoral bone marrow and umbilical cords. We established conditions required to purify and grow macaque CD34+ HSCs in vitro to maximize the efficiency of CCR5 gene disruption while minimizing any adverse effects on cell viability or hematopoietic potential. We successfully engineered CCR5-modified macaque hematopoietic stem cells that were resistant to SIVmac239 infection after in vitro differentiation and expansion on thymocytes. We demonstrated the feasibility of using ZFN technology to establish CD4+ T cells and hematopoietic stem cells resistant to SIV infection in macaque. Our data suggests that genome editing with CCR5-ZFNs may provide therapeutic benefit to HIV-infected individuals. The generation of a nonhuman primate model using this modern molecular-based strategy might significantly help in the design of new therapies to prevent viral infection and eradicate HIV infection in human.

Submission ID: 72550
Submission Title: Enhanced Maintenance of Genetic Integrity in Induced Pluripotent Stem Cells

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Topic
Basic Research, Medicine, and Health

Problem
Currently, induced pluripotent stem cells (iPSCs) are the most promising potential source of pluripotent stem cells for future clinical applications. Unlike embryonic stem cells (ESCs), which are pluripotent from the origin, iPSCs are derived from somatic cells that typically bear significantly more mutations than pluripotent cells. This raises a safety concern regarding the genetic integrity of iPSCs, which needs to be carefully addressed before applying iPSCs in the clinic.

Background
In 1991, the Big Blue Transgenic Mouse (BBM) system originally established by Stratagene (Kohler et al., 1991) was developed to screen for spontaneous point mutations in the lacI mutation-reporter transgene which can be recovered from any cell or tissue type. This allows researchers to determine the frequency and spectrum of spontaneous point mutations in various cell types. Our previously published and unpublished data show that the spontaneous mutation frequency is significantly higher in somatic cells than that in germ cells or in ESCs.

Hypothesis
We hypothesized that enhanced genetic integrity is a fundamental characteristic of pluripotent stem cells and created two aims to test this hypothesis – Aim 1: Test the hypothesis that pluripotent iPSCs function like pluripotent ESCs to maintain enhanced genetic integrity; Aim 2: Test the hypothesis that directed differentiation of iPSCs to form specific somatic cell types will result in a decrease in the level of genetic integrity maintained in these cells.

Research
Big Blue iPSCs provide an ideal cell model in which to test our hypothesis, because the lacI mutation-reporter transgene can be tracked and the spontaneous mutation frequency can be measured in starting differentiated somatic cells used to generate iPSCs, in iPSCs derived from the differentiated somatic cells, and in differentiated cells derived from the iPSCs in vitro. Thus, we generated iPSCs from adult tail-tip fibroblasts (TTFs) from mice heterozygous for two transgenes – a drug-inducible cassette encoding reprogramming factors and the BBM lacI mutation-reporter transgene. We then measured and compared the spontaneous mutation frequency in the starting TTFs, two karyotypically normal TTF-derived iPSC lines maintained over many passages, and subsequent fibroblasts derived from these iPSC lines by directed differentiation.

Observations
Our data indicate that the spontaneous mutation frequency was initially high in the starting TTFs, then was much lower in the iPSCs derived from these fibroblasts, and then rose again in the fibroblasts derived from the iPSCs to levels as high or higher than those in the starting TTFs. These results suggest that enhanced maintenance of genetic integrity is a fundamental characteristic of pluripotent cells, and that the mechanisms responsible for enhanced genetic integrity are subject to epigenetic reprogramming during the derivation or subsequent directed differentiation of iPCS. These results also provide insight into the safety of cells derived from iPSCs for potential therapeutic applications.

Submission ID: 72646
Submission Title: The human stem cell patent landscape

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Topic
Ethics, Law and Society

Problem

Stem cell and regenerative medicine technology developers must pay particular attention to patents at all stages of product R&D. Indeed, prudent consideration of patents as a component of overall R&D strategy can minimize potentially important downstream costs by ensuring that resources invested in the product have been optimized to best support freedom to operate of the product. Therefore, it is important that companies consider collecting patent information as early in the R&D cycle as possible. With regard to freedom to operate or licensing, an understanding of the stem cell patent landscape can assist a company in respectively identifying potential risks or partners. Today, stem cell players rely on patent landscapes to map scientific and legal trends. However, serious inconsistencies persist in collecting patent information as a result of reliance on methods that do not distinguish between patents claiming, or merely describing, a product (i.e stem cell) or between patent applications and granted patents. An obvious problem with patent landscape studies relates to the fact that every patent document is generally presented as a potential ownership of a product, which is a serious error.

Background

In their precursor work, Bergman and Graff (Collaborative IP management for stem cell research and development, 2007) reported that 10,681 patent documents (including 1724 granted US patents) had been published with at least one of the terms “stem cell(s), totipotent cell(s), pluripotent cell(s), multipotent cell(s), progenitor cell(s), precursor cell(s)” in a claim. In fact, this dataset refers indistinctly to patent applications or patents dealing with various stem cells such as plant stem cells, mouse stem cells, or transgenic swine, polysaccharides, antibodies, growth factors, nucleic acids, or various devices. Thus, coming up with the number 10,681 only creates a false impression that patenting activity in this field is potentially dramatic. It is true that as an enabling technology, stem cells may be particularly susceptible to the emergence of a patent thicket but no direct evidence has clearly established that the proliferation of stem cell patents or patent applications has been a bar to the development of stem cell-based tools for research and therapy. A second stem cell patent landscape concerns the work done by A. Konski and D. Spielthenner (Stem cell patents: a landscape analysis; Nature Biotechnology , 2009). Their analysis of 8000 patent applications and patents bearing the term “stem cell” in the claims lead to the listing of the top 20 “most important” patents identified as brokering knowledge in stem cell research based on cross-citation between or among the patent documents. The analysis based on patent citations used in this study yielded misleading results by failing to examine the claims of the patents.

Hypothesis

The most effective patent landscaping protocols align scope and methodology with the purpose of the specific issues to be addressed. To shed some light on the explosive growth in the number of stem cell patents being filed since the 2000s, we applied our methodology to bring clarity and to deliver useful patent information by considering patents as documents having legal significance and not only as documents with bibliographic data and citations.

Research

Thousands of patent applications and patents have been filed or granted in relation to inventions

which claim stem cells in some or other manner. Thus, to clearly define our stem cell patent landscape, we decided to specifically focus on issued patents. Only human stem cell inventions per se have been taken into consideration. The first task in the construction of the stem cell patent database was to isolate patents of interest i.e. only those claiming product or process involving stem cells per se. As there is no straightforward manner associated directly with stem cell patents per se, all retrieved patents were validated for inclusion by manual reading of the claims of all patents. Searches for patent documents were conducted in both public and commercial patent databases using combinations of more than 20 keywords in claims such as: “stem cell, pluripotent, progenitor, precursor” in conjunction with relevant patent classification groups. In order to efficiently clean our patent dataset of patents which were irrelevant to the subject matter of claiming stem cells per se but still contained one of the keywords in their claims, the patents containing one of the keywords in their claims were reviewed manually. Patents disclosing human (or primate or mammal) stem cells but, for any reason, only specifically claiming for example monkey or mouse stem cells, were not added in the patent dataset. After the manual reading of all patents that were identified by our searches, the searching portion of the study was completed. To provide further insight into each patent retrieved we have taken the step of creating our own classification scheme. Each patent of our database is classified according to the origin of the starting material to isolate or prepare stem cells. Patents were individually reviewed to determine which single stem cell category best classify the patent. This new classification, called STC for “stem cell type”, allows to identify pioneer companies involved in the development of particular technologies.

Observations

This study provides statistical analysis for all US patents identified during the searches and validated as a second step according to the study design. The statistical analysis includes the distribution of the patents based on priority dates, assignees and stem cell types. In total, 2098 US Patents were identified as specifically claiming human stem cell inventions as of June 1st, 2014. Of this total number, 1736 patents were still in force at the end of August 2014. The total of 2098 US patents are distributed over 404 private companies and 349 public organizations of which 215 are private and 164 are public US assignees. The 2098 patents currently making up the stem cell patent dataset have been segmented into more than 70 subgroups of the SCT classification. For each SCT it is possible to perform a detailed analysis including the evolution over time of patent filings, identification of the key players, collaborations, as well as identification of the key patents based on their claims. The 10 main stem cell type groups form 65% of all the relevant patents. Nearly 20% of US patents related to stem cells are directed to hematopoietic stem cells. This number is even higher if all blood product stem cells, such as umbilical cord and bone marrow stem cells, are considered. The SCT subgroup “IPSC”, accounts for 2% of patents. The “young” Induced Pluripotent Stem Cell patent landscape involves some thirty actors, like KYOTO UNIVERSITY (6 patents) and WISCONSIN ALUMNI RESEARCH FOUNDATION (4 patents). 750 different assignees are involved in US stem cell patents as of June 2014. 6 public and 5 private US organizations have been the most prolific in terms of stem cell patents granted. The top 5 assignees are WISCONSIN ALUMNI RESEARCH FOUNDATION (52 US patents), GENERAL HOSPITAL (51 US patents), GERON (43 US patents), OSIRIS THERAPEUTICS (42 US patents) and UNIVERSITY OF MICHIGAN (41 US patents). 73% of the assignees (551 actors) in the stem cell field own 1 or 2 patents. This study

constitutes an analysis of the extent and scope of patents related to stem cells. It provides accurate and high-added value information to all those working in the field who need a comprehensive patent dataset to know what is going on, who is doing it, and which may among other things be of particular interest to potential patent applicants, those responsible for monitoring and analysis of stem cell patents, and all those who need up-to-date information in this field. This patent database also offers the possibility to perform further analysis of patents of interest thanks to a searchable Excel table including, for each patent we have manually evaluated, 8 different data fields.

Submission ID: 72699
Submission Title: Co-Regulation of Plutipotency and Enhanced Maintenance of Genetic Integrity at the Genomic Level*

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Topic
Basic Research, Medicine, and Health

Problem
Determining the mechanism(s) by which pluripotent cells (ES and iPS) maintain enhanced genetic integrity compared to somatic cells. Elucidating these mechanisms could potentially assist with ensuring maximum safety of future stem cell-based therapeutic protocols.

Background
It is known that stem cells more stringently maintain genetic integrity compared to somatic cells, but the mechanisms by which this is accomplished and the manner in which enhanced maintenance of genetic integrity is co-regulated with pluripotency are not well understood.

Hypothesis
We hypothesize that the epigenetic state of pluripotency, as defined by expression of pluripotency factors including SOX2, OCT4, and NANOG, is mechanistically linked at the genomic level to a cellular state in which enhanced genetic integrity is maintained, as evidenced by differential expression of genetic integrity genes and pathways regulated directly or indirectly by pluripotency factors.

Research
Expression levels of DNA repair and cell death genes (collectively referred to as ‘genetic integrity

genes') in human and mouse pluripotent ES and iPS cells were examined by mining existing transcriptome datasets. Additional mining of cistrome datasets describing genome-wide binding patterns for three pluripotency factors, SOX2, NANOG, and OCT4 was conducted to test a potential mechanistic link between differential expression of genetic integrity genes and the presence and function of pluripotency factors. Large-scale interaction networks were generated using Ingenuity Pathway Analysis (IPA), and a subset of these computationally predicted interactions were experimentally validated in ES cells using ChIP-qPCR.

Observations
DNA repair genes are substantially up-regulated in human and mouse pluripotent cells compared to somatic cells, while cell death genes are differentially expressed overall, with both up- and down-regulation. DNA repair and cell death pathways are both significantly up-regulated in human and mouse ES and iPS cells. A substantial number of differentially expressed genetic integrity genes are directly bound by pluripotency factors in ES and iPS cells, and an even larger number of these genes are bound by factors which are, themselves, regulated by pluripotency factors. Taken together, our data support our hypothesis that enhanced maintenance of genetic integrity is mechanistically linked to the epigenetic state of pluripotency at the genomic level. In addition, these findings demonstrate how a small number of key pluripotency factors can regulate large numbers of downstream genes in a pathway-specific manner. *In press, Stem Cell Research

Submission ID: 72852
Submission Title: Mesenchymal stem cells response to different doses of ionizing radiation in vitro

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Topic
Basic Research, Medicine, and Health

Problem
The ionizing radiation (IR) used in the radiotherapy (RT) to eliminate tumor cells, does not have the capability to distinguish between normal and tumor cells, and hit the majority of the cells in the

pathway of the photon beam. Nevertheless it is one of the most effective approach for tumor cells killing. Beside technological resources to restrict the radiation field to the tumor volume, normal cells inevitably end up being affected. Thereby the comprehension of how normal cells, specially the stem cells, respond to different doses of IR is of great interest with clinical relevance.

Background

RT is one of the most currently used form for cancer treatment. Particularly, it is used in the patients conditioning as a coadjuvant of leukemias' treatment, in order to decrease residual leukemic cells, being also applied to cause the medullary ablation prior to bone marrow transplantation. Thus, IR is important to decrease body's immunoreactivity to avoid graft x host disease upon an organ transplantation. Exposure to IR may cause various types of DNA damage in a dose-dependent manner. A hallmark of IR is the formation of clustered damage sites, which include double strand breaks, characterized by two or more lesions within one or two helical turns of the DNA. In response, cells have a sophisticated machinery to promote the repair of the lesions. DNA damage sensors within the nucleus detect this damage and initiate signal transduction pathways resulting in activation of cell cycle checkpoints and DNA damage repair. The cell's response involves a number of proteins including, γH2AX, DNA-PK, ATM/ATR, Chk1/2 and p53, as well as the generation of reactive oxygen species/reactive nitrogen species (ROS/ RNS). As a result, cells can undergo different fates: survival, in case of efficiently elimination of the DNA damage; cell death, when the caused DNA damage is irreparable; or also, chromosome aberrations and gene mutations as a result of an incorrectly rejoining of the DNA double strand breaks. Cells may die by different mechanisms following irradiation. For the most of the cells, the mitotic death is the dominant mechanism; while for some cells, apoptosis is more relevant, and also, the growth arrest, which is referred as senescence, is commonly observed in various types of tissues and organs. In common, whatever the mechanism, cell loses its ability to proliferate indefinitely, such a feature explored in the RT for the eradication of a tumor.

Hypothesis

Once it remains a challenge for the radiotherapeutic protocols to restrict the incidence of the radiation in the tumor volume, leading to the spreading of radiation by the surrounding normal tissue, there is a high probability of the mesenchymal stem cells (MSC) be targeted. It is due to the incidence of mesenchymal stem cells all over the body, as in the adipose tissue, peripheral blood, umbilical blood, aminiotic fluid, adult brain and in other sites, populating various stromal compartments of a human body. Thus, it is of interest to evaluate the responsiveness of these cells to IR, in terms of radiosensitivity, since it is inherent to this kind of treatment that not only the tumor but also normal cells are be affected by the IR, which may cause cytotoxicity in both cases. In this context, the knowledge of how normal tissue cells respond to IR is relevant, in order to optimize cancers treatment, which efficacy rely on the highest killing of tumor cells than the surrounding normal tissue cells. Concerning the issue in question, the evaluation of MSC behaviour upon IR is desirable, in order to improve a clinical radiotherapeutic treatment in body areas where healthy mesenchymal cells are also founded. This kind of knowledge would be useful to minimize the damage to normal tissue, based on the tolerable dose response of healthy cells to the IR

concomitantly to its toxic effect on targeted tumor cells.

Research

The C3H10T1/2 immortalized lineage of mesenchymal stem cells, isolated from mouse embryos were used in this study. The cells were cultured in DMEM medium supplemented with 10% of SFB additionally to antibiotics (penicillin/streptomycin). For each experiment, 8x104 cells were plated in six well plates. 24 h after plating, each well medium volume were exchanged for fresh medium prior irradiation. The irradiation were performed using a Linear Photon Particles Accelerator of 6MV (Varian), in a dose rate of 300cGy/min, at the single doses of 2, 4, 6, 8, 10, 15 and 20Gy. Firslty, cells response to different doses of radiation was evaluated through the viability assay using Annexin-V and Propidium Iodide (PI) kit (BD Biosciences) according to manufacturer´ instructions. Data acquisition and data analysis was performed using a FACS Canto II Flow Cytometer and the Flow Jo Software respectively. In addition, cells´ clonogenic survival capacity was evaluated upon irradiation. For this purpose, different number of cells was plated in a volume of 6.5 mL of culture medium in 70 mm dishes, and immediately follow to the adherence of the cells in the plate (which took around 2h), cells were irradiated at the same doses described previously. Cells were maintained at the humidified incubator for 9 days. Follow this period, cells were fixed with acetic acid/methanol (1:7 v/v) and stained with 0.5% of Violet Crystal for 30 min. Colonies of more than 50 cells were counted. For the calculus of plating efficiency (PE) and survival fraction (SF), the following equations were applied: PE = 100 x (mean of colonies formed / number of cells plated) SF = mean of colonies counted / (number of cells plated x (PE / 100)) The oxidative status of the cells was also evaluated. The intracellular ROS content was verified, incubating the cells with 5 µM of CM-H2DCFDA (Invitrogen) for 40 min at 37°, followed by the analysis by Flow Cytometry. In addition, nitric oxide (NO●) production was evaluated in the supernatant of the irradiated and non-irradiated cells, using a high-sensitivity chemiluminescence technique provided by NOATM 280 apparatus, which method is based on the equimolar reduction of the stable NO● metabolites, nitrate and nitrite, in their reaction with vanadium. Statistical Analyzes were performed using Prisma 5 Software. For multiple comparisons among groups, ANOVA followed by the post hoc Bonferroni test was used. $p > 0.05$ was considered significant.

