McGill's SCRM Stem Cell Regenerative Medicine Network

Title: GPR56 as a Potential Regulator of Human Normal and Leukemic Stem Cells

Author: Heather Duncan

Laboratory Director: Dr. Kolja Eppert

Leukemic stem cells (LSCs) sustain acute myeloid leukemia (AML) and must be eliminated to cure a patient. G-protein coupled receptor 56 (GPR56) was identified among a gene expression signature common to LSCs and normal hematopoietic stem cells (HSCs). GPR56 is implicated in development and regulation of murine HSCs, although this is controversial. GPR56 is a novel marker associated with a higher LSC frequency in AML patients. This study aims to establish the functional role of GPR56 in human LSCs and HSCs, providing insight into the molecular regulation of HSCs and LSCs.

We observed high *GPR56* expression in human cell fractions enriched for LSC as well as HSC and progenitors versus mature populations in leukemic or normal blood by qRT-PCR. We found a positive correlation between *GPR56* expression and poor outcome across three cohorts of cytogenetically normal AML ($P<0.01$). In another large cohort *GPR56* was more highly expressed in poor and intermediate cytogenetic risk patient samples. Lentiviral overexpression of GPR56 in AML cell line MOLM13 resulted in increased colony formation ($p<0.01$).

To assess GPR56 function *in vivo*, GPR56 overexpressing lineage negative cord blood cells were injected into immunodeficient mice. Flow cytometry at 12 weeks revealed an increase in engraftment of GPR56 overexpressing human CD45+ cells ($p<0.0001$). This advantage was maintained in secondary engraftment ($p=0.0375$, 24 weeks). The lineage distribution and percentage of stem and progenitor cells (CD34+, CD90+) was unaltered by GPR56 overexpression, indicating that the increased engraftment is due expansion of the stem and progenitor cells.

These data suggest that *GPR56* may regulate HSCs and LSCs, and contribute to poor outcome in AML through effects on leukemic stem cells. We aim to identify a functional mechanism to explain the correlation between high levels of *GPR56* and poor outcome in AML patients, leading to potential development of GPR56-targeted therapies.

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Title: The role of spindle orientation in prostate stem cell homeostasis and lineage specification

Author: Shafer, M., Tremblay, M., Lacomme, M., Monat, C., Cayouette, M., Bouchard, M.

Laboratory Director: Dr. Maxime Bouchard

The formation of multiple lineages and the stratification of cell types during epithelial morphogenesis is controlled by both extrinsic and intrinsic cell fate determination signals. The epithelial lineages and stratified architecture of the prostate are generated during development by coordinated symmetric and asymmetric divisions in bi-potent basal stem cells. These divisions control the relative amounts of the stem-like basal, and differentiated luminal cells. Using conditional gene inactivation in the mouse, we have observed that the transcription factor Gata3 controls this process by regulating the expression and localization of the protein kinase aPKC, a member of the apical Par complex. Deregulation of aPKC by loss of Gata3 leads to spindle orientation randomization in basal stem cells, and an increase in the formation of 'double-positive' progenitor cells. These defects ultimately lead to aberrant prostate branching morphogenesis and epithelial hyperplasia. aPKC controls spindle orientation by directly interacting with the spindle complex protein LGN, which links the spindle with the apical or lateral cortical membranes during asymmetric or symmetric divisions, respectively. In many different contexts, inhibition of LGN by apically localized aPKC is thought to block asymmetric stem cell divisions, whereas interaction of LGN with INSC is hypothesized to promote asymmetric divisions. In contrast to its necessity for asymmetric cell divisions in the epidermis, we recently found that INSC is dispensable for spindle orientation in the prostate, and that LGN is required for symmetric divisions in prostate stem cells. Loss of LGN, or it is newly discovered regulator SAPCD2, leads to epithelial hyperplasia and increases in progenitor cells within the prostate. Further analysis of these proteins will shed light on the mechanisms of epithelial lineage determination and stratification, and highlight the critical role of spindle orientation within stem cells.

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Title: ***Pioneer factor action during cell fate specification***

Author: Alexandre Mayran, Konstantin Khetchoumian, Fadi Hariri, Yves Gauthier, Aurélio Balsalobre, Tomi Pastinen and Jacques Drouin

Laboratory Director: Dr Jacques Drouin

Background: The establishment and maintenance of cell identity requires stable cell-specific chromatin reprogramming. Pioneer transcription factors fulfill this role by triggering chromatin remodeling during development.

Methods: We used genome wide approaches to study pituitary cell specification and to define the salient features of pioneer action.

Results: Comparison of purified pituitary cells of different lineages showed that chromatin accessibility differs at enhancers rather than promoters. Subsequently, we found that the pioneer Pax7 specifies one pituitary lineage identity by opening a new repertoire of enhancers in a cell-specific manner. We also show that Pax7 binds its targets before gene activation and chromatin remodeling. Finally we show that Pax7 remodeling induces loss of DNA methylation and accordingly leads to long term epigenetic memory of the new cell identity.

Conclusions: The present work identifies enhancer pioneering as a critical feature for cell fate specification and maintenance.

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Title: A rapidly-gelling chitosan sponge for stem cells encapsulation

Author: Timothée Baudequin, Hadil Al Jallad, Laila Benameur, Reggie Hamdy, Maryam Tabrizian

Laboratory Director: Dr. Maryam Tabrizian

Background: Critical size bone defects (CSBD) are the smallest bone defects that will not completely heal without an external intervention. A rapidly in-situ gelling scaffold (less than 1.6 second) based on chitosan crosslinked either Guanosine-5'-diphosphate (GDP) or Adenosine-5'-diphosphate (ADP) has been developed in our laboratory as a minimally invasive system for bone regeneration in CSBD as well as for in-situ cell delivery. Over the last decade, adipose-derived stem cells (ASC) have emerged as a new source of stem cells. Thus, the main goal of this study was to investigate the potential of this sponge for ASC encapsulation. The process was optimized with the pre-osteoblastic cell line MC3T3-E1 and the optimal conditions were employed with ASC.

