UNLEASHING THE POTENTIAL OF ADULT MOUSE RETINAL STEM CELLS *IN VIVO*

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Background: In lower vertebrates, Müller glia and Retinal Stem Cells (RSCs) located in a peripheral region of the retina named the ciliary margin zone (CMZ) have the potential to generate new neurons throughout life, and regenerate damaged retinas after injury. In mammals, however, endogenous repair is not observed. Müller glia can only produce a very limited number of new neurons *in vivo*, and although the ciliary body (CB) - which develops from the CMZ - was proposed to contain adult RSCs, the existence of these cells in vivo remains controversial, and thus far, they were only shown to produce neurons when grown in culture. What prevents adult RSCs from generating new neurons *in vivo* is unknown. Here we show that the endocytic adaptor Numb, an antagonist of Notch signalling, is expressed in the developing and adult CB.

Methods: To inactivate Numb, we use a condition mouse expressing Cre recombinase specifically in the peripheral retina and CMZ.

Results: Numb inactivation results in a massive expansion of the adult CB that contains several types of differentiated neurons, suggesting that Numb plays a part in maintaining RSCs quiescence and/or in CB cell fate specification. In neurosphere assay, we also found that Numb knockout spheres are significantly larger than controls, suggesting that Numb inhibition releases proliferation of adult neurospheres-forming cells. To ask whether the cellular expansion observed was due to loss of Numb function in the CMZ, we used a tamoxifen-inducible Cre mouse line inactivating Numb specifically in the developing CMZ from embryonic day 14 (cKO). Strikingly, cell-lineage tracing in both controls and Numb cKO revealed the presence of cell clusters composed of all retinal cell types in the peripheral retina of postnatal mice, but, strikingly, significantly more clusters were detected in the Numb cKO.

Conclusion: These data indicate that cells from the CMZ normally contribute to neurogenesis in the peripheral retina, and that Numb is required to maintain RSCs quiescence and/or promote the ciliary epithelium fate at the expense of the neural cell fate.

One Step Differentiation of IPSCs into Cortical Neurons

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The ability of researchers to study the cortex has been aided by the development of methodologies for the generation of cortical neurons from induced pluripotent stem cells (IPSCs). These techniques have enabled the generation of *in vitro* models of cortical neurons from somatic cells, and represent a landmark achievement for stem cell research. However, currently published protocols for the generation of these neurons from pluripotent stem cells can be difficult to preform, with many different stages at which the cells must be exposed to diverse growth factors and media supplements over two months. These logistical barriers make it more difficult for researchers to incorporate *in vitro* generated cortical neurons into their experiments.

Here we present a simplified and accelerated protocol for the generation of cortical neurons from IPSCs. Through sequential aliquots of only two different types of media, IPSCs were able to be differentiated into cortical neurons after one month. These neurons express cortical markers, are electrically active, and form functional synapses.

This protocol represents a more time, effort, and reagent efficient methodology to generate cortical neurons from IPSCs. This research aims to lower the logistical hurdles associated with generating cortical neurons *in vitro*, and support the use of these cells in future research in neurodevelopment and neurological disease.

Basic Research

REGULATION OF P53 ACTIVITY VIA MYSM1

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p53 is a tumor suppressor protein that is mutated in up to 50% of human cancers. It regulates the expression of hundreds of genes which cause cell cycle arrest, cellular senescence, or apoptosis. We are hypothesizing that MYSM1, a chromatin interacting deubiquitinase, regulates the transcriptional activity of p53 by localizing to DNA with chromatin modifying factors, and controlling post-translational modifications of p53 or the chromatin at p53 target promoters. We recently demonstrated that loss of MYSM1 results in p53 activation in the mouse hematopoietic system, while inactivation of p53 can fully rescue the phenotypic abnormalities seen in *Mysm1*-knockouts [1]. Protein-protein interaction studies will be done in H1299 and BaF/3 cells to confirm the binding of human MYSM1 with p53 and its partners. The effects of MYSM1 on p53 post-translational modifications in BaF/3 cells will then be analyzed. Following this, chromatin immunoprecipitation studies will be done to test the effects of *Mysm1*-knockdown or overexpression on the binding of p53 and other chromatin-modifying factors to p53-target gene promoters. Overall, this work is important in understanding the regulation of the p53 pathway in hematopoietic systems. Since p53 mutations are less common in hematological malignancies than in solid tumors, activation of p53 via MYSM1-inhibition is a feasible strategy for the treatment of hematological cancers.

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LINEAGE TRACING OF MÜLLER GLIA IN THE ADULT RETINA *IN VIVO*

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The mammalian retina is incapable of regeneration after neuronal cell loss caused by injury or disease, leading to irreversible blindness. In contrast, most fishes and amphibians have the capacity to regenerate their retina through the reprogramming of Müller glia into progenitor-like cells that are able to proliferate and differentiate into all retinal cell types. Recent studies have demonstrated that Müller glia reprogramming is also possible, at least to some extent, in mammals. After artificial retinal injury and injection of growth factors in the mouse eye, some Müller glia re-entered the cell cycle and BrdU-labelled neurons were observed 5 days after growth factor treatment. Although promising, these studies did not provide direct evidence that Müller glia actually generated new neurons, as genetic lineage tracing was not performed. Here we used a Glast-Cre^{ERT2}; RosaYFP mouse line to study this question in vivo. We have shown that the RosaYFP reporter is specifically expressed in adult Müller glia 4 days after 4 intraperitoneal injections of tamoxifen, providing a highly specific and efficient genetic lineage tracing tool. To reprogram the Müller glia in a progenitor state and stimulate proliferation, we injected NMDA and epidermal growth factor (EGF) in the eyes of Glast-Cre^{ERT2}; RosaYFP mice that were previously injected with tamoxifen. Müller glia-derived progenitors and their descendants will then be followed using expression of the RosaYFP reporter. Results from these ongoing studies will be presented. We next plan to manipulate gene expression in Müller glia-derived progenitors using in vivo electroporation of a Cre-inducible expression vector. This work may lead to novel therapies for blindness by promoting endogenous Müller glia to regenerate retinal cell types lost after injury or disease.