Observations

Decrease in cellular viability at 24h and 48h post irradiation was significant only at the highest doses over 8Gy in a crescent rate, achieving around 10% of dead cells at the dose of 20Gy in 24h. In 48h, the viability percentage decreased similarly, however, the peak occurred at the dose of 15Gy achieving around 7% of dead cells. In both period of time evaluated, there was a prevalence of apoptotic cells, verified by the increase of Annexin-V + cells (around 10%), while the percentage of necrotic (PI + cells) remained unaltered in relation to the control (non-irradiated) MSC. Beside the decrease in cell viability verified by the slight increase percentage of cells in apoptosis, the clonogenicity of MSC was also evaluated using the survival curve assay. This analysis showed that the replicative capacity of MSC was significantly affected by the IR in all tested doses ranging from 2Gy to 20Gy in a dose-dependent manner, given by the diminished number of colonies formed by the irradiated cells. The PE, which is given by the number of colonies formed by the non-irradiated cells, was of 59%. From these data provided, the SF could be calculated according to the formula

described above. For non-irradiated the cells, the SF is considered 1. At the dose of 2Gy the SF was 0.68; at 4Gy the SF was 0.47; at 6Gy the SF was 0.29; at 8Gy the SF was 0.17; at 10Gy the SF was 0.10; at 15Gy the SF was 0.009 and at 20Gy the SF was 0, which means that none colony was formed upon this dose of IR. Also, extrapolating these data to the biophysical models, it was possible to qualificate the radiobiological response of MSC to IR, taking into consideration the SF obtained and its correspondent shape of survival curve. From the single/multi-target survival curve, the IR dose required to reduce the fraction of surviving cells to 0.37 (denominated by D0 or D37) was 2.09Gy for MSC, which means that cells-event killing started to occurs at the dose of around 2Gy, resulting in a decrease of cell survival to 37% (63% of cell killing). The D10 parameter, that is related to the dose necessary to kill around 90% of the cells, was of 4.80, which means that approximately 5 Gy are necessary to reduce the survival of MSC to 10%. In addition, according to the linear quadratic model, the α / β ratio for MSC was of 17Gy, that fit these cells in the category of a fast or early response tissue to IR, with a lower frequency of DNA repair. Alterations in the redox status of MSC was evaluated at a low, an intermediary and a high dose of IR - 2Gy, 6 Gy and 15Gy respectively - at the time of 24h post irradiation for analyses. Although there were an increase in the generation of ROS observed upon the tested doses, it was not significant. On the other hand, NO● production in irradiated cells was significantly lower in comparison to NO● production in non-irradiated cells. Altogether, these data shows that apoptosis is not the main responsible mechanism for cell survival decreasing induced by IR in the MSC, but other processes might be involved, e.g. autophagy, mitotic catastrophe among others. Also, Also, the high α / β ratio, put in evidence the occurrence of low rate of DNA lesions repair, which seems to have relation with the low generation of ROS (that usually induces cell death when at high concentration), and the diminished production of nitric oxide (which induces a quiescent state of stem cells conferring resistance to radiation) associated with the high repopulation capacity of these cells. Clinically, it is highly relevant that normal cells are somehow resistant to the harmful effects of IR, which allows the applicability of different treatment protocols for different types of cancers, guarantying the favorable balance between the preservation of the healthy cells over the tumor cells. In summary, these preliminary results show the behavior of cells derived from normal tissue, more specifically the primitive MSC, in response to IR. Clinically, it is highly relevant that normal cells are resistant to the harmful effects of IR, which allows the applicability of different treatment protocols for different types of cancers, guarantying the favorable balance between the preservation of the healthy cells over the tumor cells.

Submission ID: 72853
Submission Title: Endothelial-like cells derived from bone marrow Nes+ MSCs cells have a sinusoidal perivascular phenotype

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Topic
Basic Research, Medicine, and Health

Problem
Mesenchymal stem cells (MSCs) and endothelial cells are essential for the perivascular bone marrow (BM) niche function. Nestin-expressing (Nes+) MSCs perivascular cells regulate hematopoietic stem cells (HSCs) self-renewal and proliferation. Recently, two distinct perivascular Nes+ MSCs with opposite functions were described in the literature: whereas arteriolar NG2-expressing (NG2+) Nes+ MSCs induce HSCs quiescence, sinusoidal Leptin receptor-expressing (LEPR+) Nes+ MSCs promote HSCs proliferation. It is well known that MSCs can differentiate into endothelial-like cells (EL-MSCs). Despite the fact that EL-MSCs modulate hematopoiesis, it is uncertain to which perivascular niche these cells belong and, consequently, their effect on HSCs support remains unclear.

Background
HSCs mainly reside in the BM where signals generated by their niche regulate their self-renewal, proliferation and trafficking. Important components of the perivascular niche include endothelial cells, mesenchymal progenitors and Nes+ MSCs - including LepR+ cells and NG2+ cells. These cells produce several factors that have been implicated in HSCs maintenance and proliferation, including stem cell factor (SCF). Thus, molecular and cellular signals from these cells maintain the hematopoietic system homeostasis.

Hypothesis
Since EL-MSCs are commonly used to study the niche modulation on hematopoiesis in vitro, we investigated if EL-MSCs derived from Nes+ MSCs would have a sinusoidal or an arteriolar phenotype. In this way, we could estimate the role of these cells on hematopoiesis.

Research
BM MSCs were isolated from C57BL/6 mice and differentiated in vitro into EL-MSCs by a medium supplemented with 2% SBF, VEGF, FGF, IGF and EGF per 14 days. EL-MSCs were morphologically caracterized and their endothelial markers (FLT1-VEGFR1, KDR-VEGFR2 and NT5E-CD73) mRNA expression was determined by qPCR. Besides that, NES-Nes, LEPR-LepR and CSPG4-NG2 mRNA expression was quantified on both MSCs and EL-MSCs. Additionally, KITL-SCF mRNA expression was evaluated to access the stimulation of HSCs proliferation capacity from Nes+ MSCs and EL-MSCs.

Observations
We observed that all cells studied expressed NES. After the endothelial differentiation, Nes+ MSCs

acquired endothelial phenotype and morphology. Although the expression of CSPG4 mRNA was similar between Nes+ MSCs and EL-MSCs, LepR mRNA expression in EL-MSCs was higher when compared to MSCs. Furthermore, KITL mRNA expression was quite increased on EL-MSCs. Therefore, we assume that EL-MSCs present a sinusoidal perivascular phenotype and likely induce HSCs proliferation and self-renewal.

Submission ID: 72862
Submission Title: Evaluation of Human IPS Stem Cell-derived Neurons for Neuropharmacology Studies

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Topic
Basic Research, Medicine, and Health

Problem
Studies in neuropharmacology have relied almost exclusively on animal neurons. Developments in stem cell science have provided new opportunities to generate human neurons from stem cells and to use them in drug discovery and drug safety evaluation.

Background
Here, we have explored the neurophysiological and neuropharmacological properties of iCell neurons using single cell patch-clamp techniques and immunocytochemistry.

Hypothesis
Our experiments aimed to determine if iCell neurons expressed functional receptors and ion channels with properties consistent with those described for primary central nervous system neurons maintained in cell culture

Research
Whole cell recordings were obtained from iCell neurons cultured for up to 4 months in vitro. iCell neurons were also fixed and tested for the expression of the neuronal antigen, beta-III tubulin and the presence of nuclear DNA using the chemical label, DAPI.

Observations

Our experiments showed functional expression of voltage-gated sodium, potassium and calcium ion channels and GABA-A, glutamate, NMDA and glycine receptors in iCell neurons; we also recorded spontaneous and evoked action potentials. iCell neurons therefore appear to have important electrophysiological properties consistent with human neurons. These cells may be valuable tools in drug discovery, and neuropharmacology studies.

Submission ID: 72965
Submission Title: High- and multi-functional in vitro liver model derived from mouse ES/iPS cells on micro-fluidic device

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Topic
Basic Research, Medicine, and Health

Problem
Animal use alternatives, i.e., in vitro assay systems, are urgently required for drug, cosmetic, and functional dietary developments. The liver is a vital and multiple functional organ involved in metabolism, detoxification and protein synthesis. However, it is impossible to maintain multiple hepatic functions during a long culture of primary hepatocytes and also to keep structural hepatic polarity. Primary hepatocyte has neither structural polarity nor endothelial cells in the culture, despite polygonal and multipolar hepatocytes in the liver are surrounded by sinusoids, bile canaliculus, and adjacent hepatocytes.

Background
There are many reports to induce ES/iPS cells to single lineage of hepatocyte but not to liver tissue. We established a unique system of in vitro liver model derived from murine ES/iPS cells, i.e., IVL of mES/iPS. The IVL of mES/iPS, consisting of not only hepatocytes, but also endothelial networks, together with cardiac mesoderm differentiation, was induced after the embryoid body formation. IVL of mES/iPS has expression of liver-specific genes, potential of ammonia degradation, and activities of cytochrome P450s.

Hypothesis
Because the hepatic tissue structure with cellular polarities should be strongly related to the liver-specific multiple functions, to construct this architecture is important in the in vitro system. To equip the IVL of mES with medium-circulating system using micro-fluidic device mimicking in vivo liver would be expected to be close to the in vivo metabolism system.

Research
To confirm cellular polarities of the IVL of mES/iPS, first, dichlorofluorescein diacetate (CDFDA), which is incorporated into hepatocytes via organic anion-transporting polypeptide 2 (OATP2) in the basal side, hydrolyzed to green fluorescent CDF by cytoplasmic esterase, and then excreted to bile canaliculus via multidrug resistance-associated protein 2 (MRP2) in the apical side, was added into the IVL of mES/iPS. Second, we tried to activate urea cycle by the addition of L-ornithine in the IVL of mES/iPS or liver perfusion system. Third, we made an in vitro flow system to culture the IVL of mES/iPS on a micro-fluidic device (IVL of mES/iPS chip).

Observations
CDF was observed to be accumulated at the boundary of the cells in the IVL of mES/iPS, but not in primary hepatocyte culture, suggesting that MRP2 and OATP2 were confirmed to be functional in the IVL of mES/iPS, but not in the primary culture of hepatocytes. Urea production increased and ammonia concentration decreased in a dose-dependent manner with respect to the amount of L-ornithine both in the IVL of mES/iPS and the liver perfusion system, but not in primary hepatocyte culture, indicating that architectural and functional properties in the IVL of mES/iPS were quite similar to those in the liver perfusion system, but different from those in the culture of primary hepatocytes. Hepatic activities were much higher in the culture of primary hepatocytes with flow than that without flow, furthermore the activity of the IVL of mES/iPS chip with flow was the highest in others. The IVL of mES/iPS chip has great promise to be useful for drug metabolism and pharmacokinetics in liver as an alternative to animal experiments.

Submission ID: 73138
Submission Title: Human Precursor Cells of Hematopoiesis with Induced Apoptosis are Able to Kill 50% of Rat C6 Glioma Cells

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Topic
Basic Research, Medicine, and Health

Problem

Healthy individual was found to have 0.5mln (10-19M) tumor cells (TC) in a body and they are gradually eliminated by autologous anticancer regulatory systems (AARS) of immunity. Neural stem cells (SC) and precursor cells (PC) play the role of AARS.

Background

Glial brain tumors (GBT) neural SC and PC are the main source of TC and gliomaspheres, i.e. cancerous SC (CSC). Their amount can be as high as 60-96% of tumor volume . TC and CSC of GBT are little sensitive to chemo- and radiotherapy, and resistant to proapoptotic factors and apoptosis inducers (AI).

Hypothesis

To initiate apoptosis we involved a well-known mechanism of cell induction that triggers instructive apoptosis in healthy hematopoietic SC (HSC) and PC of hematopoiesis by known AI. Regulation object is not TC and CSC, but apoptosis induced multipotent SC (iMSC), able to trigger instructive apoptosis in target TC.

Research

Culture of C6 glioma cells was used for in vitro experiment. Hematopoietic (CD34+) SC (HSC) were mobilized through standard 4 days stimulation of hematopoiesis by G-CSF followed by leukapheresis, separation and culturing of CD34+, CD45- HSC. iMSC were obtained from human HSC by in vitro processing with AI phytoprotein toxin ricin. Exposition of AI processing made 10 min, then cell preparation was washed by two-times centrifuging with 0.9% NaCl solution. TC were cultured in ten samples to make a control group #1, OC and HSC were cultured in other 10 samples for control group#2. Ten samples coculturing TC and iMSC made research group.

Observations

Coculturing of iMSC and C6 glioma cells for 7 days in vitro statistically valid (p

Submission ID: 73163
Submission Title: Epithelial Cancer Stem-like Cells-expressed IgG is Involved in Cancer Metastasis

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Topic
Basic Research, Medicine, and Health

Problem
In this study, we explore the mechanisms how epithelial cancer derived IgG involved in cancer metastasis.

Background
It was a fact that Ig was overexpressed in many non B cell-derived cancer cells. Moreover, the IgG was found to be directly involved in carcinogenesis. However, the mechanism still remains unclear.

Hypothesis

Epithelial cancer stem-like cells-expressed IgG recognized by RP215 may serve as a novel marker of human stem/progenitor cells and cancer stem cells, as well as a potential target for cancer therapy.

Research
In this study, using the mAb, RP215, which specifically recognize an epitope in Fab segment of IgG, we explored the expression of IgG in cancer tissues and cells. Moreover, we compared the IgG-low and IgG-high cancer cells in the aspects of self-renewal, drug resistance, migrating and metastasis abilities.

Observations
We unexpectedly found that IgG was widely expressed in epithelial cancer cells, especially overexpressed in cancer stem-like cells, such as in CD44+/CD24- breast cancer cells as well as the leading cancer cells at front of invasive epithelial tumors in different epithelial tumors. Interestingly, the IgG was also found in normal p63+ stem/progenitor cells of stratified epithelia, but not in mesenchymal or the lymphatic lineage normal or cancer cells. Importantly, IgG-high cancer cells displayed high ability of self-renewal, drug resistance, migrating and metastasis.

Submission ID: 73696
Submission Title: Modeling of Stress Induced Premature Senescence (SIPS) using Mesenchymal stem cells

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Topic
Basic Research, Medicine, and Health

Problem
None

Background
Normal cellular growth can be divided into five distinct phases including G1, S, G2, M, and G0. Stress induced premature senescence (SIPS) occurs after exposure to kinds of sublethal stresses such as reactive oxygen species (ROS). Cells undergoing SIPS exhibit properties similar to cells in replicative senescence (RS). And their ability to the homeostatic maintenance of organs and tissues

cannot be discounted. Mesenchymal stem cells (hMSCs) are multipotent progenitors which self-renew and differentiate into multiple lineages.

Hypothesis
H2O2, has been the most commonly used inducer of SIPS, which shares features of replicative senescence (RS) including a similar morphology, senescence-associated β-galactosidase activity, cell cycle regulation, etc. Therefore, in this study, the senescence of hMSC during SIPS was confirmed using a range of different analytical methods.

Research
Several methods were utilized to induce and confirm SIPS. Among these Five differentially expressed spots were detected in the 2-DE map, which were identified as ANXA2, MLC2, ECH1, PSMA1 and mutant β-actin using ESI-Q-TOF MS/MS. Also, proton (^{1}H) NMR was used to elucidate the difference between metabolites in the control and h-MSCs treated with H2O2. Furthermore, ANXA2 was later probed for its correlation with cellular senescence and apoptosis through the pathway P21. H2O2-induced overexpressed ANXA2 directly interacted with CAV1 and down-regulation of ANXA2 induced caspase-mediated apoptosis through regulation of p21 which is a major inhibitor of p53-dependent apoptosis.