Methods: Two chitosan solutions (3 mg/mL, pH 5 (C3PH5) or 6 mg/mL, pH 6 (C6PH6)) and two cross-linkers, GDP or ADP, resulting in 4 different sponges, were studied. Chemical and structural characterizations were performed with µCT and FTIR. First, MC3T3 were encapsulated over a week to select the best parameters. Then, primary rat ASC were encapsulated and cultured in adipogenic or osteogenic media either after explants digestion or directly as fresh rat fat pads (FP). Cell metabolic activity, proliferation (SEM, Alamar blue, fluorescence microscopy) and differentiation (protein production, ALP activity) were assessed.

Results: MC3T3 were able to survive and stay active over the week. The optimal viability was found with GDP solution and was explained by the internal structure of the sponge. Chitosan concentration and pH didn't lead to significant differences. ASC and FP were thus encapsulated in GDP C3PH5 sponges. The presence of living tissues was confirmed at the end of the culture.

Conclusions: Both ASC and FP showed pros and cons regarding biological response and technical issues. Our chitosan sponge was shown as a promising scaffold for cell delivery.

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Title: Beyond Fibroblasts: Inducing iPSCs from Alternative Patient Cells

Author: Bell Scott, Peng Huashan, Ernst Carl

Laboratory Director: Dr. Carl Ernst

Background: The use of induced pluripotent stem cells (iPSCs) for clinical and basic research has exploded over the last decade. While iPSCs can theoretically be generated from any somatic cell, the vast majority are generated from fibroblasts. Fibroblasts are easy to maintain in culture, but are difficult to extract from patients and have a laborious induction process compared to more easily obtained cells, such as those found in blood and urine. These cells may offer an attractive alternative to fibroblasts for researchers seeking to produce large cohorts of patient derived iPSCs.

Methods: We collected skin punch biopsies, peripheral blood mononucleocytes (PBMCs), and urine from healthy patients. Cells were cultured, expanded, and induced to become iPSCs with an episomal vector. The suitability of these cell sources for the production of iPSCs were compared on ease of sample extraction, how long samples remained viable once extracted, time required to establish stable cultures of cells, transfection efficiency, time required for transfected cell to establish a stable iPSC colony, and the average cost per sample of procuring a iPSC culture.

Results: Cells extracted from urine were able to establish a pure and stable population in significantly less time than that either fibroblasts or PBMCs, had a transfection efficiency comparable to fibroblasts, and significantly higher than PBMC's, and formed stable iPSC colonies in much less time than either fibroblasts or PBMCs, resulting in a significantly reduced cost per iPSC culture produced.

Conclusions: Due to their improved ease of access, rapid proliferation, and fast induction into iPSCs, we found cells extracted from urine to be a substantially cheaper and less laborious alterative to fibroblasts for generating iPSCs, and would be well suited to the generation of iPSCs from a large cohort of patients.

McGill's SCRM Stem Cell Regenerative Medicine Network

Title: Ex vivo Interactions of skeletal and immune cells using contractile collagen microdroplets

Authors : Mira Abou-Rjeili[1, 2] Stephanie Mok[5] Jose Ramirez-Garcia-Luna[1, 3] Christopher Moraes[5] Janet E Henderson[1, 4] Paul A Martineau[1, 4]

[1]Bone Engineering Labs, Injury Repair & Recovery Program, Research Institute-McGill University Health Centre; [2]Experimental Medicine, [3]Experimental Surgery and [4]Division of Orthopaedics, Faculty of Medicine, McGill University; [5]Department of Chemical Engineering.

Laboratory Director: Dr. Janet Henderson

Background and rationale: Mast cells (MC) localize to fracture callus in the early inflammatory phase of bone repair. In previous work we showed impaired long bone healing in MC-null KitW-sh mice, which are also deficient in other cells that affect bone repair including osteoblasts and osteoclasts. More recent work using Cpa3Cre/+mice, which lack MC, reveals defects in bone healing associated with impaired vascularization and alterations in the balance between catabolic M1 and anabolic M2 macrophages in repair tissue. The goal of the current study is to use 3D co-culture to determine how MC influence vascular endothelial cells (VEC) and mesenchymal stem cells (MSC) in the bone repair micro-environment.

Methods: MC are differentiated from precursors in the suspension cells isolated from WT bone marrow and grown in the presence of SCF and IL3. Weekly FACS analysis identifies CD117 and FceR1 positive mast cells. VEC will be isolated from mouse aorta and mesenchymal stem cells (MSC) from bone marrow. An aqueous two-phase system of contractile collagen microdroplets will be printed with VEC or MSC and mature MC seeded in suspension around the droplet. MC will then be activated using compound 48/80 and the collagen droplets harvested at timed intervals for analyses.

Results: About 90% of cultured cells are CD117 and Fc□R1 positive after four weeks of culture. PCR analysis of RNA harvested at weekly intervals confirmed these results. In the presence of MC we anticipate VEC will form a network of vessels and MSC will differentiate into osteoblasts, evidenced by remodeling of the collagen droplet.

Conclusion: 3D collagen microdroplets mimic the in vivo micro-environment in which MC interact with VEC and MSC during bone repair. The technology provides a valuable tool to investigate the molecular pathways by which MC influence re-vascularization and repair of cortical bone defects.