Reactive Oxygen-Mediated Reduction of Immunosuppressive Capacity in Aged Human Mesenchymal Stromal Cells

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Background

Mesenchymal stromal cells (MSC) possess immunosuppressive properties and are a promising treatment option for patients with a variety of chronic inflammatory diseases. Increased donor age is associated with a pro-inflammatory MSC secretome and reduced immunopotency. The mechanisms underlying the age-associated changes in MSC's secretome remain undefined. We assessed whether age-associated accrual of intracellular reactive oxygen species (ROS) underlie their defective immunosuppressive capacity.

Methods

MSCs, defined according to the International Society of Cellular Therapy, were isolated from adult (62±7 years, n=10) and pediatric (15±3 years, n=10) adipose tissue. Immunopotency was assessed in an in vitro assay that measured allogeneic MSC-mediated suppression of CD4+ T-cells activated with anti-CD3/CD28 coated beads. MSC's intracellular ROS was determined by flow cytometry using the dye DCFH-DA. IL-6 and IL-8, key pro-inflammatory components of the MSC secretome were assessed by ELISA.

Results

Compared to pediatric MSCs, the immunosuppressive ability of adult MSC's is impaired. Intracellular ROS levels and secreted levels of IL-6 and IL-8 are higher in adult than pediatric MSCs. Treatment of pediatric MSCs with oligomycin, an inhibitor of oxidative phosphorylation, increased intracellular ROS, enhanced the secretion of pro-inflammatory cytokines and reduced MSC's immunopotency. Treating adult MSCs with the ROS scavenging enzyme N-acetyl-L-cysteine (NAC) reduced pro-inflammatory cytokine production, and improved overall suppressive capacity.

Conclusions

Aging increases intracellular ROS levels and reduces MSC's immunopotency by modulating inflammatory components of the secretome. Interventions aimed at restoring the redox state of adult MSC to enhance their in vitro immunosuppressive properties may be relevant for cellular therapy.

Inhibition of eIF2α dephosphorylation promotes skeletal muscle stem cell self-renewal and enhances their engraftment capacity.

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Disorders of skeletal muscle characterized by degeneration of muscle make up the family of muscular dystrophies, aging associated sarcopenia and cancer cachexia. Development of stem cell based therapies for the muscular dystrophies aim to replace the pool of skeletal muscle stem cells required for muscle regeneration. For long-term stem cell based therapy to be effective, transplanted stem cells must not only contribute to the regeneration of healthy muscle, but must also 'self-renew' and be present throughout the lifetime of the individual. The importance of self-renewal is illustrated by the total failure of engrafted myoblasts, which lost the stem-cell capacity to self-renew, to provide any benefit to the muscle of patients with Duchenne muscular dystrophy in early clinical trials performed in the 1990s. These failures indicate that strategies are necessary to optimize the self-renewal capacity of skeletal muscle stem cells both during *ex vivo* expansion and after their engraftment. We aim to identify small molecule compounds that enhance the self-renewal capacity of skeletal muscle stem cells, which would permit their expansion *ex vivo* and also improve their ability to restore the satellite cell compartment after their engraftment *in vivo.* We have identified phosphorylated eIF2α to be present in skeletal muscle stem cells. Upon activation of skeletal muscle stem cells, eIF2α is rapidly dephosphorylated. We show that pharmacological inhibition of eIF2α dephosphorylation promotes skeletal muscle stem cell self-renewal and expansion.

Effect of Cell-surface Interactions on Monocyte Cell Fate

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Human monocytes are currently used in regenerative medicine to obtain FDA-approved autologous immunotherapy products. To this end, monocytes, which usually give rise to macrophages *in vivo*, can also be used as progenitor cells to generate dendritic cells *in vitro.* Monocyte-derived dendritic cells exposed to antigens can be re-injected in cancer patients to trigger an immune response towards cells expressing these antigens. The clinical-scale production of monocyte-derived dendritic cells is preferably performed in closed systems such as fluoropolymer bags, rather than conventional polystyrene flasks. However, the effect of different surface types on monocyte cell fate decisions has not been well characterized.

Fluoropolymer and polystyrene surfaces differ in their chemical composition, surface topography, wettability and other physico-chemical properties. We have cultured human monocytes on both surfaces in defined, serum-free medium and induced their differentiation into dendritic cells with IL-4 and GM-CSF. The surface marker expression of the cells before and after culture was investigated via flow cytometry. A complete loss of CD14 surface expression with concomitant upregulation of the antigen-presenting cell markers CD40 and CD80 could be observed within two days of culture. Regardless of the culture surface used, cells showed comparable levels of viability, aggregation and adhesion. However, we found that proteins from cell culture medium adsorbed faster and at higher quantities to fluoropolymer surfaces compared to polystyrene surfaces. As proteins are known to facilitate cell adhesion to surfaces, we hypothesize that the composition of the protein layer forming on culture surfaces will determine cell adhesion and subsequent mechanotransduction events. Understanding cell-surface interactions at a molecular and mechanical level will allow for the tailoring of materials and surfaces for cell therapy applications.