Observations
Comparative analyses between control and premature senescent hMSCs were done. ANXA2, MLC2, ECH1, PSMA1 and mutant β-actin were identified using proteomics. Choline, leucine, glycine and proline were identified using metabolomics. In conclusion, some factors like ANXA2 should be further investigated to better study the signaling pathways that mediate h-MSC senescence.

Submission ID: 73763
Submission Title: Age of Ischemic Cardiomyopathy Patients Does Not Compromise MSC Therapeutic Efficacy

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Topic
Basic Research, Medicine, and Health

Problem
The role of patient age on the efficacy of mesenchymal stem cell (MSC) therapy in ischemic cardiomyopathy (ICM) is controversial.

Background
The role of patient age on the efficacy of mesenchymal stem cell (MSC) therapy in ischemic cardiomyopathy (ICM) is controversial, with some data suggesting a decline in responsiveness with age.

Hypothesis
We hypothesized that the therapeutic effect of culture expanded MSCs persists even in older subjects

Research
METHODS: Patients with ICM who received MSCs via transendocardial stem cell injection (TESI) as part of the TAC-HFT (n = 19) and POSEIDON (N = 30) clinical trials were divided into 2 age groups:

Observations
RESULTS:Mean 6MWD was similar at baseline and increased at 1 year post-TESI in both groups: 48.5 ± 14.6 m (p = 0.001) for the younger and 35.9 ± 18.3 m (p = 0.038) for the older participants (p = NS between groups). The older group exhibited a significant reduction in MLHFQ score (−7.04 ± 3.54; p = 0.022), while the

Submission ID: 74009
Submission Title: Exposure of Bone Marrow Derived Mesenchymal Stem Cells to Extremely Low Frequency Electromagnetic Fields (EL-FEM) promotes expression of Neural Differentiation Markers

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Topic

Basic Research, Medicine, and Health

Problem
There exist a range of both technical and ethical challenges in the usage mesenchymal stem cells. The utilization of Extremely Low Frequency Electromagnetic Fields (EL-FEM) to induce differentiation in mesenchymal stem cells decreases some of the technical hurdles and some negative effects of other methods i.e, gene manipulation etc.

Background
The study an ancillary way of inducing and influencing differentiation to BM-MSCs. Hence, this finding adds to the shortlists of methods that's being utilized to induce differentiation in mesenchymal stem cells.

Hypothesis
EL-FEM, has been already known to influence several biological functions.Hence, it could induce differentiation in mesenchymal stem cells.

Research
The cells were subjected to an ELF-EMF Helmholtz configuration oriented to produce a vertical magnetic field with parameters as following axial symmetry (2D), the distance between two coils (17.5 cm), the intensity of current (I ¼ 200 mA), and number of loops (N ¼ 1000).Proliferative capacity wasassessed through a BrdU incorporation assay after ELF-EMF exposure.

Observations
The morphology and proliferation of BM-MSCs during ELF-EMF exposure. Several protein analyses showed differential expressions of proteins: thioredoxin-dependent peroxide reductase, ferritin light chain, and tubulin beta-6 chain, wherein exposed samples showed a marked increase in expression.

Submission ID: 74030
Submission Title: Evidences on the Existence of Cardiomesenchymal Stem Cells

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Topic
Basic Research, Medicine, and Health

Problem
Ischemic heart disease is a leading cause of death worldwide, cellular therapy has emerged as a promising option for the treatment of myocardial infarction (MI) and subsequent systolic heart failure. Cardiac cell therapy comprises two essential objectives: cardiomyocyte generation and angiogenesis. Multiple types of stem cells have been used to treat systolic heart failure after MI, including fetal cardiomyocytes, skeletal myoblasts, endothelial cells, smooth muscle cells, fibroblasts, embryonic stem cells, induced pluripotent stem cells, adipose tissue derived stem cells and bone marrow progenitor cells. These explored lineages have disadvantages in relation to their differentiation potential, because they either fail to generate the required structures or achieve functional differentiation in only a small proportion. Currently there is no consensus regarding the best stem cell source for cardiac cell therapy. The ideal stem cell lineage should be easily accessible through minimally invasive procedures and have a differentiation capacity committed toward cardiac tissue. With respect to differentiation potential, cardiac stem cells (CSCs) are possibly the best option for cardiac regeneration; because their residence in the adult heart confers them a precise differentiation commitment toward cardiac lineages, nonetheless CSC therapeutic value is hindered by their scarcity and the invasiveness of their isolation procedure. Contrastingly, mesenchymal stem cells (MSCs) stand out as an encouraging option for cell therapy due to their multipotential differentiation capacity, accessible isolation, uncomplicated cellular culture, great expansion potential, and angiogenic properties, however their differentiation potential is indefinite obtaining only a small fraction of cells that adequately differentiate into cardiomyocytes or vascular endothelial cells. To achieve better therapeutic results and enhance clinical feasibility, it is essential to find easily attainable cell populations with a precise commitment to differentiate into cardiac tissues.

Background
CSCs are mainly identified by their constitutive expression of c-kit, a membrane receptor for stem cell factor, upon stimulation it increases stem cell proliferation and survival. Another prominent marker in the characterization of CSCs is CD34, a membrane protein primarily involved in adhesion to extracellular matrix. Based on the expression of KDR, a vascular endothelial growth factor receptor, CSCs can be further classified into miogenic CSCs (mCSCs) (KDR negative) and vasculogenic CSCs (vCSC) (KDR positive). MSCs are a heterogeneous population of multipotent stem cells able to differentiate into various lineages, including vascular endothelium and cardiomyocytes. Every MSC fulfills at least three criterions; first, MSCs must be plastic-adherent when maintained in standard culture conditions. Second, MSCs must express CD105, CD73, and CD90, while lacking expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-II surface molecules. Third, MSCs must differentiate into osteoblasts, adipocytes and chondroblasts in vitro. It is interesting to notice that various studies have found diverging results of when comparing

the utility of MSCs isolated through different protocols, a plausible explanation for his phenomenon is that diverse extraction protocols may favor particular subgroups within a heterogeneous population. Commitment to a particular cellular lineage restricts global differentiation capacity. In spite of appropriate induction conditions, only a small percentage of an unsorted MSC population will complete differentiation into the desired tissue, oppositely, CSC differentiate with high efficiency into cardiac tissue but have lost their ability to form other mesenchyme-derived tissues.

Hypothesis

We propose the existence of a MSC subpopulation with an intrinsic commitment to differentiate into cardiac tissues, combining the specific differentiation capacity of CSCs, with the high isolation efficiency of MSCs. Such cell line would evidently have a limited differentiation capacity toward traditional mesenchymal tissues, with an enhanced ability to differentiate into cardiac tissues. This theoretical sub-population would express a hybrid immunophenotype, characterized by the presence of both CSC and MSC surface markers. In order to emphasize the combined properties of CSCs and MSCs, a suitable name to describe this population is cardiomesenchymal stem cells (CMSCs). We are also aiming to explore the existence of a direct correlation between the expression of a given set of surface molecules and differentiation capacity, expecting that the expression of prototypical CSC markers confers an enhanced commitment toward cardiac lineage. If we consider the immunophenotype of CSCs an ideal profile of cardiac progenitors, the early expression of cardiac structural proteins could additionally be an essential factor to predict MSC fate.

Research

In order to maximize the proportion of isolated stem cell populations we implemented a modification to the classical mesenchymal stem cell extraction protocol. Whole bone-marrow cells (BM) were obtained from Wistar rat femur and desegregated with 750 µl of trypsin at 250 mg/ml in a total volume of 10 ml of DMEM high glucose at 4ºC during 16 hrs. A collagenase treatment (2 µg/ml) was carried out for 1 hr at 37 °C while shaking at 120 rpm. After enzyme inactivation cells were filtered through 70 µm and 40 µm filters and centrifuged at 1,800 rpm for 7 minutes, to be later incubated with 1 ml red blood cell lysis buffer. The cells were resuspended in complete DMEM with 10 ng/ml basic fibroblast growth factor (bFGF) and plated on two 10 cm2 cell culture plate dishes, in which they were incubated at 37 °C and 5% CO2. Twenty-four hours later non-adherent supernatant cells were recovered and seeded in a new 10 cm2 tissue dish. Seventy-two hours later adherent cells, which we called supernatant bone-marrow mesenchymal like stem cells (S-BM-MSC-like) were harvested and seeded in a 96-well plate to perform dilution cloning. We obtained 12 different clones, flow citometry was used to characterize each clone searching particularly for the surface markers CD34 and CD117. The subpopulation expressing highest levels of these markers was isolated and expanded in culture; all the following experiments were performed on this cell population, which will be referred to as CMSC-like. These cells were evaluated for the presence of the following MSC markers: CD29, CD44, CD90, CD105 and CD106. In order to determine whether CMSC-like conserved the typical differentiation potential of MSC, we evaluated their differentiation capacity for chondrocytes, adipocytes and osteocytes using a commercial kit. CMSC-like were evaluated through rt-PCR for the expression of the following cardiac genes: GATA4, MEF2, Nkx2.5, a-MHC, conexin-

43 and troponin-T, using mature cardiomyocytes as positive control and GAPDH as housekeeping control. The expression of mRNA was later verified by q-PCR for the previously stated genes. The presence of proteins coded by these genes was evaluated through western blot and immunofluorescence. In order to assess the in vitro cardiac differentiation potential of CMSC-like we performed a series of co-cultures with different population rates of isolated mature cardiomyocytes and with conditioned medium of cardiomyocyte cultures, CMSC-like were previously infected with green-fluorescent-protein (GFP) adenovirus (ad-GFP) to be distinguishable from cardiomyocytes. The follow-up time was 21 days, cells were monitored with an inverted microscope and recorded in video. We verified the expression of prototypal cardiac proteins through immunofluorescence for GATA4, MEF2, Nkx2.5, a-MHC, conexin-43 and troponin-T. To evaluate CMSC-like therapeutic potential, we used a murine model of MI. Sixty days after a MI by left descending artery ligation, we injected 5x106 CMSC-like transduced with ad-GFP directly into myocardial tissue. Cardiac function was tracked with left-ventricle ejection fraction (LVEF), measured though transthoracic echocardiography, and myocardial perfusion measured through single-photon emission computed tomography (SPE-CT) at the following time points: before MI, 60 days post-MI and 60 days post CMSC-like treatment. To demonstrate CMSC-like engraftment we sacrificed the treated rats to perform a myocardium histological analysis through the co-location of GFP and cardiac markers with an epifluorescence microscope.

Observations

We identified CD34+ and CD117+ positive clones of S-BM-MSC-like, we selected and isolated the clone with the highest expression of these markers (>80% CD34+/CD117+). The chosen clone also expressed typical MSC markers: CD29+, CD44+, CD90+, CD105+ and CD106+. Considering that this clone possesses a combined expression of CSC and MSC markers we denominated them CMSC-like. Even though CMSC-like express typical mesenchymal markers they have a limited differentiation capacity compared to classical MSC, CMSC-like are able to differentiate into osteocytes, but not into adipocytes or chondrocytes. This may suggest that the additional expression of CD34 and CD117 restricts overall differentiation capacity, directing differentiation toward cardiac lineage. In an initial screening for mRNA of specific cardiac genes, rt-PCR revealed that CMSC-like express the following genes: GATA4, MEF2, Nkx2.5, a-MHC, conexin-43 and troponin-T. Subsequently, we determined the basal expression level of the previously mentioned genes compared to cardiomyocytes using q-PCR: the expression levels of GATA4, MEF2, Nkx2.5, a-MHC, and troponin-T were below the control expression; conexin-43 reached a 60% higher expression than control. CMSC-like are capable of traducing mRNA to cardiomyocyte specific proteins, through western-blot we detected the unequivocal presence of GATA4, MEF2, Nkx2.5, a-MHC, conexin-43 and troponin-T in CMSC-like. Interestingly, when this data was verified through immunofluorescence protein detection we obtained discordant results: only a meager proportion of cells was positive for GATA4, a-MHC and troponin-T, compared to the quantity inferred by western-blot. CMSC-like cells phenotypically differentiate into cardiomyocytes when co-cultured with mature cardiomyocytes: CMSC-like presented spontaneous beating capacity at the seventh day of follow-up. Intriguingly, only cells that constitutively express Troponin-T before co-culture acquired spontaneous beating capacity when in contact with cardiomyocytes. It is important to notice that only a small proportion of cells acquired spontaneous beating capacity and conserved Troponin T expression during the whole

tracking period. CMSC-like improve cardiac function in vivo, treated rats exhibited an improvement in LVEF and myocardial perfusion. Sixty days after MI LVEF dropped form a mean of 63% to 29%, sixty days after cellular therapy with CMSC-like LVEF increased to a mean value of 56%. Myocardial perfusion doubtlessly decreased 60 days after MI, and increased significantly 60 days after cell therapy. We detected CMSC-like presence in the hearts of treated rats, epifluorescence analysis demonstrated the existence of CMSC-like expressing GFP and specific cardiac proteins around and inside the infarcted area, this suggests that CMSC-like engraftment is followed by differentiation into myocardial tissue, however, it is impossible to assert that differentiation is the sole contributor to the improvement of cardiac function, because our experiment did not assess the effect of paracrine factors in myocardial regeneration. In this work we provided evidence on the existence of a bone-marrow resident cellular subpopulation that combines the expression of CSC and MSC markers, possesses a limited differentiation capacity toward traditional mesenchymal tissues, expresses genes specific to cardiac tissue and constitutively produces prototypical cardiac proteins. The cells assessed in our experiments are able to phenotypically transform into cardiomyocytes when in contact with cardiomyocytes and a fraction exhibits spontaneous beating. This cell line is capable of significantly improving cardiac function after a MI, probably through direct myocardial engraftment and differentiation into cardiac tissue. The available data allows to infer a relation between CSC-related surface marker expression, cardiomyocyte-specific gene transcription, prototypal cardiac protein presence and a differentiation potential restricted to cardiac tissue, however it does not completely suffice to establish a direct correlation with an enhanced cardiac differentiation capacity. Our experiments provide an initial approach in the characterization of bone-marrow resident stem cells with an apparent commitment to cardiac differentiation. It is necessary to consider that even though CD117 and CD34 are prominent markers in the characterization of CSC, they are not exclusive to cardiac cell lines. Gene expression is not a flawless resource to predict cardiac differentiation in stem cells, but transcription of cardiac genes is tentatively an indicator of cardiac differentiation commitment; we are convinced that the evaluation of a broader gene spectrum, including cardiac-related and non-cardiac genes, is fundamental to create a genetic profile that accurately forecasts cardiac differentiation. Additional future challenges include the evaluation of MSCs obtained from alternative sources and multipotent stem cells extracted from other lineages, as well as the assessment of the effect of cardiac protein induction on differentiation potential. Finally, we believe our efforts render insights on the existence of cellular candidates to be possibly defined as CMSC, the complete characterization and isolation of such cells is a promising approach to increase therapeutic efficiency and clinical feasibility of cardiac cell therapy.

Submission ID: 74063
Submission Title: Carbon nanotube and silica nano-particle films for proliferation and differentiation of stem cell

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Topic
Basic Research, Medicine, and Health

Problem
Stem cell characteristics are easily changed by micro environment. Even on flat culture plates such as T-flaks, cell proliferation process on artificial structure as flat culture plate (like T-flask), there may be problem that loses their differentiation characteristics. The control of proliferation and differentiation properties of stem cells remains a challenge for therapeutic application.

Background
Cell-material interactions play an important role in controlling stem cell proliferation and differentiation. Particularly, nanostructures are known to be important cues at the cellular level and, consequently, can enhance proliferation and differentiation of stem cells.

Hypothesis
In this work, we hypothesize that thin films of single walled carbon nanotubes (SWNTs) and silica nanoparticles (SiNPs) provide suitable topographical features for controlling proliferation and differentiation of stem cells in the culture environment.

Research
The SWNT and SiNP thin films are fabricated using pre-designed surface functional groups and simple and rapid patterning methods. Systematic variation of their structures demonstrates changes in proliferation and differentiation properties of stem cells. As a result, we find that manipulation of interaction between stem cells and nanostructures can be an effective tool to provide optimal conditions of proliferation and differentiation. Besides, the films can be in other applications as well, such as in sensor. Taking as an example, SWNT-based sensor is used to measure surface charge of stem cell membranes and eventually to probe gene transfection efficiency of DNA/nanomaterials.