McGill's SCRM Stem Cell Regenerative Medicine Network

Title: The ciliary margin zone contributes to retinal neurogenesis in mammals

Author: Marie-Claude Bélanger, Benoit Robert, and Michel Cayouette

Laboratory Director: Dr. Michel Cayouette

Background: In lower vertebrates, a population of stem/progenitor cells located in the most peripheral region of the retina called the ciliary margin zone (CMZ) produce new retinal neurons throughout life, allowing for growth and even retinal repair following injury. Interestingly, self-organizing optic cups obtained from human embryonic stem cells were found to contain a CMZ-like zone that can generate some retinal neurons, and the ciliary epithelium was proposed to contain cells that can adopt some stem cell-like properties in culture. But whether the CMZ actually contributes to mammalian retinogenesis *in vivo* remains unknown.

Methods: To address this question, we carried out genetic lineage tracing of CMZ cells using a tamoxifen-inducible Msx1-Cre^{ERT2} mouse line containing the Rosa26-YFP reporter. Cre activation from embryonic day 14 (E14) led to specific YFP reporter expression only in the CMZ 24-48 hours later, identifying this mouse line as a reliable tool for lineage tracing studies.

Results: Strikingly, when we analyzed animals at postnatal stages following tamoxifen injection at E14, we found several YFP-positive cell clusters oriented radially in the peripheral retina. Co-staining with various cell type specific markers showed that the clusters contain many different retinal cell types, indicating that the mouse CMZ contains a population of progenitor cells that contributes to normal retinogenesis. We next asked what could be regulating the CMZ/retinal cell fate. In a candidate approach, we found that Numb, a well-known Notch signalling antagonist, is expressed in the developing CMZ. To investigate whether Numb controls the CMZ fate, we generated a tamoxifen-inducible Msx1-Cre^{ERT2};Rosa26-YFP;Numb$^{loxP/loxP}$ mouse line. Specific depletion of Numb in the CMZ from E14 strikingly increased the number of postnatal CMZ-derived cell clusters observed in the retina.

Conclusion: Altogether, our results identify the CMZ as a retinal neurogenic niche in mammals, and suggest a model whereby the loss of Numb in CMZ cells promotes retinal fate.

McGill's SCRM Stem Cell Regenerative Medicine Network

Title: The ciliary margin zone contributes to retinal neurogenesis in mammals

Authors : Marie-Claude Bélanger [1,2], Benoit Robert[3], and Michel Cayouette[1,2,4]

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Laboratory Director: Dr. Michel Cayouette

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McGill's SCRM Stem Cell Regenerative Medicine Network

Title: Mesenchymal Stromal Cell Derived Extracellular Vesicles: Role on T-cell Suppression

Authors: Anastasia Cheng, Natalia de França Shimabukuro, Inés Colmegna
Laboratory Director: Dr. Inés Colmegna

Background: Mesenchymal stromal cells (MSC) are assessed as therapeutic candidates in chronic inflammatory diseases due to their immune modulatory properties. Cell contact dependent and independent mechanisms mediate the MSC immunosuppressive effects. Whether extracellular vesicles (EVs) are important in mediating MSC:T-cell suppression is controversial.

Methods: Human adipose tissue derived MSC (n=6) were characterized according to the minimal criteria proposed by the International Society for Cellular Therapy. Seventy-two hour conditioned media (CM) was collected from MSC under resting and activated (IFNγ+TNFα) conditions. EVs were depleted from the CM using a 100 kDa molecular weight cut off filter. EVs were quantified by nanoparticle tracking analysis (NTA). Exosomes were purified by ultracentrifugation and characterized by flow cytometry, NTA, dot blot and transmission electron microscopy. The T-cell suppressive ability of MSC (1) CM, (2) EV-free CM, or (3) purified exosomes (EXO), was assessed in a 4-day allogeneic co-culture system.

Results: MSC activation resulted in an increased production of EV (n=6, resting MSC $2.5\pm1.1x10^9$ vs activated MSC $6.3\pm1.8x10^9$ EVs/ml, p=0.03), and greater T-cell suppression (n=6, resting MSC 28.2±8.5% vs activated MSC 41.6±8.8% T-cell inhibition, p=0.03). EV depletion reduced the suppressive ability of MSC CM (n=6, CM 35.0±5.5% vs EV-Free CM 21.0±11.2% T-cell inhibition, p=0.03), suggesting that EVs are involved in MSC:T-cell suppression. Furthermore, MSC EXO (size: 100 nm in diameter, exosomal markers: CD63, CD81, HSP70) suppressed T-cell proliferation (n=6, EXO 12.0±6.1% vs vehicle 0% T-cell inhibition, p=0.03).

Conclusions: EV production is increased in MSCs in response to pro-inflammatory cytokines. These EVs and among them purified EXO have a role in suppressing activated T-cells. This supports the therapeutic use of MSC subcellular fractions in inflammatory and/or autoimmune conditions.

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Title: Naïve to Prime Pluripotency Transition is Intermediated by Epiblast Like-Cells *in vitro*

Authors: Aaron Kwong, Nobuko Honma-Yamanaka, Yojiro Yamanaka
Laboratory Director: Dr. Yojiro Yamanaka

Nature provides us with two distinct states of cell pluripotency: naïve and primed. The naïve state is the initial pluripotent state observed in earliest in embryonic development (E3.5) which is represented by embryonic stem (ES) cells. The primed state is a slightly differentiated state of ES cells appearing later in development at the anterior primitive streak (E6.5), and are marked by epigenetic and gene expressions alterations; primed pluripotent cells are thusly termed epiblast stem cells (EpiSC). While this transition has been observed in embryo development through *in vivo* cross-sectional studies, the exact mechanism of this transition and dependency on certain signalling events remains elusive. For decades it was challenging to observe blastocyst implantation in real-time due to accessibility to the embryo *in utero*. Now, with the advances in 3D culturing techniques, *in vitro* conditions of implantation may be mimicked to induce this transition for real time observations.