FUNCTIONALIZED ELECTROSPUN SILK FIBROIN SCAFFOLDS FOR REGENERATIVE MEDICINE

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Background

Silk fibroin (SF) is frequently considered as a template for mimicking biomineralization[1]. The α-chymotrypsin digestion of aqueous SF solutions generates anionic polypeptides (Cs)[2], which rapidly induce carbonated-hydroxyapatite (CHA) formation in dense collagen gels[3,4]. The aim of this study was to induce CHA deposition on electrospun (ES)-SF and evaluate the potential role of Cs in mediating the osteoblastic differentiation of seeded mesenchymal stem cells (MSCs).

Methods

Cs[2] incorporated ES-SF scaffolds were produced[5]. Zeta potential and contact angle measurements were used to investigate the effect of Cs on scaffold surface charge and hydrophilicity. Mineralization of the various scaffolds was assessed at days 1, 3 and 7 in 1.5 X SBF[6] using ATR-FTIR, XRD and SEM.

MSCs were seeded ($5x10^3$ cells/cm^2) on ES-SF scaffolds and cultured for 21 days in basal and osteogenic media. Alamar Blue™ assay was carried out to investigate seeded MSC proliferation at days 1, 7, 14 and 21. Quantitative real-time PCR was carried out at days 14 and 21 to assess MSC osteoblastic differentiation.

Results

There was an increase in the negative charge properties of the ES-SF scaffolds with increasing Cs content. Immersion in SBF indicated CHA formation.

Seeded MSCs attached on all scaffold types with differences in metabolic activities observed when cultured in osteogenic medium. MSCs on Cs incorporated ES-SF scaffolds demonstrated higher metabolic activities compared to those seeded on neat ES-SF.

Relative to basal medium, there was an up-regulation of alkaline phosphatase, Runx2 and Osteocalcin in osteogenic medium (at days 14 and 21). Surprisingly, under basal medium, MSCs seeded on Cs incorporated ES-SF scaffolds also expressed both Runx2 and Osteocalcin.

Conclusion

The ability to incorporate Cs during SF fibre formation can induce CHA formation and control MSC osteoblastic differentiation, providing a basis for future optimization of ES-SF scaffolds for potential bone regenerative medicine applications.

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Oxygen delivery to the pancreas during preservation prior to islet isolation: Report from two clinical islet transplant laboratories

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Background: For the last half-century, organ preservation prior to transplantation has relied primarily on standard cold storage (SCS) in a sealed container packed on ice. This dramatically lowers the metabolic rate of the organ, but does not ameliorate the effects of ischemic insult. The βcells within the pancreas are highly vascularized and are thus susceptible to ischemia. The scope of this work focuses on diminishing the effects of prolonged cold ischemia (CIT) using concentrated oxygen generated by the Giner P3S Persufflation System (P3S) for the improvement of outcomes in clinical islet transplantation.

Methods: Pancreases are retrieved from multi-organ donors. A back table dissection is necessary to aseptically connect the P3S to the splenic and superior mesenteric arteries for the delivery of humidified and concentrated gaseous oxygen (40%). Pancreases on the P3S are transported to the MUHC Human Islet Transplant Laboratory, Montreal, QC, or Clinical Islet Transplant Laboratory, Edmonton, AB. Islet isolation is performed and the islets are assessed for insulin secretion, oxygen consumption rate (OCR) and viability staining prior to release for clinical islet transplantation.

Results: Using this protocol, the time between aortic cross clamp and initiation of oxygen delivery is minimized to 30min. Glucose stimulated insulin secretion is maintained in islets from pancreases with prolonged CIT on the P3S vs. SCS. Islets isolated at the HITL using the P3S displayed greater islet equivalents per cc and a higher post-culture recover than SCS. The greatest mean OCR resulted from P3S islets at the HITL under shorter mean CIT than the CITL. At both centres, mean OCR for islets on P3S was greater than SCS.

Discussion: Our protocol facilitates rapid oxygenation of the pancreas during retrieval. Most centers rely on SCS resulting in longer CIT and poor outcomes following islet transplantation. Greater OCR in P3S islets is indicative of greater islet functionality prior to transplant. Early results of this pilot trial indicate that a greater proportion of patients may be able to stop exogenous insulin administration after infusion of P3S islets compared to SCS.

Hematopoietic Stem Cells and Genome Engineering using the CRISPR/Cas9 System

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Human umbilical cord blood is a rich source of stem cells that could be used in hematopoietic transplantation. Recently, gene therapy approaches using the CRISPR/Cas9 system to modify genes have come to light and the potential clinical applicability is becoming increasingly evident. However, the effect of modifying hematopoietic stem cells must be studied in the laboratory setting before gaining widespread clinical use. Modified hematopoietic stem cells could be used to treat a spectrum of hematological diseases. One focus of our group is using such genome engineering strategies for the treatment of sickle cell anemia by inducing HSCs to re-express fetal hemoglobin, which studies indicate is a promising approach. The aim of this talk is to discuss the various aspects required to obtain modified HSCs and the use of these in downstream applications.