Observations
The line width of the SWNT and SiNP thin films is patterned within 3 μm, and adhesion properties and shape of the stem cells are observed. ECM-like network structure of SWNT enables culture of adipose derived-mesenchymal stem cell (ADSC). On the other hand, electric current through aligned monolayer of SWNT induces neural differentiation of PC-12 cell line by potential change of cell membrane in real time. Also, it is observed that the SiNP monolayer can differentiate bone marrow derived-mesenchymal stem cells (BM-MSCs) to chondrocyte-like cells by influencing the film

structure only.

Submission ID: 74391
Submission Title: Evaluation of Drug-Host interactions on enhanced targeted MSC Homing induced by pulsed focused ultrasound (pFUS): implications for cellular therapy and regenerative medicine

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Topic
Basic Research, Medicine, and Health

Problem
Homing of exogenous stem cells as part of cellular therapy to pathological loci is frequently required for therapeutic efficacy and the homing processes are inefficient and depend on short-lived pathological inflammation. Drug-host interactions with cell therapies remain unexplored and are not controlled for during clinical cell therapy trials and may interfere with homing of cells to targeted tissues.

Background
Recent studies have demonstrated the utility of noninvasive pulse focused ultrasound (pFUS) in enhanced homing permeability and retention (EHPR) of stem cells to targeted tissues. pFUS targeted exposure to skeletal muscle (M) esults in micro-environmental changes associated with increased expression cyclo-oxygenase (COX2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) in tissues along with a cascade of cytokines, chemokines and trophic factors (CCTF) and cell adhesion molecules (CAM) initiating within 10 minutes and lasting up to 48 hours. Therefore, pFUS exposures can be used to induce sites of CCTF and CAM that are important in stem cell homing and the effects of drugs on cellular therapy can be evaluated

Hypothesis
Maximal homing of infused stem cells to diseased tissue is critical for regenerative medicine. Do clinical relevant drugs, such as ibuprofen (non-specific COX inhibitor) or etanercept (TNFα inhibitor), inhibit molecular signaling and stem cell homing to target pathology following pulsed focused ultrasound exposure?

Research

This study sought to characterize the molecular responses from pFUS in skeletal muscle that enable EHPR of MSC and identify key signaling contributors to the evolution of a molecular zip code. Characterization implicated TNFa and COX2 as major mediators of the molecular zip code and subsequent MSC homing in mouse models. 10e6 human MSC were administered IV to immuno-competent mice following pFUS to muscle and animals were euthanized 24 hours later to determine cell homing to target tissue. Ibuprofen and etanercept were administered prior to pFUS treatment to examine whether the molecular zip code and MSC homing could be suppressed in healthy tissue.

Observations
pFUS induces a unique expression of CCTF for either M with an increase in TNF (4.5x) at 10min following exposure without elevation in Heat Shock Protein 70. Both pro and anti-inflammatory CCTF remained elevated for 48 hour pFUS along with cell adhesion molecules. Pretreatment of mice prior to pFUS with Ibuprofen (IB)or Etenercept (ET) resulted in significant reduction in CCTF, COX2 and NFkB protein expression when compared results in pFUS only treated mice. Treatment with IB or ET prior to pFUS and IV MSCs resulted in significant (ANOVA p

Submission ID: 74461
Submission Title: Multiple miRNA-mRNA regulatory networks in colon cancer stem cells

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Topic
Basic Research, Medicine, and Health

Problem
Cancer Stem Cells (CSC) is the subpopulation of tumor cells which is believed to resistant conventional therapies and leads to recurrence. Currently, the molecular changes that occur in colon CSCs compared to non-CSCs are not fully understood.

Background
In Colon cancer, CD133 expression has been reported to be associated with CSC phenotype. Although significant amount of research has been conducted on colon CSC, the miRNA/mRNA regulatory networks in colon CSCs has not been characterized in full.

Hypothesis
Therefore, the goal of this study is to characterize the deregulated microRNA/mRNA networks in colon CSCs. Understand the molecular changes in CSCs might provide novel insight into tumorigenesis and might help us design more efficient targeted therapies for this disease..

Research
Colon cancer cell lines (CD133+: SW620, HCT116, HT115, WiDr, and SW403; CD133-: RKO, HCT8, SW480, and LS174T) were maintained in complete DMEM medium. Total RNA was extracted using Total RNA Purification Kit (Norgen-Biotek Corp., Canada) according to the manufacturer's instructions and RNA quality was checked using Agilent 2200 TapeStation. Extracted RNA was labeled and then hybridized to the Agilent Human SurePrint G3 Human GE 8x60k microarray chip (Agilent Technologies, Santa Carla, CA,USA). Data analyses were conducted using GeneSpring 12.0 software (Agilent Technologies) while Percentile Shift was used for data normalization.

Observations
Our data revealed 271 upregulated and 644 downregulated genes in CD133+ cell lines. Similarly, we found 9 upregulated and 4 down regulated miRNAs. In silico analysis of predicted micoRNA targets revealed 161 potential regulatory networks comprising upregulated miRNAs and downregulated gene in CD133+ cells. Similarly, 39 regulatory networks were found which compromise downregulated miRNAs and upregulated genes in CD133+ cell. The biological relevance of those identified miRNA/mRNA networks in relation to colon CSCs is currently being investigated. Conclusions: Our data revealed multiple miRNA/mRNA regulatory networks in colon cancer stem cells, which could potentially be targeted as therapeutic modality for this disease. Acknowledgment: This work was supported by grants 11-MED1582-02 from the National Plan for Sciences and Technology, King Saud University.

Submission ID: 74474
Submission Title: Data integration tools for regulatory comparison of components on technology platforms based therapies: the case of MSCs platforms based therapies

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Topic
Industry Infrastructure

Problem
The traditional regulatory pathways for clinical licensing of a medicament are challenged by the increased complexity and uncertainty of cell therapies products and procedures. Such uncertainty requires clinical trials with many times unfeasible large quantity of patients, with promoter facing significant hurdles in an era of personalized medicine, where therapies are even more focused in specific groups of patients. But with promising clinical results for still unmeet needs, the challenges on regulatory evaluation are impacting the patients' access to breakthrough therapies. Different mixes of procedures, technologies and techniques are giving rise to several early stage clinical trials addressing similar cell types and/or indications, such in the case of Mesenchymal Stem Cells (MSCs), yet the regulatory analysis end being mainly a case-by-case approach. Promoters and regulators bear with the lack of standards for safety and efficacy. Cell therapy technologies are still in their infancy promising significant improvements on production technologies, and understanding of therapeutic and cellular mechanisms. Thus, it is expected that current proposed therapies suffer significant innovations towards safety and efficacy, challenging how it can be accommodated in previously approved therapies. So, there is a need for addressing the traditional case-by-case approach on dealing with clinical uncertainty of new cell therapies and introduced innovation by increasing the available knowledge of the drivers of safety and efficacy in a final cell therapy.

Background
Cells produced for a cell therapy are characterized by significant variability. As so the regulatory clinical analysis has been extended from the notion of a final and defined product to include the production process. With small molecules, the quality of the final product allows to access different production setups, similar to what is still possible in most of available biological, supporting the arrival of generics and biosimilar drugs based in more efficient production, which have huge impact in economies and health care systems. However, the final quality of a cell can only be tested on a clinical setup, since it is unknown most of the individual impact of techniques on the safety and efficacy of a cell therapy. Thus, comparing similar therapies for the same indication extends cells comparison, to include also production, administration and even monitorization setups. As so, they may have clinical safety and efficacy profiles very distinct, needed to be assessed new clinical trials. Understanding what impacts on the clinical therapy outcomes may provide invaluable knowledge about the underlying complexity of a cell therapy, and guide regulators and promoters towards standards, specific areas of risk or improvement, and support the future development of biosimilars. New adaptive regulatory and reimbursement frameworks propose continuous collection of data on the therapies' life cycle to acknowledge the existing uncertainty and manage the risks and benefits while knowledge of a therapy is gathered during clinical usage. However, the application to cell therapies with a certain degree of similarity, such the case of MSCs, can benefit from avoiding a case-by-case approach, by increasing prediction of clinical outcomes using the knowledge gathered in the evaluation of other cell therapies if one can compare their technology setups. It would allow better accommodations of continued innovations of production setups in cell therapies life cycle.

Hypothesis

This work is based in the hypothesis that a cell therapy can be seen at the light of the definition of technological platform, being the result not only of innovations arising from cell biology developments, but also from production, administrations or even monitorization technologies. So, it is proposed a regulatory analysis based on a technological platform, comprising several proposed therapies with high level of similarity, but distinct innovations on one or more steps, thus requiring individual assessment of clinical safety and efficacy. This work considers that can be possible to use the atomized information of each therapy setup of procedures and techniques, including the used technologies and workflow, to compare different proposed therapies on a given technological platform. Such comparison of several therapies may allow to model them and extract meaningful information about individual steps comprised in a given cell therapy, identifying sources of risks or needed improvement, when considering the clinical data gather for each one. By considering a continuous gathering of clinical data, and increase in the knowledge of the impact of techniques in the overall safety and efficacy profile of a therapy, it is expected the possibility of better predicting the impact of introducing new technologies allowing the reduction of needed clinical assessment before patients' access. This would provide a model-based tool for a more efficient usage of adaptive licensing approaches on therapies that arise from the dynamism of technological platforms.

Research

This poster presents an ongoing research. As a start base, Cell therapies can be composed by several technology platforms, characterized by a specific cell type. Multiple clinical outcomes are common to several indications, such as those related with safety and distribution on the human body. These commonalities may provide additional source of information to the setups around a certain cell type. To support this first definition of a cell therapy technological platform, the example of MSCs was chosen, due being a cell type widely used on the ongoing clinical trials of cell therapies. MSCs based therapies will be then used on this research to better define what is a cell therapy technology platform, namely its extension and relevance at the clinical and regulatory level. The next step consist on the analysis of several case studies to identify the core elements of a MSCs therapy setup using a system analysis approach of steps since the collection of biological material until final intervention of therapy. This analysis allows the development and proposal a model of the setup for a cell therapy, which ultimately will enable the comparability tool between changes introduced by the usage of different sources, technologies or techniques. Each of this will have a predictable or unknown impact at parameters relevant to the clinical outcomes of the cell therapy, namely its safety and efficacy. The use of available literature will provide meaningful variables to account at the development of a biological model. Some of the variables are already identified include, for instance, the probability of mutation or contamination associated to each technique. The literature of each technology is critical to better understand the impact of different characteristic factors, such as cell stress, doubling number, etc. on the final cell characteristics. As a model, it is expected that the relevance of several relations to be unknown. Different levels of detail for model will be considered, in order to represent alternative setups meaningfully while providing a basis for feasible comparison between cell therapies in a proposed technological platform. Although,

it is expected that the level of detail and atomization of each step influences the feasibility of usage of existing clinical data about different setups, as an increase level of detail may require a larger set of different cell therapies to acquire the intend knowledge of the model relations and impacts. A quantification of the information included in each model will be balanced with a feasible number of cell therapies. The next step would be to group existing registered clinical trials on MSCs under a proposed definition of a technology platform, which degree of similarity must be defined based on the clinical coherency and the capability to provide meaningful data in a feasible way. For this purpose, an indicator of the similarity of different therapies represented in the model will be proposed given the different technologies, techniques and parameters used, supporting the grouping of identifies MSCs therapies. This framework will be then used to evaluate the ability of extracting meaningful causality information on impact of each step on the clinical outcome, and assessment of the contribution of certain unknown variable affected by a technology. It is expected that with the increase of clinical data and cell therapies the knowledge about certain variables and the impacts of each technology increase. The ultimate step will be the prediction of a clinical outcome given the model representation of a cell therapy. As so the capability of predicting such outcome will be measured against a dataset of clinical evaluated therapies to assess if the propose model is capable to provide a robust tools to regulatory analysis.

Observations
n/a

Submission ID: 74578
Submission Title: miRNA profiling as a tool for developmental neurotoxicity pathway analysis in human in vitro model

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Topic

Basic Research, Medicine, and Health

Problem

miRNAs are abundant in the central nervous system (CNS) and their expression is tightly regulated during brain development . They are implicated in many developmental processes such as neurogenesis, neuronal differentiation, neurite outgrowth and synaptic plasticity. Synaptic formation and plasticity play central roles in neuronal connectivity that is associated with learning and memory. Dicer ablation that results in the absence of all mature miRNAs has been used as a valuable tool to study the general role of miRNA regulatory pathways in the CNS. In this study we analysed whether miRNAs expression profiles in a human pluripotent derived neuronal cell model can be useful tool for a more mechanistic approach for developmental neurotoxicity evaluation.

Background

MicroRNAs (miRNAs) are endogenous, small and noncoding RNAs that introduced an additional layer of regulatory control of gene expression. They regulate most of cell processes from development, differentiation to cell death. It has been suggested that each miRNA could target up to a few hundred target genes resulting in the regulation as much as one third of the protein expression coding in animals. One third of miRNAs are highly conserved across different specious and in the case of mouse and human mature miRNAs 60% of conservation has been identified. Due to the ability of miRNA to recognize imperfectly matched sequences, each single miRNA can bind to several mRNA targets, thus amplifying the area of action from one to many regulated genes. Differentiating human pluripotent cells towards neuronal phenotypes were repeatedly treated for five weeks with non-cytotoxic concentrations of methyl mercury chloride, a well known neurodevelopmental toxicant and the expression of miRNAs was determined and compared with the control cell culture.

Hypothesis

It is expected that miRNA profiling could provide simplified functional evaluation of the cellular pathways involved in the developmental neurotoxicity mechanisms in comparison with the transcriptomics analysis were thousands of mRNAs expression is analysed.

Research

The main aim of this study was to evaluate whether miRNA profiling could be used as a tool for developmental neurotoxicity testing (DNT) by identifying the miRNAs profile expression that could be used as biomarker for DNT. Therefore, to identify the changes in miRNAs expressions during the process of neuronal differentiation we have compared the profile of miRNAs expression in the control culture of undifferentiated human NT2 cells versus the cells differentiated into neuronal phenotype after 5 weeks of the exposure to retinoic acid (RA). At the second stage of the studies the NT2 cells were exposed to MetHgCl, well known DNT compound, during the 5 weeks of neuronal differentiation in the presence of RA. Differentiating cells were repeatedly treated with non-cytotoxic concentrations of MetHgCl and the induced toxicity mechanisms were evaluated based on the miRNAs expression in comparison with the control culture (exposed only to RA). NT2 cells were

exposed to MetHgCl at the initial stage of cell differentiation during the first 36 DIV when the neuronal differentiation was triggered by the simultaneous presence of RA. MetHgCl is one of the best known DNT compound that affects neuronal differentiation, synapse formation and causes the damage to the process of learning and memory. The available databases of miRNAs/mRNA gene ontology and bioinformatics tools were used to link miRNAs expression to their mRNA targets to be able to interpret the obtained changes in miRNAs expression in terms of their effect on cell function modification.

Observations

The results obtained from the miRNA expression analysis have identified the presence of a miRNA signature which is specific for neural differentiation in the control culture and another for the response to MeHgCl-induced toxicity. In differentiated neuronal control cultures we observed the down-regulation of the stemness phenotype-linked miR-302 cluster and the over-expression of several miRNAs specific for neuronal differentiation (e.g. let-7, miR-125b and miR-132). In the cultures exposed to MeHgCl (400 nM) we observed an over-expression of a signature composed of 5 miRNAs (miR-302b, miR-367, miR-372, miR-196b and miR-141) that are known to be involved in the regulation of developmental processes and cellular stress response mechanisms. Using gene ontology term and pathway enrichment analysis of the validated targets of the miRNAs deregulated by the toxic treatment, the possible effect of MeHgCl exposure on signaling pathways involved in axon guidance and learning and memory processes was revealed. The obtained data suggest that miRNA profiling could provide simplified functional evaluation of the toxicity pathways involved in developmental neurotoxicity in comparison with the transcriptomics studies.

Submission ID: 74667
Submission Title: 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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Topic

Basic Research, Medicine, and Health

Problem

Current methodologies employed to generate induced pluripotent stem cells are problematic. They require complicated and unreliable protocols resulting in poor reprogramming efficiency, reproducibility, clonal heterogeneity, and poor differentiation potential.

Background

Induced pluripotent stem cells (IPSCs) are invaluable tools for translational research. Despite recent developments in multiple reprogramming methods, most strategies still have significant limitations. These obstacles include complicated and unreliable protocols, undesirable genomic integration of reprogramming vectors, imbalanced expression of reprogramming factors, and heterogeneity in resultant IPSC quality. These blocks lead to poor IPSC reprogramming efficiency, maintenance of pluripotency, and differential potential.