The main objective of my project is to investigate how naïve ES cells exit self-renewal and transition into the primed state via 3D culturing techniques. We report a novel intermediate state of pluripotent stem cells that are developmentally analogues to the primitive ectoderm epiblast cells of E5.5 embryos. These intermediate embryonic have been characterized to have downregulated expression of key naïve markers (FGF4, Rex1), indicating they are not naïve; similarly they do not exhibit upregulated expression of key primed markers (FGF5, Brachyury, Nodal), indicating they are not primed. When primitive streak formation was induced via exogenous Activin A and CHIR99021 to upregulate the Nodal pathway, these intermediate stem cells demonstrated upregulated FGF5 and Brachyury expression suggesting primed ES cell differentiation. Morphologically these intermediate stem cells form an epithelial monolayer, similar to that of EpiSC and E5.5 primitive ectoderm. The experiments thus far have shown the potential for a novel intermediate stem cell population which can be differentiated from naïve ES cells, and then further differentiated into primed-like EpiSC. These cells show competency to exogenous factors for differentiation, and thus may play a key role in how naïve ES cells exit self-renewal.

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Title: Arginine Methylation in Neuromuscular Maintenance

Author: Daryan Chitsaz, Romeo Blanc

Laboratory Director: Dr. Stephane Richard

Background: Protein arginine methyltransferases (PRMTs) have innumerable well-characterized roles in regulating stem cell differentiation and proliferation during development, but have hardly been studied in the context of adult neurons. Motor neurons in the spinal cord extend axons during embryogenesis to form specialized synapses called neuromuscular junctions (NMJs), which become dysmorphic in aging and numerous neurodegenerative and muscular diseases. Prmt7$^{-/-}$ mice generated by our lab show accelerated aging, cachexia, and a progressive motor "clasping" phenotype often observed in models of such disorders, prompting us to investigate the central and peripheral nervous systems, including NMJs. We recently reported Prmt7's requirement in adult muscle stem cells (Blanc *et al*., 2016 Cell Reports), which may contribute to the neuromuscular defect, as these cells regulate NMJ regeneration.

Methods: Prmt7$^{-/-}$ mouse muscles and spinal cords are assayed via immunofluorescence for gross- and fine morphology or cell identity abnormalities, or degeneration. Clasping defects are scored as "present" or "absent" by a blinded observer.

Results: The clasping phenotype is age- and sex-linked, and present to a reduced degree in heterozygotes. Little-to-no markers of degenerating inputs or abnormal motor control circuits can be identified in Prmt7$^{-/-}$ spinal cords. These mice also lack global motor deficits associated with damage to higher motor function areas, tentatively ruling out the central nervous system. In the periphery, however, hindlimb muscles display striking abnormalities in NMJ morphology. Despite the absence of muscle deinnervation, a switch of myofibers from type I to II, characteristic of reduced neuronal input, can be observed.

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Title: An iPSC-based Model of Lymphangioleiomyomatosis (LAM)

Author: Alexander Goldberg

Laboratory Director: Dr. Arnold Kristof

Background: Lymphangioleiomyomatosis (LAM) is a rare disease caused by the abnormal proliferation of smooth muscle-like cells (LAM cells), which form destructive microscopic tumors in the pulmonary interstitium. Histopathological features indicate that the cause involves hyperactive mTOR signaling due to loss of *TSC2* gene function, β-estradiol (E2), neural crest differentiation, and neoplastic metastasis. Focusing on neural-crest specific mechanisms of oncogenesis, we previously identified a role for the neuropeptide urotensin-II (UII), and its receptor (UT). Attempts to further study the role of oncogenic signaling in LAM have been hindered by the lack of valid patient-derived cell culture model. Therefore, we developed iPSC-derived model LAM cells in order to recapitulate the mechanisms of LAM pathogenesis.

Methods: We characterized induced pluripotent stem cell (iPSC)-generated smooth muscle cells derived from a patient with LAM for common markers of LAM pathogenesis. We also measured, by wound healing assay, the migration of these cells in response to UII, E2 and the UT antagonist, SB657510. Finally, we measured UII and UT mRNA expression in response to E2.

Results: LAM-SM cells showed increased mTOR activity, increased expression of neural crest markers (GD3), and reduced autophagy and Akt activation. Migration of LAM-SM cells was inhibited by SB657510, and this effect was reversed by TSC2 expression. E2 induced the migration of LAM-SM cells, but not control cells, and this effect was additive upon treatment with UII. Furthermore, E2 increased mRNA expression of UII and UT.

Conclusions: LAM-SM cells show common hallmarks of LAM pathogenesis. These cells are sensitive to the effects of UT antagonism, and UII signaling is potentiated by E2. Therefore, E2 and UII signaling are key mediators of LAM cell migration. iPSC-derived smooth muscle cells represent a novel and validated cellular model for studying the molecular mechanisms that mediate crosstalk between E2 and UII oncogenic signaling in LAM.