AN INTERLEUKIN-6-INDUCED THERAPY FOR MYOCARDIAL REGENERATION

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Background:

Given the limited healing ability of adult myocardium, the development of an effective treatment for myocardial infarction is warranted. Mesenchymal stem cell (MSC) therapies have been explored and found to be effective in some applications, but their primary mechanism of repair remains unconfirmed. A paracrine hypothesis suggests MSCs adjust to the microenvironment of the infarct zone, and respond by secreting a specialized profile of paracrine messengers (secretome) which facilitate myocardial repair. Interleukin-6 (IL-6) – a cytokine abundant in the infarct zone – is hypothesized to stimulate this MSC paracrine activity.

Methods:

Through cell culture, an IL-6-induced secretome was generated from rat bone marrow MSCs (rBM-MSCs) and assessed *in vivo* and *in vitro*.

In vivo, the secretome was injected into surgically-simulated infarct zones of Lewis rats. Fractional shortening (FS) of the hearts was evaluated by echocardiogram 3 weeks after treatment, and heart tissues were collected for histology.

In vitro, western blots were performed to identify key components of the secretome, and to check for markers of cardio-protection in secretome-treated cardiomyocytes. Chemo-attractive properties of the secretome toward rBM-MSCs was evaluated via trans-well migration assay.

Results:

After 3 weeks, the FS of hearts treated with the IL-6-induced secretome was roughly 10% greater than those of negative controls ($p=0.0197$). Histological analyses revealed these hearts to heal with significantly less fibrotic scar tissue as well.

Western blots revealed IL-6-conditioned rBM-MSCs to be expressing agiopoietin-1/2, fibroblast growth factor-2/7, vascular endothelial growth factor-1, and transforming growth factor-β – cytokines strongly linked to neovascularisation, cardiomyocyte survival, and anti-inflammatory properties.

Compared to a negative control, the IL-6-induced secretome was also found to be significantly chemo-attractive to rBM-MSCs ($p=0.012$).

Cardio-protective assays are currently ongoing.

Conclusions:

The IL-6-induced secretome is shown to acutely stimulate myocardial regeneration following an infarction through paracrine activation of several pro-regenerative processes, and the potential recruitment of additional MSCs.

MAPK/ERK Pathway as a Possible Regulator for Naïve to Primed Pluripotency Differentiation and Rosette Formation

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Pluripotency can be defined as the ability of a cell to generate all cell types in the body. Naïve pluripotency is the ground state of pluripotency, which allows cells to stably self-renew. Primed pluripotency is an intermediate phase between naïve pluripotency and cell specialization which is marked by x-chromosome inactivation, genome-wide epigenetic alterations, and gain of cell polarization. The naïve-primed pluripotency transition (NPPT) occurs at ~E4.5 and initiates cell cluster reorganization from a disordered inner cell mass into a rosette which expands to form the egg cylinder cone. While it has been previously demonstrated in 2D culturing that the MAPK/ERK pathway is required for NPPT, it is unknown how this pathway regulates the morphological changes and progression of pluripotency during embryogenesis.

We hypothesize that activation of MAPK/ERK pathway is required for ES cell specialization into primed pluripotent cells and reorganization into a rosette structure linking to epithelia formation. Here we describe our 3D culturing system for observing NPPT in vitro from single cell naïve pluripotent stem cells. At 48hour of development, 6 classes of morphology were identified. Clusters were either an EpiLC monolayer or a cluster with or without an organized nuclei ring, and an actin foci. Cells may also aggregate to form superclusters. Cell clusters were also found to commonly exhibit blebbing, occasionally causing cell extrusion from the cluster. A minority of cell clusters were seen demonstrating whole cluster migration and targeting neighboring cell clusters for aggregation. Cell clusters were also found to exhibit aberrant cluster morphology at 72h, losing normal cluster organization and developing highly invasive cell branching. Our 3D culture system demonstrates a viable option at investigating NPPT in vitro. Our next step is to generate a Grb2 knockout line to examine cessation of MEK/ERK pathway from growth factors to examine pathway importance in cell cluster organization.

HYPOXIA MODULATES hPD-MSCs MIGRATION IN VITRO FOR CARDIAC REPAIR

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Background: Mesenchymal stem cells (MSCs) from different sources have been evaluated in cell therapy for myocardial infarction (MI). MSCs derived from human bone marrow (hBM-MSCs) have been extensively studied in clinical trials. However, readily available - placenta derived MSCs (hPD-MSCs) appears to be an effective and efficient alternative. In this study, we have analyzed the stromal cell derived factor 1α (SDF-1α)/CXC Receptor Type 4 (CXCR4)-mediated cell migration and proliferation in both hPD-MSCs and hBM-MSCs.

Methods: Cell surface localization of CXCR4 was detected by immunofluorescence. Cell viability and MTT cell proliferation assays were performed for optimizing cell culture conditions. CXCR4 cDNA was detected by semi quantitative RT-PCR. To understand cell signaling, protein expression of CXCR4, HIF-1α, IL-6, IL-10, Akt, p-Akt, ERK, and p-ERK were analyzed by western blot. CXCR4 positive cells were sorted and analyzed by FACS.

Results: CXCR4 was expressed by both hBM-MSCs and hPD-MSCs at basal level. hPD-MSCs had a significantly greater cell migration potential than hBM-MSCs towards SDF-1α in a dose dependent manner. The expressions of CXCR4 were significantly increased ($p<0.05$) after the treatment of $CoCl_2$ than the treatment of SDF-1α and glucose in both hPD-MSCs and hBM-MSCs. In hypoxic condition, the expression of CXCR4 was significantly increased ($p<0.0001$) in hPD-MSCs, compared to hBM-MSCs. CXCR4/MEK/ERK pathway was significantly activated ($p<0.05$) in hPD-MSCs, whereas, CXCR4/PI3K/Akt pathway was significantly activated ($p<0.01$) in both cell types in hypoxic conditions.