Hypothesis

Progenitor Life Science's Matisse™ Reprogramming Technology overcomes impediments of traditional iPSC methods through an all-in-one episomal reprogramming vector, pPuro(CMVmt1), that delivers OKSM reprogramming factors (OCT3/4, KLF4, SOX2, and c-MYC, and) each under independent control of separate attenuated CMV promoters. The Matisse™ System provides coordinated expression of all four factors upon transfection into human somatic cells, bypassing the multi-step screening procedures required to ensure homogeneous culture-wide gene transfer associated with other systems. The Matisse™ Reprogramming System improves efficiency and reduces investigator work-load during the reprogramming process.

Research

The Matisse™ episomal reprogramming vector was transfected into BJ human skin fibroblasts (BJ cells) and EBV transformed lymphoblastoid cells (LB cells). Ectopic expression of the OKSM reprogramming factors from the Matisse™ episomal vector was then assessed by RT-PCR. Cultures were monitored for the appearance of IPSC colonies which were subsequently isolated one month post-transfection. Live immunostaining of surface markers was regularly performed to verify continued pluripotent status. Cells derived from independent colonies were then assessed for their potential to form embryoid bodies and to terminally differentiate into specific cell types.

Observations

Electroporation or liposomal transfection of BJ human skin fibroblasts resulted in culture-wide balanced expression of the OKSM factors. We observed the formation IPSC colonies within 6-9 days and 15-20 days in LB and BJ cultures respectively without the necessity feeder cells. BJ-derived iPSCs exhibited ES cell morphology and expressed characteristic pluripotency genes. These cells

demonstrated efficient embryoid body formation, differentiation potential into endodermal, ectodermal, and mesodermal germ layers as well as the ability to continue differentiation into terminal cell types.

Submission ID: 74722
Submission Title: Enhanced Genetic Integrity in Mouse Embryonic Stem Cells

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Topic
Basic Research, Medicine, and Health

Problem
Due to the ability of pluripotent inner cell mass (ICM) cells to give rise to all cell types in the organism, robust mechanisms have evolved to prevent the expansion of mutations within the genomes of these cells. Embryonic stem cells (ESCs) provide an in vitro model of such pluripotent cells that can be maintained indefinitely in culture.

Background
ESCs can potentially be used in cell-based therapeutic approaches to treat disease or severe injuries. Excessive mutations in ICM cells can potentially compromise proper development and function in the organism, while excessive mutations in ESCs can lead to unsafe transplantations of ESC-derived differentiated cells for therapeutic uses.

Hypothesis
We hypothesized that pluripotent cells, including ESCs, should display enhanced genetic integrity relative to differentiated derivatives of these cells.

Research
We analyzed the frequency and spectrum of spontaneous point mutations in three ESC lines over time and compared these to ESC-derived differentiated cells produced from these same lines. We hypothesized that pluripotent cells, including ESCs, should display enhanced genetic integrity relative to differentiated derivatives of these cells. To test our hypothesis, we used the Big Blue Mouse® mutation-reporter transgene system developed by Stratagene (La Jolla, CA) to compare the frequencies of point mutations in ESCs and spontaneously differentiated cells, respectively. This

system utilizes a blue-clear color selection system to identify mutations in the lacI mutation-reporter gene carried in the Big Blue transgene.

Observations
ESCs show a low mutation frequency ranging from to 4.8E-06 to 1.05E-05 over 194 doublings. Spontaneously differentiated cells without retinoic acid have a mutation frequency that ranges from 1.1E-05 to 1.9E-05. Spontaneously differentiated cells induce with retinoic acid (1.0E-6 per ml) show a mutation frequency ranging from 1.0E-05 to 1.08E-04, which is substantially higher than the ESC mutation frequency. Our results suggest that multiple ESC lines do in fact maintain a lower mutation frequency than spontaneously differentiated cells induced with or without retinoic acid when maintained over a similar number of cell doublings. Mutation frequencies observed in ESCs also suggest potential selection against cells bearing higher frequencies. Similarly cells spontaneously differentiated from ESCs in the absence of retinoic acid also showed evidence of selection against cells with higher mutation frequencies, however cells induced to spontaneously differentiate in the presence of retinoic acid did not appear to show such stringent selection mitigating the accumulation of new mutations. Taken together, these results indicate that ESCs do maintain enhanced genetic integrity relative to differentiated cells over numerous cell doublings and therefore support our central hypothesis that enhanced maintenance of genetic integrity is a fundamental characteristic of pluripotent cells.

Submission ID: 74880
Submission Title: TCF7L2 in Adult Hypothalamic Neurogenesis in Mice and Men

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Topic
Basic Research, Medicine, and Health

Problem

Obesity and type 2 diabetes (T2D) are growing health concerns with worldwide impact. Elucidation of novel targets for intervention is needed. Growing evidence indicates that adult hypothalamic neurogenesis is necessary for regulation of energy balance, and this process is regulated by diet, hormones and energy-related signals such as leptin. Genetic variants associated with T2D or obesity may affect this process.

Background

Recent studies have shown that turnover of arcuate nucleus neurons is reduced in high fat diet-induced obese mice, and that leptin is required for maintenance of hypothalamic stem cells. In addition, partial loss of Sox2-positive hypothalamic neural stem cells in adult mice resulted in weight gain and glucose intolerance. These studies all indicate that hypothalamic neurogenesis is a dynamic response to changing dietary and environmental conditions and therapeutic interventions upon this process may be beneficial for metabolic disorders. The gene transcription factor 7-like 2 (TCF7L2) is part of the WNT signaling pathway, and intronic variants of TCF7L2 are associated with risk of type 2 diabetes (T2D). In the brain, TCF7L2 exists in 3 distinct isoforms that show differential expression during development. In murine embryonic development 34kD, 58kD, and 75kD isoforms of TCF7L2 are expressed while in only the 58kD and 75 kD isoforms are expressed at mature stages. Although TCF7L2 is known to be involved in embryonic neurogenesis, it is unknown whether it is critical for adult neurogenesis. Such a role may provide a connection between age-related decline in neurogenesis and increased susceptibility to T2D.

Hypothesis

We are investigating a potential role for T2D-associated genetic variants of (TCF7L2) in adult hypothalamic neurogenesis using human induced pluripotent stem cell derived neurons and murine neural stem cells.

Research

We have assessed TCF7L2 expression in human iPS-derived hypothalamic NPY/AgRP neurons. We next conducted siRNA knockdown of adult isoforms of TCF7L2 followed by gene ontology analysis of the sets of either up- or down-regulated genes. Additionally, we have isolated adult neural stem cells from the murine hypothalamus and assessed TCF7L2 expression.

Observations

The 34kD, 58kD and 75kD isoforms of TCF7L2 were observed in the early stage human iPS-derived neurons; however, the 34kD form is no longer present in mature neurons. Thus, a similar expression pattern to mouse is observed in human neuronal development. Preliminary gene ontology analysis of differentially expressed genes in our human model system suggest these TCF7L2 isoforms may be necessary for expression of myosin networks which in turn affect neuronal migration and plasticity. Also, knock-down led to an upregulation in genes involved in synaptogenesis and neuropeptide release. In murine hypothalamic adult neural stem cells, we found that they express

high levels of the 58kD and 75kD isoforms of TCF7L2 and weak expression of the 34kD isoform, suggesting that TCF7L2 is also important in adult neurogenesis.

Submission ID: 74894
Submission Title: Construction of high-sensitivity detection system for Mouse Feeder Cell Quantification by qPCR method

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Topic
Basic Research, Medicine, and Health

Problem
Mouse feeder cells are used to support the growth and cultivation of stem cells including hESC (human embryonic stem cells) and hiPSC (human induced pluripotent stem cells).

Background
For certain stem cell applications such as regenerative medicine, removal of heterologous mouse feeder cells is critical. Therefore, a system to detect any remaining mouse cells is required to assess stem cell culture quality.

Hypothesis
The Mouse Feeder Cell Quantification Kit is designed to facilitate highly sensitive detection and quantification of genomic DNA derived from residual mouse feeder cells using real-time PCR to analyze genomic DNA extracted from stem cell cultures (e.g., hESC, hiPSC).

Research
The kit includes primers for real-time PCR that were designed to detect a mouse mitochondrial (mt) DNA sequence, allowing detection of mouse feeder cells with high sensitivity. The copy number of cellular mtDNA is known to vary depending on cell type, cell line, and differentiation state, and there is a possibility of fluctuation between mouse feeder cell lots. The primer set for quantification of mouse mtDNA copy number included in this kit can be used for lot-to-lot correction* of mouse feeder cells.

Observations
When genomic DNA derived from different lots of mouse feeder cells are quantitated using the same standard curve, it is necessary to confirm that there is no difference in mtDNA copy number between each lot. When a standard curve is prepared for each lot of mouse feeder cells used, this correction

is not necessary.

Submission ID: 75003
Submission Title: Impact of Tissue Origin, Donor and Culture Conditions on Mesenchymal Stem Cell Characteristics

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Topic
Basic Research, Medicine, and Health

Problem
Clinical trials involving mesenchymal stem cell (MSC) therapy have highly variable outcomes.

Background
Better understanding of the factors involved and how they affect stem cell behavior may prove invaluable for the more appropriate selection of specific stem cell products for cell therapy applications.

Hypothesis
We hypothesize that these variable outcomes are largely attributed to variations in MSC functional characteristics, which are impacted by the tissue of origin, donor-to-donor variability, and culture conditions.

Research
We examined proliferation characteristics, cytokine secretion profiles and differentiation capabilities of bone marrow (BM-MSC) and adipose tissue-derived MSC (AD-MSC) from 23 donors. Seventeen donors were female and six were male, with a median age of 47 years (range: 3 – 70). Sixteen samples studied were derived from adipose tissue and seven from bone marrow. MSC were isolated and expanded to passage 3 or greater. Proliferation characteristics were studied by analyzing population doubling times for each MSC type. Conditioned culture medium was collected and analyzed for the presence and relative concentrations of 41 different cytokines and growth factors using the Luminex multiplex bead platform. Osteogenic, adipogenic and chondrogenic differentiation capabilities were assessed.

Observations
We found significant differences in proliferation characteristics between BM-MSC and AD-MSC. AD-MSC were shown to have the capacity to undergo 30 or more population doublings, while proliferation rate was found to slow considerably in BM-MSC by 20 population doublings. We also observed more donor-to-donor variability in proliferation characteristics of BM-MSC than with AD-MSC. Cytokine analysis revealed that 4 cytokines [TGFα, GM-CSF, IL-3 and MIP-1B] were consistently secreted by only BM-MSC, while 3 cytokines [Eotaxin-1, GRO and RANTES] were secreted by AD-MSC exclusively. Analysis of cytokine profiles over time revealed that 13 cytokines increased in concentration with increasing passage number during BM-MSC culture, while this trend was not observed with any cytokine during culture of AD-MSC. Contrarily, 8 cytokines were found to decrease in concentration with increased passaging in AD-MSC, while only platelet-derived growth factor AA (PDGF-AA) followed this pattern in BM-MSC. Although both AD-MSC and BM-MSC exhibited the capacity for osteogenic and chondrogenic differentiation, AD-MSC appeared to display a more enhanced inclination toward adipogenic differentiation, as expected. While overall differentiation potential remained strong in AD-MSC until at least passage 5, this potential began to decline in BM-MSC as early as passage 3. In summary, MSC characteristics are significantly influenced by their tissue of origin, donor and culture conditions. Further studies are necessary to evaluate how these variations might affect the intended function of MSC in the therapeutic setting, and how this knowledge can be exploited for the development of novel cell therapy applications.

Submission ID: 75015
Submission Title: Frontotemporal Dementia-associated MAPT N279K Mutation Disrupts Subcellular Vesicle Trafficking and Induces Cellular Stress in iPSC-derived Neural Stem Cells

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Topic
Basic Research, Medicine, and Health

Problem
New insights of disease etiology have been revealed using iPSC technology, which has facilitated its exponential use as a novel tool to understand the pathogenesis of multiple neurodegenerative diseases. However, the exact mechanism owing to the cell death of specific neuronal populations in FTDP-17 remains to be identified.

Background
Pallido-Pontal Nigral Degeneration (PPND) belonging to frontotemporal dementia with Parkinsonism related to chromosome 17 (FTDP-17) is a progressive and terminal neurodegenerative disease caused by mutations in the MAPT gene encoding microtubule-associated protein tau. The N279K-associated lysine substitution at residue 279 in exon 10 of the MAPT locus is one of the most commonly possessed mutations in FTDP-17 patients.

Hypothesis
Neural Stem Cells (NSCs) derived from patient-specific iPSCs can be used to model neurodegenerative diseases and uncover cellular dysfunctions associated with a disease-causing mutation.

Research
We investigated the underlying disease mechanism associated with the MAPT N279K mutation using NSCs derived from patient-specific induced pluripotent stem cell (iPSC) lines. Furthermore, we examined if the phenotypes seen in iPSC-derived NSCs reflect what happens in the diseased brain with biochemical analysis of autopsy brain samples.

Observations
In iPSC-derived neural stem cells (NSCs), the MAPT N279K mutation induced an increased ratio of 4-repeat to 3-repeat tau and elevated cellular stress. More significant, NSCs derived from patients with MAPT N279K mutation displayed impaired endocytic trafficking as evidenced by accumulation of endosomes and exosomes, and a reduction of lysosomes. Consistently, the levels of an intracellular/luminal vesicle marker Flotillin-1 were significantly increased in the frontal and temporal cortices of FTDP-17 patients with the MAPT N279K mutation.

Submission ID: 75016
Submission Title: The Optimization of Human Embryonic Stem Cell Differentiation toward Definitive Endoderm

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Topic
Basic Research, Medicine, and Health

Problem
Various protocols have been developed to generate definitive endoderm by recapitulating in vivo embryogenesis, but the efficiency varies dramatically among protocols. Validation and improvement of these protocols is needed.

Background
Endoderm lineages, such as pancreatic and hepatic cells, are in high demand in regenerative medicine. The definitive endoderm generation is the first step of pluripotent stem cell differentiation toward mature and fully functional endoderm lineages.

Hypothesis
In this study, we aim to assess 7 published definitive endoderm differentiation protocols in parallel as well as modify some of them to achieve the best cell survival rate and the most DE progenies.

Research
A human embryonic stem cell (hESC) line with an enhanced green fluorescent protein (eGFP) targeted to the SOX17 locus, an endodermal marker, was used to monitor the progress of definitive endoderm differentiation. The differentiated DE cells expressed SOX17 and were detected by the eGFP signal. The same number of undifferentiated hESC were plated and differentiated with different protocols. The percentage of eGFP positive cells at the end of each differentiation procedure were analyzed via flow cytometry.

Observations
After comparing these protocols on the total cell number and the percentage of eGFP positive cells, we modified Teo and D'Amour protocols by doubling Activin A concentration and/or supplementing

cells with B27. Three 2-step, 4-day modified protocols produced the most eGFP positive cells. These protocols produced over 70% eGFP positive cells, and the overall cell survival rate reached over 60%. Similar high definitive endoderm differentiation efficiency of these modified protocols was observed in another hESC line (H1 cells) and an induced pluripotent stem cell line. When further differentiating the endoderm towards insulin producing cells (IPC), we observed higher expression of Pdx1 and insulin, IPC specific markers, suggesting that the modified protocols not only generate more endoderm cells but also more endoderm progenies. Using these optimal differentiation conditions, the most possible DE cells can be obtained for the next differentiation step.

Submission ID: 75033
Submission Title: Exploring the immune population hierarchy using novel interactive visualization tools, a comparison of methods: sunburst, viSNE, and SPADE on mass cytometry data

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Topic
Basic Research, Medicine, and Health

Problem
The development of new technologies for high-parameter data has resulted in a critical bottleneck: identification of immune subsets is restricted to expert-based analysis, focusing on post-acquisition characterization of cell populations. Identification of cell subsets in flow cytometry has primarily focused on manual analysis, despite the fact that computational tools have proven useful for high-parameter and cross-sample comparisons. The lack of adoption of new computational tools often stems from the inability of new methods to recapitulate results from manual analysis.

Background
In the last few years, new computational methods such as SPADE (Qiu et al, 2011) and viSNE (Amir et al, 2013) have leveraged machine learning methods for demystifying complex biological data sets with applications in next-generation single cell technologies including CyTOF. These tools are not always readily accessible to biologists, due to lack of access to computational infrastructure and expertise.

Hypothesis
We hypothesize that new methods such as SPADE and viSNE can be leveraged to recapitulate as

well as augment traditional manual expert gating analysis for discovery of known biological subsets as well as rare cell subtypes.