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Title: Hippo-YAP and oxidative stress-induced cardiomyocytes

Author: Kashif Khan and Prashant Jaswal

Laboratory Director: Dr. Renzo Cecere

Background: After myocardial infarction, the cardiac tissue undergoes a series of alterations including the production of reactive oxygen species, cardiac fibrosis and scar tissue formation. The Hippo pathway is an evolutionarily conserved mechanism that is involved in regulation of cardiomyocyte proliferation and regeneration. Yes-associated protein (YAP) is a co-transcriptional regulator that acts as the main effector protein in this pathway, binding to TEA domain family member (TEAD) in the nucleus for transcription. Upregulation of YAP *in vitro* and *in vivo* has been shown to promote cardiomyocyte proliferation in mice subjected to myocardial infarction, while cardiac-specific knockouts of YAP show impaired heart growth. Here, we propose that YAP plays a protective role in reducing oxidative stress in cardiomyocytes when subjected to oxidative stress.

Methods: We have exposed control and YAP-overexpressed human left ventricular cardiomyocytes (AC-16 cells) to H_2O_2 for 6 hours. YAP was overexpressed using two different methods: YAP transfection and a YAP-specific S127A mutation. We also decreased YAP expression using YAP-TEAD inhibitory peptide (YTIP). We assessed the extent of protection against oxidative stress and apoptosis using biochemical assays such as western blotting and immunocytochemistry.

Results: YAP-overexpressed cardiomyocytes showed increased protein expression of ERK, JNK, and IGF-1. AKT signaling proteins were also found to be overexpressed compared to control.

Conclusions: Our study indicates that overexpressing YAP in AC-16 cardiomyocytes protects against oxidative stress and reduces apoptosis compared to control through AKT-related mechanisms. Future experimentation will investigate the downstream mechanisms of YAP including genomic and transcriptomic analysis.

McGill's SCRM Stem Cell Regenerative Medicine Network

Title: Towards functional mapping of male germline stem cell fate

Author: Joelle Desmarais, Xiangfan Zhang, Makoto Nagano

Laboratory Director: Dr. Makoto Nagano

Background: Male germline stem cells, or spermatogonial stem cells (SSCs), are expected to become an important resource to restore male fertility through regeneration of spermatogenesis. Yet, fundamental characteristics of SSCs remain elusive, largely because they represent only a minute population of seminiferous epithelial cells, causing difficulties in their analyses. With a lack of appropriate tools, the process of commitment of SSCs also remains unknown. Our ultimate goal is to draw a fate map of SSCs during steady-state spermatogenesis and to visualise SSCs.

Methods: To detect SSCs in intact testes of adult mice, we first performed negative selection with Percoll and immunomagnetic selection for ITGA5 and MHC-I. Second, we applied negatively sorted cells to a positive selection, based on cell size, granularity, and antigenic profiling of THY1, GFRA1, KIT, and CDH1. We then transplanted each cell fraction into sterile recipient testes and determined the levels of regenerative capacity/SSC enrichment in each fraction.

Results: Transplantation of each fraction into sterile recipient testes showed that SSCs were highly enriched in the $THY1^{+}GFRA1^{-}$, $THY1^{+}KIT^{-}$, and $THY1^{+}CDH1^{-}$ fractions (24-, 32-, and 31-fold enrichment respectively, compared to the fraction after negative selection). $THY1^{+}GFRA1^{+}$, $THY1^{+}KIT^{+}$, and $THY1^{+}CDH1^{+}$ fractions showed a medium level of SSC activity (2.2-fold, 9-fold and 3.1-fold). The $THY1^{-}$ fractions were virtually devoid of SSCs.

Conclusions: Our results suggest that the loss of THY1 demarks the exit from the SSC state. Surprisingly, antigens believed to be SSC markers (GFRA1 and CDH1) appear to be associated with the transition to commitment, while KIT, believed to be expressed in non-SSCs, was found in a proportion of regenerative cells. Our approach allowed the highest SSC enrichment thus far reported for adult intact testes.

McGill's SCRM Stem Cell Regenerative Medicine Network

Title: Osteoblast menin and bone mass: ex vivo studies in knockout mice

Author: Ildi Troka

Laboratory Director: Dr. Geoffrey N. Hendy

Background: Menin, the product of the MEN1 tumor suppressor gene, facilitates the cell proliferation control and differentiation induced by the transforming-beta (TGF-β) superfamily of ligands essential for bone development and maintenance. *In vitro* studies showed that menin is critical for the action of bone morphogenetic protein (BMP)-2 in directing mesenchymal stem cells to the osteogenic lineage and to become preosteoblasts. Our previous *in vivo* studies showed the importance of menin for proper functioning of the mature osteoblast and maintenance of bone mass. Here, we have examined the role of menin at earlier stages in the osteoblast lineage by conditional knockout of the *Men1* gene.

Methods: This was implemented through the Cre-LoxP recombination system and *Prx1-Cre;Men1f/f* and *Osx-Cre;Men1f/f* mice represent knockout of the *Men1* gene in the mesenchymal stem cell and the preosteoblast, respectively. Mice were analyzed at 6 months of age. *Ex vivo* studies were performed on primary calvarial osteoblasts and bone marrow mesenchymal stem cells.

Results: *Prx1-Cre;Men1f/f* and *Osx-Cre; Men1f/f* mice were smaller, femur lengths were shorter, and bone mineral density (by DEXA) reduced relative to wild-type littermates. *Men1* knockout mice had decreased trabecular bone volume, altered trabecular structure, and decreased cortical bone thickness (3-dimensional micro-CT imaging of femur). Trabecular number and spacing were decreased and increased, respectively. Mineralization and differentiation of the primary calvarial osteoblasts and the mesenchymal stem cells of the knockout mice were deficient relative to those of wild-type mice as assessed by Alizarin red, von Kossa and alkaline phosphatase staining. Gene expression profiling revealed reduced osteoblast markers, increased cell proliferation markers and increased RANKL/OPG ratio that would favor osteoclastogenesis.