Conclusions: The sensitivity to SDF-1α of both hBM-MSCs and hPD-MSCs indicated that there might be other SDF-1α-mediated pathways involved in regulating the cell homing activities of both cell types. Complex downstream signaling cascades of SDF-1α/CXCR4 axis are activated, including PI3K/Akt and/or MEK/ERK/IKKαβ pathways in the hypoxic condition. Therefore, it can be concluded that hPD-MSCs could represent an effective and efficient alternative to hBM-MSCs for experimental studies and clinical trials for MI treatment.

CARDIOMYOPLASTY USING AMNIOTIC STROMAL CELLS IN A CHITOSAN SCAFFOLD

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Background: Stem cell cardiac regenerative therapy is limited due to low engraftment rates and modest improvements in ventricular function. Accordingly, a multifaceted approach regenerating the cardiac myocytes and the extracellular matrix is essential. Here, we present the preliminary results of a novel composite inserting human amniotic mesenchymal stromal cells in a chitosan/hyaluronic acid (C/HA) based scaffold to combat heart degeneration.

Methods: Human amniotic stromal cells were scanned for a battery of cardiac-specific genes. In parallel, a re-designed C/HA based scaffold crosslinked with β-Glycerophosphate disodium salt hydrate and genipin and mixed with cell culture medium at 1:1 volume ratio was prepared. Thermo-gelling properties reflecting structural characteristics were determined using a rheometer. Subsequently, to examine cellular viability, Alamar blue assay was conducted on human amniotic stromal cells embedded in C/HA scaffold for 15 days. To elucidate its cardiac impact, the composite was injected into induced myocardial infarction rat model. Cardio-protective analysis is conducted using echocardiography and histological markers.

Results: Gene expression analysis revealed that human amniotic stromal cells constitutively express ample cardiac-specific genes such as ATP2A2, Desmin, GJA1, TNNT2, ACTN2, MYL2, ACTC1, and MYH7. Mechanical characteristics of C/HA platform indicated a bulk gelation time at 45 minutes. Moreover, temperature sweep assay indicated a swift elastic conversion at 40°C. Alamar blue metabolic activity showed a healthy and multiplying human amniotic stromal cells after 15 days in culture with C/HA. *In vivo* cardiac analysis assays is ongoing.

Conclusions: To date, the limited and ineffective cell-based cardiac regeneration therapies incites the pursuing of novel and advanced cellular delivery platforms. Our intriguing system of a readily available stem cell expressing a pool of cardiac-specific genes mixed in a chitosan based biological platform is a conceivable candidate to restore cardiac function and reduce remodeling.

PLACENTAL STEM CELLS FOR CARDIAC REPAIR: *IN VITRO* AND *IN VIVO* ASSESSMENT

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Background: Cardiac myocytes are understood to possess a limited regenerative capacity. Any myocardial insult leads to an irreversible injury. Mesenchymal stem cell differentiation into cardiac myocyte-like cells stands as one of the leading experimental therapies. However, a candidate cell source has yet to be defined. Here, we examined the *in vitro* and *in vivo* cardiac differentiation potential of human placenta derived stem cells (hPDSCs); a unique, abundant, and non-immunogenic cell source.

Methods: H9c2(2-1) rat cardiac cell culture medium was applied to hPDSCs at different ratios for a period of 4 weeks. In parallel, hPDSCs, human bone marrow stem cells, or cell free culture medium (n=28) were injected in peri-infarcted regions induced in rat hearts.

Results: *In vitro*, hPDSCs pre-conditioned with H9c2(2-1) cell culture medium over-expressed proportional levels of alpha sarcoplasmic actinin and displaced connexin 43 proteins from the cytoplasm to the cell membrane. Additionally, pre-conditioning promoted hPDSCs survival and triggered vascular endothelial growth factor dependent angiogenesis by activating the pAkt and p38/pSTAT3 pathways respectively. *In vivo,* echocardiography analysis showed a significant improvement in cardiac parameters in the rats injected with hPDSCs, similar to the human bone marrow stem cells injected group. Moreover, hPDSCs detected within rat cardiac tissues expressed troponin I and myosin heavy chain. In accordance with the pre-conditioning *in vitro* findings, vascular endothelial growth factor positive neovessels were observed in hearts injected with hPDSCs.

Conclusions: hPDSCs have the potential to differentiate into cardiac-like cells and induce angiogenesis via paracrine effects. With the advantages of easy availability and young age, these cells could be more suitable for clinical translation.

ROLE OF THE NUCLEUS IN AGING HUMAN MESENCHYMAL STEM CELLS

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Mesenchymal Stem Cells (MSCs) are multipotent cells that can be isolated from adipose, bone marrow and other tissues. They are characterized by their plastic adherence when cultured *ex vivo*, their multilineage differentiation potential and the expression of specific surface antigens. MSCs have great potential for health-related applications, including regenerative medicine. However, MSC homeostasis declines during tissue and cellular aging, leading to the impairment of self-renewal and differentiation capacity. At the cellular level, aging is characterized by the general loss of functions; the nucleus in particular is a major target of these changes. Located in the inner membrane of the nuclear envelope, the lamina forms a scaffold for proteins that organize the nuclear interior, and which is composed of intermediate filaments, lamins A, B and C. At present, the mechanisms underlying the age-related changes in nuclear homeostasis are not fully understood.