Research
We compare the ease of discovery of immune subsets by comparing analysis through the use of three visualization tools: the sunburst hierarchy, the SPADE tree, and dimensionality reduction using viSNE, as well as their ability to recapitulate manual expert analysis on data set collected from a CyTOF using 26 surface markers on PBMCs. The sunburst hierarchy is a visual and interactive representation of traditional manual gating, whereas the SPADE tree is a semi-automated clustering and visualization tool for identification of cell subsets. viSNE allows interaction with high parameter data in the context of two-dimensional space where gating can be accomplished. In this study, we demonstrate the ability to automatically elucidate many immune subsets using Cytobank via an iterative analytic approach, combining computational tools (viSNE and SPADE) to recapitulate manually derived cell subsets.

Observations
We retrieve known cell subsets using semi-automated tools in Cytobank, providing global views of 26-parameter data with confidence that manually derived cell subsets can be retrieved using a combination of these methods. More rare populations, less than 1% of the total (e.g. pDCs), were discovered easily using a combination of both viSNE and SPADE.

Submission ID: 75171
Submission Title: Enhancing Mesenchymal Stromal Cell Functions via Targeted Activation and Alginate Encapsulation

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Topic
Basic Research, Medicine, and Health

Problem

Mesenchymal stromal/stem cells (MSCs) are attractive as a cellular therapeutic due to their non-immunogenicity and many beneficial effects, including immunomodulation, achieved primarily via paracrine mechanisms. Despite promising clinical trial results, the inefficient homing of these cells to target tissues and their rapid clearance necessitates the use of very large and often repeated cell doses. Additionally, the exploitable therapeutic effects of MSCs are not constitutive, but must be induced by external activating factors. Pre-clinical evidence further suggests that the identity, timing, and concentration of these activating factors can result in a spectrum of functional MSC phenotypes.

Background

Alginate encapsulation is a well-established technique whereby cells are suspended in a semipermeable hydrogel which maintains cell viability, paracrine communication and prevents migration. The MSC function we are particularly interested in is the modulation of inflammatory macrophage function, which is critical in almost all tissue repair processes. We and others have shown that MSC secreted prostaglandin E2 (PGE2) is a potent regulator of macrophage behavior.

Hypothesis

We hypothesize that the aforementioned challenges of MSC therapy can be addressed using, 1) alginate encapsulation for cell immobilization and 2) targeted exogenous MSC activation. The goal of our studies is to explore MSC activation with a panel of inflammatory factors in order to identify stimuli that enhance both monolayer MSC and encapsulated MSC (eMSC) PGE2 secretion and macrophage modulation.

Research

MSC activation was investigated with a screening assay developed using fractional factorial design of experiments (FFDOE) and an in vitro macrophage model of inflammation. Briefly, human bone marrow-derived MSCs were cultured in medium containing the combinations of activating factors dictated by the FFDOE. The MSC conditioned media were analyzed for secreted PGE2 or were transferred to pro-inflammatory macrophages. After culture, the media were analyzed for macrophage secreted tumor necrosis factor (TNF)-α. This conditioned medium approach was also used in comparing the effects of activation with identified optimal factors on MSC and eMSC function.

Observations

We observed that of the panel of factors examined, lipopolysaccharide (LPS) and interleukin (IL)-1β activation of MSCs increased PGE2 secretion. In addition, activated-MSC conditioned media exhibited enhanced downregulation of the macrophage pro-inflammatory phenotype, as evidenced by a reduction in macrophage secreted TNF-α. These effects were more dramatic for IL-1β-activated MSCs than for LPS-activated MSCs. A slight synergistic effect on the upregulation of MSC PGE2 secretion was also observed when both activation factors were used together. Furthermore, when these factors were used to activate eMSCs, we observed that the trends in MSC PGE2 secretion

and macrophage TNF-α secretion were greatly enhanced, particularly in IL-1β-activated conditions. In summary, we demonstrated that, 1) FFDOE can be used to identify factors that enhance MSC immunomodulatory function and 2) the effect of IL-1β on MSC activation was enhanced post-encapsulation. This versatile platform can be easily adapted and expanded to identify activating factors that selectively promote other therapeutic MSC functions. The combination of cell activation and encapsulation may contribute to the development of MSC therapeutic protocols to enhance efficacy in vivo.

Submission ID: 75204
Submission Title: Improved Cryopreservation and Recovery Solutions for Pluripotent Stem Cells & Difficult-to-Preserve Primary Cells

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Topic
Basic Research, Medicine, and Health

Problem
While many cryopreservation reagents afford high viability immediately post-thaw, significant apoptosis and necrosis is often observed following the first 24 hours post-thaw, decreasing the effective viability, reducing cell numbers and adding additional stress and selective pressure to cultures. Further, this extends the time post-thaw cells must be cultured prior to use in downstream experiments.

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

Hypothesis
In this research we sought to generate optimized cryopreservation and recovery solutions which

maximize post-thaw viability and recovery of PSCs and difficult-to-preserve primary cells.

Research
Using a series of Design of Experiments (DOE) and mathematical modeling methods, we describe the development of a xeno-free cryomedium for use in cryopreservation of PSCs and ESCs, and a chemically defined, animal origin free, post-thaw recovery supplement for use in recovery of PSCs, ESCs, as well as difficult-to-preserve primary cells.

Observations
When used together, we show this system provides >80% direct post-thaw viability of PSCs with >70% cell survival following 24 hours post-plating. As a result of increased post-thaw survival rate, cells recover faster and are ready to passage sooner than with current solutions, while maintaining pluripotency and normal karyotype over 10 passages. Additionally, the post-thaw recovery supplement was tested in combination with other cryopreservation reagents which lead to markedly improved 24 hour post-thaw viability of difficult-to-preserve primary cells, including primary cortical neurons and human corneal epithelial cells.

Submission ID: 75298
Submission Title: Differentiation and characterization of dopamine neurons derived from baboon induced pluripotent stem cells

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Topic
Basic Research, Medicine, and Health

Problem
Stem cell transplantation therapy provides a viable option for the treatment of neurodegenerative disorders, such as Parkinson's disease. However, despite an abundance of success in preclinical trials, human clinical trials failed to reach a significant benefit.

Background
The failure to translate preclinical studies into humans may be a result of divergent neuroanatomical and immunological characteristics pertinent to transplantation therapy that exist between humans

and rodents. A nonhuman primate model may be better suited to these studies. To determine this, protocol need to be developed that can efficiently convert nonhuman primate induced pluripotent stem cells (iPSCs) into neurons.

Hypothesis
We chose the nonhuman primate, Papio Anubis (olive baboon), as our model, because it is displays a high degree of similarity to the human, especially in regards to neuroanatomy and immunology . We hypothesized that iPSCs derived from the baboon can be efficiently converted into neurons using directed differentiation protocols.

Research
We differentiated baboon iPSCs into neurons using protocols previously shown to efficiently convert human iPSCs into dopamine neurons. We used immunocytochemistry, RT-qPCR, and electrophysiology to assess the degree to which baboon iPSC-derived neurons represent dopamine neurons in vivo.

Observations
We demonstrated that baboon iPSC-derived neurons express markers indicative of dopamine neurons in vivo. We also found that, compared to the parent stem cell line, baboon iPSC-derived neurons upregulated transcripts typical of the dopamine neuron phenotype. Lastly, biPSC-derived neurons fired spontaneous rhythmic action potentials and stimulation-induced high-frequency firing activity.

Submission ID: 75303
Submission Title: Cooperative target gene regulation by PLZF and SALL4 in mouse spermatogonial stem cells.

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Topic
Basic Research, Medicine, and Health

Problem
Spermatogonial stem cells (SSCs) maintain spermatogenesis throughout adulthood through

balanced self-renewing and differentiating fate decisions, yet little is known about how these fate decisions are controlled.

Background
The transcription factors Sal-like 4 (SALL4) and zinc finger and BTB domain containing 16 (ZBTB16, aka: PLZF) are known to be required for normal SSC self-renewal and differentiation. We previously identified 3,075 PLZF binding sites and 3,490 SALL4 binding sites in undifferentiated spermatogonia by chromatin immunoprecipitation and next-gen sequencing (ChIP-Seq). In general, PLZF bound proximal promoter regions of annotated genes, while SALL4 binding was biased towards intronic sequences. Shared binding sites more closely mirrored PLZF promoter binding.

Hypothesis
To validate the importance of these binding sites to gene transcription, we utilized siRNA transfection to knock down each transcription factor in undifferentiated spermatogonia.

Research
This project utilized chromatin-immunoprecipitation and next-gen sequencing (ChIP-Seq) to access the binding repertoire of PLZF and SALL4 in mouse SSCs. To validate if PLZF/SALL4 were required for maintenance of mRNA levels of binding targets we used a knockdown approach with siRNA in cultured Thy1+ spermatogonia.

Observations
Forty-eight hours after siRNA transfection, SALL4 and PLZF mRNA levels were reduced to 63% and 74% of control (non-targeting siRNA). Similarly, mRNA levels of PLZF target genes c-Fos and Uchl1 were reduced to 50 and 55% of control, respectively. Likewise, mRNA levels of SALL4 target genes Tlr3 and Egr4 were decreased to 76 and 45% of control, respectively. Interestingly, two genes bound by both PLZF and SALL4, Etv5 and Fox01, exhibited reduced mRNA levels (>50%) with loss of either PLZF or SALL4, suggesting a cooperative PLZF-SALL4 relationship.Thus, these represent the first functional connection between PLZF/SALL4 binding and regulation of gene expression in undifferentiated spermatogonia and suggest that both factors cooperate to positively regulate gene expression with PLZF playing a dominant role.

Submission ID: 75322
Submission Title: Novel Peptide Hydrogel Scaffolds for 3D Cell Culture and Anti-Cancer Drug Efficacy Screening

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Topic
Basic Research, Medicine, and Health

Problem
Cells in living organisms are organized in spatially complex arrangements, supported by extracellular matrix (ECM) and surrounded by other cells or migration. The drug efficacy testing result in a 2D cell culture system is often misleading because the flat surface of the traditional 2D cell culture plate fails to provide such topological approximation of the complex 3D matrix.

Background
Cell culture is the key to life science research involved in drug efficacy screening, regenerative medicine, tissue engineering or organ regeneration. Low correlation of drug efficacy test using 2D cell culture system between in vitro and in vivo has motivated scientists switch to 3D cell culture system as in vitro. Most existing 3D scaffolds are more complicated for cell encapsulation and also low cell reproducibility. The objective of this research is to develop a simple and reproducible drug efficacy testing tool with 3D cell culture system.

Hypothesis
In this research a novel peptide hydrogel (PGmatrixTM , PepGel LLC) was applied for 3D cell culture scaffold. The PGmatrix is a new product derived from a self-assemble peptide hydrogel consisting of 19 amino acids. PGmatrix has a nanofiber size of 5-15 nm and pore size of 50-200 nm that is similar to natural ECM geometry. This hydrogel also possesses shear-thinning and reversible sol-gel phase transition at cell philological conditions. We expected that this 3D scaffold system would provide a simple and reliable tool for drug efficacy screening test.

Research
Hela cell encapsulation was performed at room temperature and neutral pH environment. Since the PGmatrix hydrogel possess sheering-thinning and reversible sol-gel phase transitions, the 3D cell matrix system can be transferred by pipetting from container to the dish. Camptothecin, one of the most anti-cancer drugs, was used for drug diffusion kinetics and drug efficacy screening test with hela cancer cell in the 3D PGmatrix system. Hela cells were embedded in the PGmatrix hydrogels simultaneously with the initialization of hydrogelation with camptonthecin drug solution on top of the

matrix surface. Fluorescence measurement was used to determine if there is any interaction between camptothecin and PGmatrix.

Observations
No significant interaction between the drug and the matrix in the gel concentration studied meaning that camptonthecin has no effects on the gel strength, sheer-thinning, and reversible sol-gel transition properties. About 80% of camptonthecin can diffuse in or out through the gel within 5 hours, and diffusion rate was independent of fiber concentration in the range studied here but slightly affected by temperature and reached highest at temperature 30°C. Cell viability over three days showed that the camptonthecin reached effectively to the cells. Cell responses were drug dose-dependent, and not significantly affected by the fiber concentration of the PGmatrix nor gel strength in the testing range studied here. The results provided further evidence that PGmatrix is a promising 3D cell culture scaffolds for drug efficacy screening test. Other cells (i.e., stem cells, skin cells, liver cells) have also been successfully cultured in the PGmatrix system.

Submission ID: 75344
Submission Title: Stem Cells from the adipose tissue as allogenic player in cartilage repair: in vivo performance

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Topic
Basic Research, Medicine, and Health

Problem
While global population is progressively ageing, maintaining physically active, osteoarticular ailments arise in incidence, causing reduced quality of life not only of the elderly, but also young sportsmen. Cartilage lesions, if not treated at an early stage, commonly progress to a highly degenerative scenario, such as osteoarthritis, currently difficult to manage. Clinical outcomes of current standard

of care procedures for cartilage repair, where microfracture is the most used, are not yet satisfactory. In a best case scenario, outcomes result in a fibrocartilaginous tissue that does not fulfil biomechanical demands of hyaline articular cartilage tissue.

Background
Stem cell therapies have gained interest for cartilage repair, due their therapeutic and regenerative potential. Among diverse stem cell sources, adipose tissue seems very attractive as it fulfils demanding requirements: significant chondrogenic potential; high yield of regenerative cells can be obtained per gram of tissue; considerable amounts of tissue can be sourced as it constitutes a surgical waste, and ease of access is tremendously simple as compared to, for instance bone marrow. These cells are further attractive for cartilage repair, as they constitute a potential allogenic therapy. Regenerative cells from the adipose tissue have demonstrated to be immunoprivileged and, in this particular application, will be used in a tissue shortly exposed to immunological reactions, due to its avascular nature.

Hypothesis
Herein, stem cells from the adipose tissue, chondrogenically validated, are proposed as major player in a combined therapy system as a treatment for focal cartilage lesions, in an allogenic context. Cells are delivered to the cartilage lesion in a non-animal origin hydrogel that support their chondrogenic development.

Research
Regenerative cells from the adipose tissue were xeno-free cGMP manufactured and extensively characterized for their cell surface markers, stemness and chondrogenic differentiation potential, as well as validated for absence of microbial contamination and recommended bloodborne pathogens. Cells were further combined (10 million cells/mL) with a methacrylated gellan gum-based hydrogel (2%w/V) and cultured in chondrogenic in vitro conditions to validate the system. Cartilage regeneration potential was tested by the use of a rabbit knee model. Mature New Zealand White rabbits were subjected to 4mm diameter cartilage lesion, and the cellular hydrogel was prepared in the operating room at room temperature, injected into the lesion site and instantly solidified using saline. A sub-group of animals was treated with microfracture technique (current standard of care), while others were left untreated. Regeneration was allowed for eight weeks, after which, cartilage explants were analyzed by immunohistochemistry for collagen type I and II, safranin-O staining, and evaluated according to O'Driscoll and ICRS-II scoring systems.

Observations
The combined therapy system resulted in chondrogenic development proven by histological detection of extensive hyaline cartilage matrix deposition. Collagen type II was highly expressed, while low evidences of fibrocartilage markers (collagen I) were visible. By histological scoring evaluation of the regenerative outcomes, the combined therapy outperformed microfracture

Submission ID: 75377
Submission Title: Human iPS cell-derived in vitro model for Hepatitis B virus

infection and proliferation

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Topic
Basic Research, Medicine, and Health

Problem
Hepatitis B virus, HBV, is associated with severe liver diseases and is one of the world's most widespread pathogens. Despite more than 2 billion people have been infected and about 600,000 people die annually due to the consequences of acute or chronic HBV hepatitis, cirrhosis, and hepatocellular carcinoma, progress in HBV research has been hampered by the lack of sufficient in vitro models of HBV infection and proliferation.

Background
HBV studies using the primary hepatocytes or differentiated HepaRG which is a human hepatic progenitor cell line have been restricted because the cells cannot proliferate and survive for long periods in vitro. Those two systems are also very costly, limiting their use in vaccine or drug screenings. In recent, a specific receptor for HBV infection was discovered to be Na+/Taurocholate Co-Transporting Polypeptide (NTCP), and no or very low expression of NTCP was in the human primary hepatocytes and human induced pluripotent stem cell (hiPS)-derived hepatocyte-lineage cells.

Hypothesis
Because NTCP expresses in the basal membrane of hepatocytes in the liver, specific cellular polarities of hepatocytes should be constructed in the in vitro culture for establishment of HBV infection system. We tried to reconstruct hiPS-derived liver tissue in vitro model having the specific cellular polarities.