Conclusions: Menin plays a crucial role in the development as well as maintenance of bone mass and is a potential gain-of-function therapeutic target for treatment of low bone mass disorders.

McGill's SCRM Stem Cell Regenerative Medicine Network

Title: Scaffolding Wnt-5a Pre-Treated Amniotic Stem Cells for Heart Failure

Authors: Makhoul G, Jaiswal PK, Yu B, Ghulam J, Cerruti M, Schwertani A, Cecere R

Laboratory Director: Dr. Renzo Cecere

Background: Stem cell cardiac regenerative therapy is limited due to low engraftment rates and modest improvements in ventricular function. Here, we present the results of a novel composite inserting wingless-5a (Wnt-5a) pre-treated human amniotic stromal cells (ASCs) in a chitosan/hyaluronic acid (C/HA) based platform to combat heart degeneration.

Methods: Cardiac gene expression was conducted on ASCs pre-treated with Wnt-5a in concentration and time dependent assays. Intra-cellular calcium was subsequently labelled in Wnt-5a pre-treated ASCs and observed under live imaging. In parallel, a re-designed C/HA platform was synthetized and its structural and cellular preservation characteristics were analyzed. The cardiac impact of ASCs-C/HA composite was then functionally and histologically assessed when injected in an induced rat model of myocardial infarction.

Results: A myriad of cardiac specific genes was constitutively expressed in ASCs. Wnt-5a treatment of 100 and 200 ng/ml for 3 days induced an optimal cardiac gene expression in ASCs. Interestingly, intra-cellular calcium influx was synchronized and rhythmical in ASCs pre-treated with 100 ng/ml of Wnt-5a. Mechanical characterization of the C/HA platform indicated a swift elastic conversion at 40°C. Moreover, at 37°C, the sol-gel transition time occurred rapidly. When mixed in the C/HA, the ASCs were viable and metabolically active for 15 days. *In vivo*, the ASCs-C/HA composite was abundantly detected 5 weeks following its intra-myocardial implantation. Moreover, ASCs-C/HA injected hearts reduced fibrosis at the infarction site, triggered a massive *in situ* neovascularization, and limited the deterioration of the cardiac function. Wnt-5a pre-treated ASCs injected rats are underway.

Conclusions: Our findings indicate that Wnt-5a pre-treated ASCs can acquire cardiac cell-like traits. In addition, ASCs-C/HA composite helped maintaining the cardiac function. We believe that once optimized, the potential of concurrent implantation of Wnt-5a pre-treated ASCs in a C/HA biological scaffold is a conceivable candidate to restore cardiac function and reduce remodeling.

McGill's SCRM Stem Cell Regenerative Medicine Network

Title: Characterization of Injectable Purine/Chitosan/Ceramic (ACC) Sponge for Cellular Encapsulation in Bone Repair Applications

Author: Kaushar Jahan

Laboratory Director: Dr. Maryam Tabrizian

Background: Bone defects result from injuries that do not repair without medical intervention. Autologous bone graft, the gold standard for treating bone defects, is challenging due to (a) donor scarcity and (b) donor site morbidity that follows the procedure. Regenerative medicine has shown potential as an alternative intervention; it is based on the use of scaffolds which mimic the structure of the tissue that requires repair and simultaneously supports, reinforces and organizes the regenerating tissue. An injectable purine/chitosan/ceramic (PCC) sponge with rapid gelation time has been previously developed in our lab showing to be a biocompatible, biodegradable, and potentially osteoconductive scaffold. Based on these results, the current project is focused on the encapsulation of cells (pre-osteoblasts and other bone cell precursors) within the PCC scaffold. Methods: The physico-chemical and in vitro characterization of the cell-laden sponge was done through Scanning Electron Microscopy and micro Computed-Tomography. Moreover, proliferative quantification was assessed through Alamar Blue assay. A live/dead analysis was performed to determine the viability of the cells inside the sponges. Results: The scans showed the internal structure of the scaffold and the viability assays show that the scaffold is not toxic to the cells. Conclusions: Subsequently, the loaded scaffold can then be implanted in a mice fracture model to study its bone repair potential. Ultimately, this sponge may be a clinical alternative to bone graft by decreasing the burden of complications associated with graft donor sites while simultaneously delivering cells able to deliver therapeutic agents at the site of injury.

McGill's SCRM Stem Cell Regenerative Medicine Network

Title: Application of Mesenchymal Stem Cells in Intervertebral Disc Tissue Regeneration

Authors: Rayan Fairag, Samy Bencherqui, Derek Rosenzweig, Janet Moir, Michael Weber, Lisbet Haglund.

Laboratory Director: Dr. Lisbet Haglund

Background: Low back pain is a major health and economic problem. Intervertebral Discs (IVD) lack intrinsic repair mechanism and are often removed surgically in order to alleviate associated pain. Significant promises have been shown with treatment of early stage degeneration by implantation of nucleus pulposus or stem cells which can slow the degenerative process and enhance regeneration. Here, we aim to determine and assess the influence of hydrogels on stem cells and nucleus pulposus cells in isolation or in co-culture and to investigate the effects of physiological culture (low nutrient and mechanical loading) on matrix production.

Methods: Human nucleus pulposus cells were isolated from healthy organ donors. MSCs were obtained from RosterBio. Cell differentiation was determined within a physiological dynamic culture system. Cells (nucleus pulposus, MSCs, or a combination) were encapsulated within two different injectable hydrogels and embedded within agarose. Constructs were cultured with or without loading for 3 weeks. Cell localization, viability and matrix proteins were determined.