My research hypothesis proposes that nuclear compartments control aging of MSCs and other cells. It is my objective to define nuclear changes that occur when cells age *ex vivo*. To achieve this goal, I developed and analyzed two model systems that employ cultured kidney cells. These systems are based on the (a) chemical induction of senescence and (b) drug-induced senescence.

My results show that the nuclear envelope organization is significantly affected when cells age in culture. Particularly, the concentration and distribution of Lamin A change during cellular senescence.

Based on my research, I am currently identifying new biomarkers for cellular aging. This panel of biomarkers will be applied to score the aging-dependent decline in nuclear homeostasis in MSCs. MSCs are used in regenerative medicine and other applications, however their use is limited due to their aging-related functional deterioration. Therefore, I expect that my work will provide measurable parameters that evaluate the quality of MSCs for therapeutic intervention.

MAPPING THE MYSM1 TRANSCRIPTIONAL NETWORK

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Background: MYSM1 is a chromatin-interacting deubiquitinase that acts as a transcriptional co-factor. Loss of *Mysm1* expression in mice results in severe deficiencies in hematopoietic stem cell (HSC) function and lymphopoiesis. P53 is a central regulator of cell stress responses and is essential in maintaining HSC homeostasis; it acts primarily via transcriptional regulation.

Results: In recent work we established that hematopoietic defects in *Mysm1*-deficiency are associated with an upregulation of p53 levels within all affected lineages. Furthermore, loss of p53 in our *Mysm1*$^{-/-}$*p53*$^{-/-}$ mice completely rescued bone marrow failure exhibited in *Mysm1* knockouts [1]. *In vitro* knockdown of *Mysm1* showed that it specifically regulates p53-mediated transcription of the *Bbc3* gene, which encodes a key mediator of p53-dependent apoptosis, PUMA [2]. Phenotypic characterization of *Mysm1*$^{-/-}$ *Puma*$^{-/-}$ mice revealed a restoration of hematopoietic progenitor numbers and a significant rescue of the HSC dysfunction seen in *Mysm1*-deficiency [2].

Conclusions and Future Directions: We thus establish a role for MYSM1 in regulating p53-mediated stress responses in the hematopoietic system by antagonizing p53-driven transcription of *Bbc3*/PUMA, and possibly other p53-target genes. In future work transcriptional profiling using RNA-sequencing will provide insights into the global role of MYSM1 in the transcriptional networks of hematopoietic stem and progenitor cells.

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ROLE OF HYALURONIC ACID IN STEM CELL THERAPY IN CARDIAC REGENERATION

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Background: Ischemic heart Disease is one of the leading causes of death worldwide. Myocardial damage resulting from ischemia leads to an irreversible loss of cardiomyocytes. Mesenchymal stem cells (MSCs) are multipotent cells that can differentiate into cardiomyocytes, providing a potential therapy for cardiomyopathy. Hyaluronic acid (HA) is a major component of the extra-cellular matrix and acts as a ligand to cell receptors, which stimulate cellular activity most notably through its major receptor CD44.

Methods: Proliferation was assessed using the MTT assay after exposure to HA for 72 hours while the involvement of CD44 receptors were tested by treatment with an anti-CD44 antibody (CD44 mAb). Migration was tested using the trans-well assay by counting the cells that migrated after 12 hours through either the non-coated membranes or the HA-coated membranes. FACS analysis of the Annexin V assay was used for apoptosis measurement after exposing the cells to different conditions including HA and Cisplatin.

Results: Low molecular weight HA increased cellular proliferation in contrast to the effect of high molecular weight HA which inhibited MSCs proliferation. HA showed a cytoprotective role by increasing live cell population treated with Cisplatin from 74.5% to 85.9%. HA-coated membranes have significantly increased migration of MSCs, while blocking CD44 receptors have significantly decreased cellular migration.

Conclusions: The use of MSCs in cardiac regeneration has been so far limited due to low survivability and engraftment rate of MSCs. To address these limitations, the use of scaffolds was considered to increase the efficiency of cellular therapy. As our data suggests, HA contributes an important role in proliferation, migration and cytoprotection, indicating that HA hydrogels not only acts as a scaffold holding these cells, but also providing a stimulating niche at the site of injury which might increases the effectiveness of MSCs therapy.

TNF-α INDUCED CELL FREE THERAPY FOR CARDIAC REPAIR AND REGENERATION

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Background: Tumour necrosis factor-α (TNF-α) is a pleiotropic cytokine present in ischemic cardiac regions, but the concrete roles it plays in cardioprotection and regeneration have yet to be established. The purpose of this study is to interrogate the regulatory and regenerative effects of TNF-α on rat Bone Marrow Mesenchymal Stem Cells (rBM-MSC) following a myocardial infarction (MI); and to identify the critical growth factors and cytokines these cells express to initiate and sustain the process of cardiac recovery and regeneration at the site of infarct.

Methods: Secretome from rBM-MSC cultures treated/untreated with either conditioned rat cardiomyocyte (rCM) medium, TNF-α, and/or normoxia/hypoxia in various combinations were collected. Western blot analyses, trans-well migration, and cardioprotective assays were conducted. *In vivo*, echocardiography was performed on induced infarcted rats at three weeks following their treatment with a control or TNF-α hypoxia induced secretome. Histological analyses were further conducted.

Results: Western blot analyses on rBM-MSCS and rCM lysates treated with TNF-α and hypoxia showed overexpression of TGF-β, FGF-2, VEGF-1, and Ang-2. The migration assay showed that TNF-α hypoxia induced secretome exhibits chemotactic properties. The cardioprotective assay revealed overexpression of p-38. I*n vivo*, the treated rats had a higher left ventricle fractional shortening (FS) than the control, while trichrome staining showed a decrease in the size of infarct.