Research
HiPS cell-derived hepatic lineage cells were co-cultured with human umbilical vein endothelial cells (HUVECs) networks on Engelbreth-Holm-Swarm (EHS) gel, and the hepatic sinusoid-like structure, IVL of hiPS could be formed. We tried to infect HBV to IVL of hiPS. The pre-genome RNA of HBV in

the cells was quantitated by RT-PCR and HBV DNA in the particles which were released from the cells in the culture medium.

Observations

NTCP expression was strongly higher and had kept much longer (2 weeks) in the IVL of hiPS than that in the hiPS-derived hepatocyte single culture and hepatoma cell line Huh-7. Pre-genome RNA could be detected in the IVL of hiPS but not in hiPS-derived hepatocyte single culture. HBV DNA could be also detected in the medium of the IVL of hiPS but not in the medium of hiPS-derived hepatocytes. These results suggest that we succeeded to establish an in vitro HBV infection and proliferation system which was derived hiPS cells co-cultured with HUVEC networks on EHS gel.

Submission ID: 75419
Submission Title: Attaching Cells to Naturally Occurring Extracellular Matrices Prior to Cell Delivery

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Topic
Basic Research, Medicine, and Health

Problem
Cell therapy is showing promise for efficacy in the treatment of a variety of conditions. Yet the almost immediate decline in the number of viable cells targeted for a specific tissue or location in the body is troublesome.

Background
The pursuit of a delivery mechanism that increases the survival of cells and sequestration of cells after local implantation has led to investigations utilizing naturally occurring extracellular matrices (ECM), such as small intestinal submucosa (SIS). Adherent-dependent cells, such as mesenchymal stem cells (MSC), undergo anoikis when no attachment sites are available. Combining such cells with an ECM containing native cell attachment binding sites should enhance the survival of implanted cells.

Hypothesis
Cell/ECM combinations have been implanted before, but an injectable cell-supporting ECM form would be more compatible with transcutaneous or minimally invasive treatments.

Research
The data presented here utilizes an intact, solid-phase configuration of SIS that can be delivered via a catheter or needle. Therapeutic cell types, such as placenta-derived MSCs, readily attach to this configuration of SIS, which maintains its native three-dimensional structure and composition. We investigated the combination of cells and SIS in a quick assay to simulate bedside usage and to avoid the need for prolonged co-culturing. We demonstrated that 5 x 10^5 skeletal muscle-derived cells will attach to SIS in 1 hour in a delivery volume of 0.25 ml. The attachment rates and the number of cells that attach vary for each cell type.

Observations
The attachment of cells to SIS prior to delivery will provide the cells with a healthy microenvironment within a damaged or ischemic tissue. Studies to determine if the combination of the SIS and therapeutic cells enhances cell survival after implantation are ongoing. Fewer cells may be required for clinical efficacy if the number of cells that survive increases by co-delivering cells and SIS.

Submission ID: 75444
Submission Title: An Improved Cost Effective Preparation of Purified Paenibacillus polymyxa Neutral Protease (DispaseTM Equivalent Enzyme)

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Topic
Industry Infrastructure

Problem
An evaluation of Dispase™ from multiple vendors indicated variable purity and specific enzyme activity which leads to variable performance in cell culture and isolation applications.

Background
Dispase™ (Trademark of Godo Shusei, Co. Ltd) is a P. polymyxa neutral protease (PPNP) initially manufactured to replace trypsin for adherent cell harvest from tissue culture vessels. For many cell types, PPNP was as effective as trypsin but also possessed a unique characteristic for removing sheets of epithelial cells from culture vessels. This unique characteristic was subsequently used to recover intact sheets of human epithelial cells from skin tissue. These studies indicated the PPNP disrupted the connective bonds between the epidermis and dermis. PPNP has also been used in combination with Clostridium histolyticum collagenase to recover cells from a wide variety of mammalian tissues including adipose, liver, muscle and pancreatic tissue.

Hypothesis
Improved purification of PPNP will lead to a more consistent enzyme with higher uniform specific

activity.

Research
These data indicate two prominent PPNP molecular forms could be resolved using anion exchange chromatography. The VitaCyte PPNP preparation included almost exclusively the earlier eluting molecular form whereas Dispase™ from other vendors was observed in the later eluting peak in varying ratios. The earlier eluting molecular form had a substantially higher specific activity explaining the increased activity of the VitaCyte preparation. The significant proportion of the later eluting and less potent molecular form in the Dispase™ preparations explained the lower specific activity in those products.

Observations
An overview of the literature on the specificity of this enzyme for extracellular matrix proteins and different biomedical applications illustrates the broad applicability of PPNP for manipulating cells used in therapeutic procedures. Utilizing a more defined and consistent PPNP enzyme will correlate to improved performance in clinical applications.

Submission ID: 75499
Submission Title: Differentiation of Human Adipose Mesenchymal Stem Cells into Parathyroid Hormone Synthesizing Cells

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Topic
Basic Research, Medicine, and Health

Problem
Hypoparathyroidism is characterized by insufficient levels of parathyroid hormone (PTH) in the blood, causing hypocalcemia or decreased levels of calcium. Hypocalcemia can cause convulsions, arrhythmias, as well as numbness in the hands, feet, and mouth. No effective cure for dysfunction of

the parathyroid currently exists. Hypercalcemia can have serious effects including muscle and cardiac dysfunction.

Background
In functioning tissues, PTH is secreted by chief cells in the parathyroid when calcium levels decline, based on the function of calcium sensing receptor proteins (CaSR). No effective cure for dysfunction of the parathyroid currently exists, however, standard treatment includes daily oral calcium supplements which are difficult for patients to maintain for life and include complications such as hypercalcemia, or calcium levels above normal. here are various types of stem cells that have potential to differentiate into mature cell types in the body, giving rise to potential for replacement of damaged cells or tissues with newly differentiated, functioning ones. The use of adipose mesenchymal stem cells (hADSCs) has increased recently because of their autologous potential and high differentiation ability.

Hypothesis
It was hypothesized that after 26 days of treatment human adipose mesenchymal stem cells would differentiate into parathyroid-like cell clusters, secreting PTH and expressing a CaSR message.

Research
All hADSCs were treated with FBS and experimental groups were treated with either 100ng/mL activin A, parathyroid recombinant protein (PTHrP), or human parathyroid cell conditioned media. Cells treated with PTHrP or activin A formed clusters containing parathyroid-like cells after 26 days. Based on rt PCR and immunocytochemistry analysis human adipose MSCs treated with activin A or PTHrp expressed both PTH and CaSR message and protein. Parathyroid conditioned media treatment did not show expression of parathyroid specific cell characteristics.

Observations
Human adipose mesenchymal stem cells successfully differentiated into parathyroid hormone secreting cells in two of the three experimental groups. This could provide a new mechanism of treatment for patients with various types of parathyroid dysfunction, without risk of rejection due to the nature of these stem cells. Future studies will be conducted to test the differentiation method on other stem cells types.

Submission ID: 75508
Submission Title: Identification of Germ Stem Cells in Regenerating Axolotl Ovary

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Topic
Basic Research, Medicine, and Health

Problem
The axolotl salamander, known for its ability to regenerate complex structures completely, may possess ovarian GSCs as it can regenerate the entire ovary after injury or removal.

Background
It is known that germline stem cells (GSCs) in the testes will continually produce gametes through adulthood. However, whether GSCs are present in the ovary remains controversial and largely unexplored question in the axolotl model. Since the axolotl is the only vertebrate that lays externally developing eggs and induced PGC development like mammals, this could be a powerful tool to determine genetic factors critical for proper GSC development and regeneration.

Hypothesis
To identify the germ stem cell, we used the following criteria: Large nucleus with “loose” chromatin structures, very little cytoplasm Positive for germline specific protein, Vasa Mitotically active, with a BrdU label retaining subpopulation

Research
A population of stage 0 oogonia were found to stain more brightly with DAPI, suggesting their DNA consisted of a higher percentage of euchromatin which is characteristic of stem cells. To test if these oogonia were mitotically active, we stained for Histone 3 phosphorylated at Serine 10, a mitotic marker. A BrdU pulse/chase experiment was also implemented to determine how quickly BrdU was taken up and subsequently removed from these proposed GSCs as well as mitotically active follicle cells. First, the posterior end of the left ovary was removed through keyhole surgery. 24 hours post injury, 1mg/mL BrdU was administered to the intraperitoneal cavity through the incision. 1-2mm of the injury plane was collected, then ovaries were sectioned at 5um and co-stained for BrdU and Vasa.

Observations
In injured juvenile ovary, the H3+ oogonia could be found in germinal cradles with light Vasa staining in the cytoplasm, though the incidence was low, roughly

Submission ID: 75516
Submission Title: UTF1 is induced during early differentiation of Thy1+ spermatogonia.

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Topic
Basic Research, Medicine, and Health

Problem
Undifferentiated embryonic cell transcription factor 1 (UTF1) is a transcription factor expressed in human and mouse pluripotent stem cells which is rapidly suppressed upon stem cell differentiation. Studies have shown that UTF1 is expressed in the testes. However, it is unclear whether UTF1 is present in spermatogonia stem cells and its role in spermatogenesis has not yet been described.

Background
Recent studies using PSCs have demonstrated UTF1 is required for maintenance of pluripotency and is essential for proper differentiation of derivative cells.

Hypothesis
In the rat testis, UTF1 expression is limited to undifferentiated spermatogonia, suggesting that UTF1 may play a role in regulating the undifferentiated state.

Research
We examined UTF1 expression in mouse testes and ex vivo in cultures of Thy1+ spermatogonia (which contain SSCs). We also forced spermatogonia differentiation by exposing cultures to all-trans retinoic acid (AtRA) and determined Utf1 transcript level by Real-Time PCR.

Observations
Whole-mount staining for UTF1 was observed in Asingle, Apaired and Aaligned spermatogonia which partially overlapped with PLZF, a consensus marker of undifferentiated spermatogonia. UTF1 and PLZF labeled a substantially similar population of cultured undifferentiated spermatogonia, suggesting that UTF1 is a marker of undifferentiated spermatogonia. Surprisingly, when we forced spermatogonial differentiation by exposing cultures to all-trans retinoic acid (AtRA), Utf1 transcript levels increased 8-fold, consistent with other genes induced during differentiation (cKit, Stra8) and opposite to the decline observed for other known undifferentiated markers (Plzf, Gfra1). In a reciprocal manner, knockdown of UTF1 via siRNA transfection in cultured undifferentiated spermatogonia led to reduced cKit mRNA levels and increased Plzf mRNA levels 48 hours after transfection. Subsequently, levels of cKit and Plzf mRNA returned to normal when Utf1 mRNA levels

rebounded at 72 hours. These results provide the first evidence that UTF1 may play a novel role in undifferentiated spermatogonia by helping to poise these cells for robust differentiation.

Submission ID: 75674
Submission Title: Evaluation of Optimal Intrathecal Mesenchymal Stem Cell Dose for Intracerebral Hemorrhage Application

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Topic
Basic Research, Medicine, and Health

Problem
Intracerebral hemorrhage (ICH) is the least treatable form of stroke and is associated with high morbidity and mortality. Currently there is no effective treatment for ICH.

Background
Mesenchymal stem cells (MSCs) are multi-potent cells with the capacity to induce tissue repair and modulate immune responses. We have previously developed an animal model for ICH and have shown intravenous MSCs infusion can reduce inflammation, induce neuroregeneration and improve limb function following ICH.

Hypothesis
This current study evaluates the safety and effectiveness of delivering MSCs intrathecally.

Research
We purified and characterized MSCs from bone marrow of a healthy donor. MSC expressed triple positive membrane markers (CD 73, CD 90 and CD 105) and could differentiate into cartilage, adipose tissue, and bone lineages. We have chosen three MSC doses (2x10^5, 5x10%5 and 1x10^6) for intrathecal administration.

Observations
. 2x105 MSC/rat was found to have higher therapeutic efficacy than the 2 higher doses, however, all the 3 doses are safe with no demonstrable toxicity. Our results showed 3 days after intrathecal administration of low cell dose (2x105 MSC), the treated rats started to demonstrate superior forelimb use asymmetry recovery. Similarly, at 3 weeks post MSC treatment, rats that received the lowest MSC cell dose showed significantly lower hematoma induced injury volume and expression of pro-inflammatory cytokines such as IL-1α, IL-6 and IFNγ. All MSC treated rats, regardless of cell dose, showed enhanced expression of neurogenesis marker Doublecortin (DCX). Cells were labelled with CM-Dil CellTracker and were tracked after intrathecal administration. No labelled cells were observed outside of the ventricles which were the site of injection. In summary, intrathecal administration of MSC to treat ICH appears safe, feasible, and therapeutically effective. Lower MSC dose appeared to be more therapeutically effective than higher MSC doses. Further studies will be needed to fully understand this dose-response relationship.

Submission ID: 76013
Submission Title: The Power of The Patient Advocate

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Topic
Patient Advocacy and Communications

Problem
This poster will illustrate the role of the patient advicate utilizing new-age multimedia communications techniques together with grassroots efforts. With the internet and cutting edge technology at the fingertips, there are many new ways to communicate and advocate bridging

patients with science and industry. We will show that the role of the patient advocate can be ramped up to increase visibility of their cause through learning new techniques for communication and advocacy.

Background

Many disease and injury categories, especially the more rare conditions, could benefit from leaning new skills and techniques in communication. Through communication and education of scientific research, clinical trials and industry, we can impart hope to an otherwise hopeless community.

Hypothesis

Every disease and injury category could benefit from learning organized new-age multimedia communications. With medicine rapidly changing and the potential it holds for humanity, we need to keep our community updated by educating them on the process to help excellerate bringing this promising research to market.

Research

We launched a radio show to educate the community about clinical trials, research and care that now has 89,000 listeners. We have an online news paper to promote informative reporting. We've run campaigns to educate our community about clinical trials and reasearch. We have run surveys that the community has participated and shown interest in wanting to learn and support the effort. We partner with other organizations to fortify our advocacy and educational efforts. We utilize social newtorking and multimedia to educate our community on what is happening in science and research. We launched a patient registry based on surveys. This imparts hope and gives the community a sense of empowerment which translates to participation and advocacy for science and research. Through these means of communication we have found that our community wants to know more and participate more to bring new therapies and treatments to their loved ones.

Observations

Since 2010, when we launched the first radio show, we've seen an increase of interest in clinical trials and scientific research in the community. We ran a survey on why people are not enrolling in clinical trials. The majority of answers were that they were not aware of any clinical trials in their area. We have been able to bridge the communication gap throuigh these efforts. Through our communications platform we have also been able to bridge science and industry with our community and we have found these relationships have brought great benefits to all parties involved.

Submission ID: 76056
Submission Title: Huntington's Disease - 21 Years of Fast-Track Stem Cell Research Makes the Hope for a Treatment a Reality

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Topic
Patient Advocacy and Communications

Problem
Huntington's Disease (HD) is a devastating genetic and always fatal. Children of a parent with Huntington's Disease have a 50/50 chance of inheriting the disease. Juvenile onset has become more frequent in the last 20 years. There is currently no cure or treatment, but aggressive research taking place through induced pluripotent stem cells gives affected family members much hope. Through their partnership, Huntington's Disease researchers, HD families and patient advocates work to make this the last generation of Huntington's Disease.

Background
In 1993, after a ten year search, scientists identified the defective gene that causes HD and genetic testing is now available. Given the current absence of an effective treatment or cure, many people “at risk” for HD see no benefit in knowing they may develop the disease while others want to know so they can make informed choices about their futures. More recently, an international consortium of HD experts, including several from the Sue and Bill Gross Stem Cell Research Center at the University of California Irvine, has generated a human model of the deadly inherited gene from the skin cells of affected patients.

Hypothesis

Scientific research has made great strides in understanding Huntington's Disease. With continued investments in HD research we are encouraged that a treatment or a cure is imminent.

Research
Research is taking place to understand how the presence of the Huntington's Disease (HD) mutation affects cells in the brain and to determine ways to slow or prevent the onset and progression of the disease. The goal is to identify ways to treat HD. At the forefront of these are strategies to restore the ability of HD neurons to prevent the accumulation of the toxic protein that causes HD, to make appropriate levels of important brain proteins, to restore the activity of the normal Huntingtin protein, to harness the power of stem cells for transplantation into the brain, and to use stem cells derived from skin samples from HD patients to generate the "disease in a dish" to better understand HD and test drugs.