Results: Stem cells cultured in chondrogenic media differentiated to a nucleus pulposus like phenotype as evidenced by producing collagen type II and proteoglycan. Isolated nucleus pulposus cells maintained their cellular phenotype. Combined cells maintain localization within specified layered regions of hydrogels. All cells seeded within hydrogels maintained viability and proliferation in the presence or absence of dynamic load.

Conclusions: In conclusion, dynamic culture system simulates the microenvironment of the IVD and is suitable for culturing and potentially assessing MSC differentiation. Further investigations need to be done in determining the regenerative capabilities of this study.

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Title: Induced pluripotent stem cells (iPSC) derived dopaminergic neurons as a tool to study Parkinson's disease

Author: Frédérique Larroquette, Nadine Lauinger, Xiuquing Chen, Thomas Durcan

Laboratory Director: Dr. Edward Fon

Background: Parkinson´s disease (PD) is a progressive neurodegenerative disorder, characterized by the development of motor and non-motor symptoms that include bradykinesia, tremors and cognitive decline. The classical pathological hallmarks of this disorder are the loss of midbrain dopaminergic neurons (DA) and the presence of proteinaceous inclusions termed Lewy bodies, with α-synuclein fibrils as the primary component. While the underlying mechanisms behind this neuronal loss are unclear, the differentiation of human induced pluripotent stem cells (hiPSCs) into dopaminergic (DA) neurons provides us with a powerful tool to elucidate the mechanisms through which PD develops.

Methods: Midbrain dopaminergic neurons were generated from hiPSCs, using small molecules that inhibit or trigger specific cellular pathways during differentiation. In order to characterize those neurons, immunofluorescence and immunoblotting for specific DA markers were performed. Furthermore, cell functionality was verified through electrophysiological measurements using patch clamp recordings and calcium imaging techniques. Finally, to replicate the main cellular hallmark of Parkinson's disease, we studied the uptake of pre-formed α-synuclein fibrils (PFFs) within those neurons. Neuronal lines derived from healthy or Parkinson´s patients were exposed to PFFs for 24h and the absolute uptake into TH-positive cells was quantified.

Results: In our cultures, we could achieve a high yield (70-80%) of neurons positive for tyrosine hydroxylase (TH) in immunostaining and we demonstrate the expression of forkhead box protein A2 (FoxA2) and G protein-activated inward rectifier potassium channel 2 (GIRK2) in immunoblotting. Furthermore, we showed the electrical excitability of those cells, and slow pacemaking activity, characteristic of DA neurons. In the PFF uptake assay, we report that 90-100% of 3-4 weeks old neurons take up the fibrils.

Conclusion: Altogether, iPSC-derived DA neurons provide us with a novel model to study the mechanisms underlying Parkinson's pathology, with alpha-synuclein propagation being the first pathway examined in this system.

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Title: Apoptotic Cell Signaling in Prostate Stem Cell Stem Homeostasis

Author: Sophie Viala, Mathieu Tremblay, Maxwell Shafer and Maxime Bouchard

Laboratory Director: Dr Maxime Bouchard

Prostate cancer (PCa) is the most commonly diagnosed cancer in Canadian men. One of the standard treatments for advanced cases of the disease is androgen deprivation therapy (ADT), which essentially blocks the supply of testosterone to the prostate. As most prostate cells are androgen-dependent, this leads to cell death and PCa regression. Although ADT is initially effective, a recurring and more aggressive form of PCa eventually develops. Its growth is thought to be driven by cancer stem-like cells that are resistant to ADT (ie castration). Normal prostate stem cells are also resistant to castration, but it is unclear what mechanisms confer castration resistance to those cells. Castration resistance could be either an intrinsic property of stem cells or be induced by extrinsic signals. We hypothesize that apoptotic cell signaling influences stem cell survival during castration. Signaling by apoptotic cells has been shown to influence tissue homeostasis and processes such as cell death, survival and proliferation in both normal and cancerous contexts. Phosphatidylserine (PS), a membrane protein that flips from the cytosolic side of the plasma membrane to the extracellular space during apoptosis, is one of the main mediators of apoptotic signaling. To test our hypothesis, PS will be blocked during castration to prevent apoptotic signaling. Stem cell numbers will be evaluated using FACS and sphere-forming assays. Lineage-tracing experiments will be performed using mouse lines that specifically target luminal or basal cells to assess stem cell activity. Our preliminary results show that blocking PS during castration decreases basal cell survival, a lineage known to harbour a stem cell population. Comprehending how apoptotic signaling can activate survival pathways will be a crucial step in understanding the acquisition of castration resistance by stem cells. These results will aid the development of targeted therapies to prevent PCa recurrence.

McGill's SCRM Stem Cell Regenerative Medicine Network

Title: Elucidating the effects of substrate curvature in pancreogenesis

Authors: Raymond Tran, Si Da Ling, Christopher Moraes, Corinne A. Hoesli

Laboratory Director: Dr. Corinne Hoesli / Dr. Christopher Moraes

Background: Cellular therapies targeting degenerative diseases, like type 1 diabetes, require an economically feasible cell source to reach widespread adoption. Pancreatic beta cells can be produced from human induced pluripotent stem cells (iPSCs) via timed addition of soluble factors. However, these protocols are inefficient, yielding ~10% beta cells with dysfunctional glucose sensing abilities. In these systems, developmental-associated biophysical forces occurring *in vivo* are not recapitulated. Cellular endogenous tension and biochemical cues synergistically regulate cellular programs such as proliferation, differentiation, and morphogenesis. Pancreogenesis is a complex process where pancreatic progenitor cells differentiate concurrently with rearrangement of surrounding tissue. We hypothesize that endogenous tension within pancreatic progenitor cells induced by culture on a curved substrate will affect downstream lineage specification.