Conclusion: TNF-α in hypoxic conditions overexpress proteins in rBM-MSC and rCM lysates that induce neovascularization and MSC proliferation. Furthermore, TNF-α hypoxia induced secretome plays a role in the migration of BM-MSCs and triggers a signaling pathway that initiates angiogenesis in hypoxia induced cardiomyocytes. A higher FS and the decrease in the size of infarct in the TNF-α hypoxia secretome treated rats shows that cardiac repair and regeneration maybe occurring. These results shed light on a potential cell free secretome therapy for cardiac repair in ischemia induced MI patients.

PAX2 MEDIATED LINEAGE SPECIFICATION AND TRANS-DIFFERENTIATION IN EMBRYONIC KIDNEY DEVELOPMENT

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Embryonic development requires the successive induction of new cell lineages. Previous studies from our lab have shown that Pax2/8 genes are necessary and sufficient for lineage commitment and differentiation of embryonic mesonephros (primitive kidney). However the exact process by which Pax2 and the renal fate is induced from intermediate mesoderm progenitors still remains elusive. Studies have shown that signals from surrounding tissues (paraxial mesoderm, lateral plate mesoderm and surface ectoderm) are important for renal cell lineage induction, but the exact upstream regulator of Pax2 in renal system has yet not been identified. *Given the significance of Pax2/8 genes in renal system, we hypothesize that its upstream activator(s) should be sufficient to induce renal specification of ES cells as well as transdifferentiation of fibroblasts to form renal progenitors.* To examine this hypothesis, we have developed a primary culture system of purified mouse embryonic fibroblasts (MEFs) from mice expressing a Pax2-GFP transgene for genome-wide sh*RNA* screens. Importantly, these MEFs are selected and purified for non-GFP expressing cells, such that novel targets that activate Pax2-GFP expression can be identified. We are using this system to screen for unique chromatin regulators/modifiers that can enable the genetic regulation of Pax2 and change fate of both MEFS and ES cells to renal progenitors. Further, we plan to validate the activation of Pax2 through these novel targets by whole genome shRNA screens on constitutively active Pax2 expressing cells. Our long-term goal is to obtain a complete genetic analysis of novel renal lineage regulators and to assess their function using cell culture studies and gene knockout analyses in mice.

GATA3, A MAJOR REGULATOR OF STEM CELL FUNCTION IN THE PROSTATE

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Loss of the tumor suppressor PTEN is a common occurrence in prostate cancer. 75% of the more aggressive hormone-resistant human prostate tumors show loss of active GATA3. Here, we show that the transcription factor Gata3 is progressively lost in Pten-deficient mouse prostate tumors. Using both conditional loss- and gain-of-function approaches, we found that the enforced expression of GATA3 in Pten-deficient tissues markedly delays tumor progression. This effect is associated with a correction of the aberrant sphere-forming potential of cancerous stem cells to wild-type levels by re-expression of Gata3. Moreover, deletion of GATA factors in normal prostate stem cells enhanced their long term self-renewal capacities. Using RNAseq, we found that GATA3 act through different cellular pathway to mediate this function. Together, these data establish Gata3 as an important regulator of prostate cancer progression through a role for Gata3 in prostate stem cell homeostasis.

PRMT7 Preserves Satellite Cell Regenerative Capacity

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Regeneration of skeletal muscle throughout the lifetime of an individual requires the continued presence of quiescent muscle stem cells (satellite cells), which become activated in response to injury. Herein we report that a full body deletion as well as an inducible Pax7-CreERT satellite cell specific deletion of PRMT7 displays a significant reduction in satellite cell function, as PRMT7-deficient mice had defects in satellite cell regenerative capacity upon muscle injury. We observed that PRMT7 is preferentially expressed in activated satellite cells and, interestingly, PRMT7-deficient satellite cells underwent cell cycle arrest and premature cellular senescence. PRMT7-deficient satellite cells express elevated levels of the CDK inhibitor p21 and low levels of its repressor, DNMT3b. Restoration of DNMT3b expression in PRMT7-deficient cells rescued the senescence induced by the loss of PRMT7. Our findings define PRMT7 as an epigenetic regulator of the DNMT3b/p21 axis required to maintain muscle stem cell regenerative capacity in mice.

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Pre-Treatment of Human Adipose Tissue-Derived Mesenchymal Stromal/Stem Cells with Melatonin Increases their Pro-survival and Protective Effects on Human Kidney Cells

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The efficacy of cell therapy for many diseases can be limited by the poor survival of implanted cells in an environment of tissue injury. Melatonin has been reported to have anti-oxidative and anti-apoptotic effects. Adipose tissue-derived mesenchymal stromal/stem cells (ASCs), cells easily obtained in high amounts and with minimal discomfort, have shown great promise in cell therapy applications, such as in acute kidney injury. We hypothesized that melatonin pre-treatment of human ASCs (hASCs) would improve their pro-survival and protective effects. We therefore investigated the action of melatonin on hASCs, as well as the effect of the resulting hASCs conditioned media (CM) on human kidney cells exposed to oxidative and apoptotic injury-provoking doses of cisplatin. Our results demonstrated that pre-treatment of hASCs with melatonin, 100mM for 3 hours, significantly increased their proliferation and their expression of pro-survival P-Erk1/2 and P-Akt, and of anti-oxidative enzymes catalase and heme oxygenase (HO)-1. In addition, the CM from hASCs pre-treated with melatonin provoked a significantly higher proliferation and migration of HK-2 human kidney epithelial cells. Furthermore, this CM exerted significantly greater pro-survival and anti-apoptotic actions on HK-2 cells exposed to cisplatin *in vitro*. Western blot analysis showed higher expression of P-Erk1/2, Bcl-2, superoxide dismutase-1 (SOD-1) and HO-1 in the HK-2 cells exposed to cisplatin in the presence of CM from melatonin-pre-treated hASCs. In sum, our study revealed that *in vitro* pre-treatment of hASCs with melatonin may significantly enhance their survival and their therapeutic effectiveness on injured tissue.