Observations
For the various strategies, there are experimental results in HD model systems that demonstrate their promise, such as in cells and mice that take on disease "symptoms." We can now test drugs in HD mice that "target" cell processes that the group has studied over the years. Testing in HD mice and in human stem cells may provide key information about whether a given drug or approach may ultimately benefit patients. There is considerable hope on the horizon to treat this devastating disease.

Submission ID: 76186
Submission Title: Tissue-specific ECMs Form the Stem Cell Niche and Display Differences in Physical, Mechanical, and Chemical Properties

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Topic
Basic Research, Medicine, and Health

Problem

Mesenchymal stem cell (MSC) self-renewal and differentiation are regulated by cues imbedded in the extracellular matrix (ECM). This local microenvironment, or niche, anchors the cells and provides a physical scaffold which influences cell morphology and the transduction of mechanical stimuli to cells.

Background

Previously, we demonstrated that extracellular matrix (ECM) synthesized by human bone marrow stromal cells (BM), skin fibroblasts (SK) and adipose fibroblasts (AD) direct differentiation of bone marrow-derived MSCs into tissue-specific lineages. To understand the underlying mechanisms, here we characterize the mechanical, molecular and structural properties of these three ECMs to identify the tissue-specific physical signals received by stem cells.

Hypothesis

The goal of this study is to determine the physical and mechanical properties of each tissue-specific ECM and determine if each displays a “physical signature”. This information will be critical for designing tissue engineering scaffolds, using natural or synthetic materials, for stem cell-based therapeutics.

Research

The three ECMs displayed differences in thickness (i.e. swelling) with a maximum of 90 μm for the AD-ECM to a minimum of 30 μm for BM-ECM. The hierarchy of stiffnesses for the ECMs was AD<SK

Observations

Each tissue-specific ECM was found to display a unique range of mechanical stiffness and have an effect on hMSC spreading (circularity). While the 3 tissue-specific ECMs did not exhibit dramatic differences in collagen composition, imaging data suggest that the collagens in each ECM are organized differently and assembled in unique patterns. Mass spectrometry data supports the hypothesis that distinct compositional ratios of collagens and linkers such as biglycan suggest a molecular basis for different collagen organizations observed in the three types of tissue-specific ECM. Ultimately, collagen polymerization patterns may prove crucial in defining physical and mechanical cues in the ECM and thus play a key role in regulating self-renewal and differentiation in the tissue-specific hMSC niche.

Submission ID: 76188
Submission Title: Metabolism of purine nucleotides in human adipose derived stem cells and its application for monitoring of cell growth on bio-scaffolds.

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Topic
Basic Research, Medicine, and Health

Problem
Ectoenzymes with nucleotide hydrolyzing activity have an essential role in controlling homeostasis and physiologic concentration of extracellular nucleotides. Ectonucleoside triphosphate diphosphohydrolase (eNTPDase1, CD39) plays crucial role in dephosphorylation of ATP and generation of ADP and AMP while ecto-5'-nucletidase (CD73, e5NT) converts AMP to adenosine. CD73 has been also identified as the marker of mesenchymal stem cells. This activity could be measured without cell destruction by brief exposure to the substrate.

Background
Multipotent stem cells derived from adipose tissue are promising stem cells population. They are characterized by easy accessibility and biocompatibility. Adipose derived stem cells are potentially good source for regeneration of damaged organs. For non-destructive analysis of e5NT activity, cells incubated on Cormatrix or in a standard culture flask. AMP was added to the medium and after 5-30 min small aliquot was collected for analysis using HPLC.

Hypothesis
The aim of our research was to analyze concentrations of nucleotides and activities of intra and extracellular enzymes of nucleotide metabolism in human adipose derived stem cells (hADSCs). Activity of e5NT was evaluated in the context of non-destructive assessment of cells growth on bio-scaffolds such as Cormatrix.

Research
In this study human adipose derived stem cells (hADSCs) were used. Cells were incubated for 24h in Mesenchymal Stem Cell Growth Medium DXF at 37°C, 5% CO2. After incubation cells and medium were separated and analysed for concentration of ATP and NAD as well as for activities of intra and extracellular enzymes (e5NT), ecto adenosine deaminase (eADA), intracellular adenosine deaminase (ADA), ectonucleoside triphosphate diphosphohydrolase (eNTPDase1), purine nucleosie phosphorylase (PNP), AMP deaminase (AMPD). For non-destructive analysis of e5NT activity, cells incubated on Cormatrix or in a standard culture flask. AMP was added to the medium and after 5-30 min small aliquot was collected for analysis using HPLC. Concentrations of ATP in human adipose derived stem cells was 22.0±0.84 nmol/mg protein while NAD concentration was 13.1±1.1 nmol/mg protein highlighting higher NAD concentration than in the other types of cells. The activity of e5NT (13.5±2.5 nmol/min/mg) was much higher than in other types of cells while activity of eADA was 1.1±0.57 nmol/min/mg, eNTPD1 was 0.23±0.05 nmol/min/mg, PNP was 25.7±2.9 nmol/min/mg, ADA was 16.1± 6.2 nmol/min/ml AMPD was 38.8±16.7 nmol/min/mg [±SD, n=9]. In the growing culture of hADSC initial activity of e5NT was 0.30±0.05 nmol/min/ml and increased to 0.68±0.28 nmol/min/ml after 24h growth on scaffold.

Observations
We have demonstrated exceptionally high NAD concentration and e5NT activity in hADSC. Considering ease of the procedure and its non destructive character measurement of activity of e5NT could be a convenient marker to follow human adipose derived stem cells growth on bio-scaffolds.

Submission ID: 76251
Submission Title: Rescuing Regenerative Potential of Human Mesenchymal Stem Cells from Elderly Donors

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Topic
Basic Research, Medicine, and Health

Problem

The overall quality of human mesenchymal stem cells (MSCs) decreases with age. This is both a cause and result of organismal aging. As MSCs age, damage accumulates resulting in intrinsic defects. Damaged cells then emit factors that inhibit the function of healthy cells via cell extrinsic mechanisms. This age-related functional decline of MSCs represents a major obstacle to autologous MSC therapies.

Background

For stem cell therapies to realize their promise, a strategy to enrich “healthy” MSCs from aged MSC populations is critical. Here, we identify markers defining aged MSC populations and propose a novel method to enrich “healthy” MSCs from those populations.

Hypothesis

Bone marrow-derived MSCs from elderly donors contain a subpopulation of “youthful” cells, characterized by small size, that are unable to proliferate due to inhibitory factors released from neighboring senescent cells; these cells can be rescued by separation from the original population and culture on extracellular matrix produced by young bone marrow stromal cells.

Research

Bone marrow MSCs from young and aged donors were cultured under standard conditions. To analyze phenotype, we measured cell morphology, proliferation rate, surface markers and intracellular ROS. Data was analyzed to determine what markers displayed the greatest variation between young and aged cell populations. Next, we enriched “healthy” cells in aged populations by sorting for small cells. Small, large, and unsorted cell fractions were then compared. Additionally, we examined the effect of conditioned media from all of these populations on young MSCs in order to examine paracrine effects in these populations.

Observations

We characterized aged MSCs as having decreased aspect ratio, doubling time, and SSEA-4 expression, and increased size and expression of HLAdr and ROS. Small-size MSCs from elderly donors displayed properties more similar to MSCs from young donors than the unsorted population. Furthermore, we have shown that the function of young cells is inhibited by exposure to CM from aged cells. This suggests that “healthy” cells may be inhibited within aged populations by paracrine effects. This work will serve as a foundation for future work aiming to rejuvenate aged MSCs for autologous therapies.

Submission ID: 76309
Submission Title: Recombinant Growth Factors expressed in Mammalian Cells as an alternative approach to the use of PRP (Platelet-Rich Plasma) for Cell Therapy and Regenerative Medicine.

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Topic
Basic Research, Medicine, and Health

Problem
Platelet-rich plasma (PRP) has been employed to facilitate the healing process. Its major components include: PDGF, TGF-beta, FGF and VEGF. By applying PRP at the site of injury, a robust healing response is achieved. PRP also attracts mesenchymal stem cells (MSCs) to the injury site, enhancing cell proliferation and migration. PRP is relatively simple and inexpensive to prepare; however, no controlled and reproducible protocol is yet available, each preparation displaying a

different growth factor composition, affecting the clinical outcome. In addition, regulatory guidelines require animal serum replacement for cell expansion in Cell Therapy protocols, leading to the use of allogeneic PRP, which may elicit antibodies formation.

Background
Tissue Repair involves three different phases, namely: blood clotting and inflammation, cell proliferation and tissue remodeling. Exogenous growth factors may be used for wound repair. By manipulating the growth factors composition, it is possible to accelerate or modify the process of regeneration and remodeling of damaged tissues. During inflammation, neutrophils and macrophages are recruited to produce TGF-beta1 and G-CSF. Critical roles are played by PDGF and TGF-beta1 in recruiting fibroblasts (proliferation phase). VEGF is essential for angiogenesis. During remodeling, epidermal proliferation is mediated by GM-CSF and TGF-beta3. While TGF-beta1 is directly involved in cutaneous scarring, TGF-beta3 antagonizes this effect, avoiding excessive scarring.

Hypothesis
An adequate combination of recombinant peptide growth factors is likely to substitute the platelet-rich plasma (PRP) preparation avoiding the problems of pooled plasma from different individuals and allogeneic transplantation.

Research
We have produced human PDGF-BB, TGF-beta1 and VEGFs in mammalian cells. The cDNAs were amplified from an in-house constructed Human cDNA Bank and then cloned into the pGEM®-T-Easy vector. E. coli transformants were screened by colony PCR. Upon DNA sequencing, the cDNA inserts were transferred to an expression vector. HEK293 or 293T cells were transfected with recombinant plasmids containing PDGF-B, VEGF or TGF-beta cDNAs. PDGF-B and VEGF were co-transfected with a hygromycin resistance (Hygr) vector for clone selection, at a 40:1 ratio. Cell clones were selected with 100ug/mL hygromicin.

Observations
Overproducing cell clones were selected and gene expression was assessed by Western blotting. Specific in vitro biological activity assays were employed for each growth factor. PDGF and VEGF were purified using heparin affinity chromatography. FGF production and TGF-beta1 purification are underway. Different concentrations of each factor will be tested to compose a special growth factor cocktail to treat an animal model displaying a tissue injury, in comparison with the PRP treatment. This safe and alternative approach could greatly contribute to the Brazilian Cell Therapy Network, avoiding the use of PRP in Cell Therapy and Regenerative Medicine. Support: BNDES, CNPq, FAPESP, FINEP, MCTI, MS-DECIT

Submission ID: 76333
Submission Title: Cytogenetic Analysis of Fibroblasts from the NINDS

Repository Collection and its Importance for Banking of Control and Neurodegenerative Disease Fibroblasts and iPSCs

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Topic
Basic Research, Medicine, and Health

Problem
Skin fibroblasts are a widely used source of somatic cells for the reprogramming of induced pluripotent stem cells (iPSCs). Access to a high-quality and well-characterized source of patient-derived fibroblasts is essential for successful iPSC reprogramming and the implementation of disease-in-a-dish models. However, there are no in- depth studies revealing the extent of chromosomal abnormalities present in primary fibroblasts after extensive culturing.

Background
The NINDS Repository at the Coriell Institute (http://catalog.coriell.org/1/NINDS) has established a large collection of fibroblasts derived from patients diagnosed with amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, frontotemporal degeneration or Huntington's disease, as well as neurologically normal population controls. The NINDS Repository at the Coriell Institute houses de-identified samples and complies with federal regulations for subject protection. In an effort to ensure the quality of these valuable resources, the NINDS Repository has established a vigorous quality control regimen on primary fibroblasts derived from skin explants, including testing recovery and viability of frozen distribution lots, sex and ID verification, DNA extraction, and G-band karyotyping.

Hypothesis
In an effort to ensure the quality of these valuable resources, the NINDS Repository has established

a vigorous quality control program onregimen on primary fibroblasts derived from skin explants, including testing recovery and viability of frozen distribution lots, sex and ID verification, DNA extraction, and G-band karyotyping.

Research

The NINDS Repository has observed chromosomal instability in primary fibroblasts and here we describe karyotyping of more than 80 skin-derived primary fibroblasts submitted to the NINDS Repository. This cohort includes samples from control subjects or donors harboring different mutations representing varies neurodegenerative diseases. Our results demonstrated that approximately ~75% of the patient-derived primary fibroblasts are karyotypically normal, as indicated by G-banding analysis, whereas the remaining fibroblasts displayed various karyotypic abnormalities, independent of the mutation, age, sex, and disease diagnosis. Among the fibroblasts with abnormal karyotypes our analysis revealed cases of trisomy, monosomy, and chromosomal derivation, deletion, addition and/or translocation involving various chromosomes. Our results indicate that that a fraction of primary fibroblasts obtained from skin of either neurodegenerative disease patients or healthy controls has either an intrinsic degree of chromosomal aberration and/or a propensity to drift once placed in culture. Karyotyping analysis of fibroblasts at different passages numbers may be useful in assessing de novo genomic instability.

Observations

In addition, we have found iPSCs reprogrammed from a mosaic fibroblast population that display normal karyotype and remain pluripotent, likely due to the nature of clonal selection process of iSPC reprograming. This observation confirms the value of primary fibroblast from subjects with a unique clinical manifestation and/or mutations even if they display abnormal karyotypes and mosaicism. Finally, our findings suggest the cytogenetic analysis is a critical step for quality control of fibroblasts derived from skin biopsies, and should be performed by biobanks before making them available to the scientific community; the NINDS Repository now routinely karyotypes its incoming fibroblast submissions.

Submission ID: 76401
Submission Title: Current Status of Human Pluripotent Stem Cells Banking in Korea: National Stem Cell Bank of Korea

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Topic
Basic Research, Medicine, and Health

Problem
There are many hurdles in the clinical use of human pluripotent stem cells (hPSCs) before they can be used in regenerative medicine. First of all, hPSCs should be derived and expanded through quality-controlled and well-characterized process for high-quality research and clinical application.

Background
Human pluripotent stem cells have a wide variety of possibilities in regenerative medicine. hPSCs have two advantageous characteristics that can be distinguished from adult stem cells: (1) they can differentiate into any type of cell or tissue; and (2) they can proliferate indefinitely under optimal conditions.

Hypothesis
To overcome the hurdles we, Korea National Institute of Health (KNIH), launched National Stem Cell Bank of Korea in 2012 to provide high quality stem cells and related information.

Research
As a national bank for hPSCs we have registered human embryonic stem cell (ESC) lines and have established human induced pluripotent stem cell (iPSC) lines for distributing well-characterized hPSCs and their information to the scientific community. We are also developing culture and QC

procedures compliant to international standards.

Observations

Here we report current progress of hPSCs characterization and banking. KNIH is planning further step to support stem cell research. National Center for Stem Cell and Regenerative Medicine will be established and start to work on 2016 as a key infrastructure including GMP facility.

Submission ID: 76420
Submission Title: Microparticle immunosuppression in swine composite allograft transplants

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Topic
Basic Research, Medicine, and Health

Problem
Efforts to ameliorate the lethal impact of ballistic and improvised explosive devices have resulted in the development of modern body armor which effectively shields the torso but leaves the extremities and maxillofacial regions vulnerable. Combat related extremity and maxillofacial trauma results in functional deficits or limb loss in deployed service members, which often cannot be reconstructed by conventional techniques.

Background
Reconstructive transplantation, or vascularized composite allotransplantation (VCA) offers a potential solution to this problem. Using a validated swine model of VCA based around the gracilis muscle, we evaluated the efficacy of local immunosupression to delay the onset of rejection in the absence of systemic immunosupression.

Hypothesis
Our hypothesis is that pretreatment of transplant grafts with drug eluting microparticles loaded with immunosuppressive agents can reduce or obviate the requirement for systemic immunosuppression in reconstructive transplantation, thus reducing the morbidity and mortality associated with the procedure. This will enable more wounded service personnel to benefit from potentially life-changing reconstruction.

Research

A donor gracilis myocutaneous flap (200-300g) is procured from Yorkshire swine (70-90kg). Prior to transplantation into a recipient Yorkshire swine, drug eluting microparticles loaded with IL-2, TGF-β, and rapamycin are injected subdermally into donor tissues. Microvascular anastomoses are then performed to a recipient animal using the right external carotid artery and internal jugular vein. Serum markers of tissue injury and tissue biopsies are taken for a 14-day survival period, and rejection graded using the Banff rejection scale from 1-4. Eight treatment animals are compared with 8 control animals which receive transplantation without immunosupression.

Observations

Subdermal application of drug eluting microparticles with IL-2, TGF-β, and rapamycin significantly delays the time to acute rejection compared to controls.