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Bone marrow mesenchymal stem cells conditional medium repress cancer stem cells through inhibition of the epithelial mesenchymal transition

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ABSTRACT

Background: There is increasing evidence that a sub-population of cancer cells with stem cell-like properties known as cancer stem cells (CSCs), may be responsible for tumor growth and metastasis. Bone marrow derived mesenchymal stem cells (BM-MSCs) are multipotent stromal cells that can differentiate into a variety of cell types and have been shown to play seemingly paradoxical roles on epithelial cancer progression, with both stimulatory and inhibitory effects on tumor cells, raising controversy on the role of BM-MSCs in cancer progression. However, whether secreted factors from BM-MSCs can regulate cancer stem cells and metastasis progression, has not been yet fully explored. The aim of the present study is to examine the potential of such secreted factors of BM-MSC to regulate cancer cell proliferation, apoptosis and stemness in squamous cell carcinoma.

Methodology: In the present study, we have used conditioned medium derived from human BM-MSCs and from placental MSCs as the source of secreted factors**.** A431 squamous cell carcinoma cells were cultured in the conditioned medium or 10%FBS as control medium. Cell proliferation was analyzed by trypan blue stain cell counts. Cellular apoptosis was assessed by flow cytometry using Annexin V and propidium iodide labeling. The cancer stem cell population was determined by a tumor spheroid assay.

Results: We found CM-BM-MSCs exert significant anti-proliferative and pro-apoptotic effect on human skin cancer and breast cancer cells whereas the placenta-MSC conditioned medium showed no such effect. Furthermore, exposure of tumor cells to CM-BM-MSCs leads to a dramatic down-regulation of the expression of EMT markers and stem cell markers on those cells. Concomitant with these observed changes, a decrease in the cancer stem cell population, a reduction of spheres formation, indicating that BM-MSC derived conditioned medium attenuated cancer stem cell population.

Conclusion: Our findings suggest that the BM-MSC conditioned medium contains soluble anti-tumor factors that are able to inhibit tumor cells growth, to promote their apoptosis and to decrease cancer stem cell population**.** Understanding this anti-tumorigenic potential and determining the factors involved in this process might provide new therapeutic tools for cancer treatment.

Differentiation of human embryonic stem cells into cone Photoreceptors through simultaneous inhibition of BMP, TGFβ and Wnt signaling

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Cone photoreceptors are required for colors discrimination and high-resolution central vision and are lost in macular degenerations, cone and cone/rod dystrophies. Cones transplantation could represent a therapeutic solution. However, an abundant source of human cones is not available. Work performed in model organisms suggest that anterior neural cell fate is induced "by default" if BMP, TGFβ and Wnt activities are blocked. Likewise, photoreceptor genesis operates through a S-cone default pathway. Using a soluble Cerberus-like BMP, TGFβ and Wnt antagonist, we report on the differentiation of human embryonic stem cells into S-cone photoreceptors with an unprecedented efficiency. Differentiated cells express cone photoreceptor transcripts and proteins, develop a cone-like morphology *in vitro*, and degrade cGMP when exposed to light. Our work provides a platform to produce human cones for developmental, biochemical, and cell therapy studies.

SKELETAL MUSCLE STEM CELL HOMEOSTASIS IS REGULATED BY THE PHOSPHORYLATION OF FRAGILE X MENTAL RETARDATION PROTEIN

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Adult tissues regeneration depends on stem cells primed to enter a differentiation program, while remaining quiescent. How these two characteristics can be reconciled is exemplified by skeletal muscle where the majority of quiescent satellite cells (SCs) transcribe the myogenic determination gene *Myf5*, without activating the myogenic program. We have previously shown that the microRNA pathway regulates the *Myf5* mRNA translation, which is subsequently sequestered in RNA granules present in the quiescent SCs. Furthermore, microRNA-dependent regulation of *Myf5* mRNA translation is reversible. In activated SCs, RNA granules are dissociated, *Myf5* mRNA is released to the translation machinery, Myf5 protein rapidly accumulates. The microRNA silencing is lifted by the dephosphorylation of fragile X mental retardation protein (FMRP) in activated SCs. In this work, we have further analyzed the FMRP role in SC homeostasis. Skeletal muscles of *Fmr1*$^{-/-}$ mice have reduced numbers of quiescent SCs and exhibit a regeneration defect after injury. We show that the Ang1/Tie2 signaling pathway, previously implicated in maintaining SC quiescence, functions partially through the FMRP phosphorylation. Culture in the presence of Ang1 inhibits entry into the myogenic program of skeletal muscle stem cells isolated from the muscle of wild-type, but not *Fmr1*$^{-/-}$ mice. We therefore propose a model in which post-transcriptional mechanisms hold quiescent stem cells poised to enter a tissue specific differentiation program.

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