Methods: Human iPSCs were differentiated to produce pancreatic progenitor cells. To recapitulate developmental geometry, curved microchannels were fabricated by using SU-8 mold lithography with radii of curvature ranging from 50 – 2000 microns. Viscous finger patterning was used to create cylindrical hydrogel channels of 100 micron diameter. To isolate effects of endogenous tension, cells were spatially confined via microcontact printing of extracellular matrix proteins. Human iPSC-derived pancreatic progenitor cells were then cultured on these curved surfaces to determine the effect on differentiation into pancreatic and other endodermal lineages.

Results: The differentiation protocol yielded 72.6% $Sox17^{+}/Foxa2^{+}$ cells at the definitive endoderm stage and 10% $Pdx1^{+}$ at the posterior foregut stage. Tightly curved cylindrical microchannels were successfully fabricated according to design with no defects. In future experiments, pancreatic progenitors will be loaded into the microchannel at confluency and monitored for 3 days to quantify the effect of substrate curvature on lineage specification.

Conclusions: The work in this study provides a framework to understand the effects of endogenous tension in development. This fundamental research can inform design of novel bioreactors to efficiently manufacture cellular therapy products.

McGill's SCRM Stem Cell Regenerative Medicine Network

Title: Control of embryonic stem cell self-renewal and differentiation via coordinated alternative splicing and translation of YY2

Authora: Soroush Tahmasebi[1,2,3], Seyed Mehdi Jafarnejad[1,2,3], Ingrid S. Tam[2,3], Thomas Gonatopoulos-Pournatzis[4], Edna Matta-Camacho[2,3], Yoshinori Tsukumo[2,3,13], Akiko Yanagiya[2,3], Wencheng Li[5], Yaser Atlasi[6], Maxime Caron[7,8], Ulrich Braunschweig[4], Dana Pearl[2,3], Arkady Khoutorsky[9], Christos G. Gkogkas[10], Robert Nadon[7,8], Guillaume Bourque[7,8], Xiang-Jiao Yang[2,3,11], Bin Tian[5], Hendrik G. Stunnenberg[6], Yojiro Yamanaka[2,7], Benjamin J. Blencowe[4,12], Vincent Giguère[2,3,11] and Nahum Sonenberg[2,3,*]

Laboratory Director: Dr. Nahum Sonenberg

Translational control of gene expression plays a key role during early phases of embryonic development. Here we describe a transcriptional regulator of mouse embryonic stem cells (mESCs), Yin-Yang 2 (YY2) that is controlled by the translation inhibitors, 4E-BPs. YY2 plays a critical role in regulating mESC functions through control of key pluripotency factors, including *Oct4* and *Esrrb*. Importantly, overexpression of YY2 directs the differentiation of mESCs into cardiovascular lineages. We show that the splicing regulator PTBP1 promotes the retention of an intron in the 5´ UTR of *Yy2* mRNA, which confers sensitivity to 4E-BP-mediated translational suppression. Thus, we conclude that YY2 is a major regulator of mESC self-renewal and lineage commitment, and document a multilayer regulatory mechanism that controls its expression.

McGill's SCRM Stem Cell Regenerative Medicine Network

Title: Arginine methylation by PRMT1 regulates muscle stem cell fate

Author: Roméo S. Blanc , Dr. Xing Li , Dr. Shawn Li and Dr. Stéphane Richard

Laboratory Director: Dr. Stéphane Richard

Quiescent muscle stem cells (MSC) become activated in response to skeletal muscle injury to initiate regeneration. Activated MSCs proliferate and differentiate to repair damaged fibers or self-renew to maintain the pool and insure future regeneration. The balance between self-renewal, proliferation and differentiation is a tightly regulated process controlled by a genetic cascade involving transcription factors such as Pax7, Myf5, MyoD, and MyoG. Recently there have been several reports about the role of arginine methylation as a requirement for epigenetic-mediated control of muscle regeneration. Herein we report that the protein arginine methyltransferase 1 (PRMT1) is expressed in MSCs and that conditional ablation of *PRMT1* in MSCs causes impairment of muscle regeneration. Importantly, PRMT1-deficient MSCs have enhanced-cell proliferation after injury, but are unable to terminate the myogenic differentiation program, leading to regeneration failure. We identify the co-activator of Six1, Eya1 as a PRMT1 substrate. We show that PRMT1 methylates Eya1 and loss of PRMT1 *in vivo* prevents Eya1 methylation. Moreover, we observe that PRMT1-deficient MSCs have reduced expression of Eya1/Six1 targets *MyoD* due to disruption of Eya1 recruitment at *MyoD* promoter and subsequent Eya1-mediated co-activation. These findings suggest that arginine methylation by PRMT1 regulates muscle stem cell fate through the Eya1/Six1/MyoD axis.

McGill's SCRM Stem Cell Regenerative Medicine Network

Title: miR-378a is a myotome-specific modulator of early myogenesis

Author: Nicholas Anthony

Laboratory Director: Dr Colin Crist

Background: The vast majority of all skeletal muscle in the mouse and human arises from a population of epithelial cells called the dermomyotome, which forms on the dorsal aspect of the somites following neurulation. The dermomyotome, marked by the expression of Pax3, delaminates shortly after formation, creating the myotome, a myogenic mesenchyme within the somite. The myotome is marked by downregulation of Pax3 and upregulation of Myf5, which leads to upregulation of MyoD and MyoG, considered to be markers of myogenic specification. Critical to proper myogenic differentiation are short, non-coding RNAs called microRNAs (miRNAs), which modulate post-transcriptional gene expression by destabilizing transcripts to which they bear partial complementarity. Many such miRNAs exist in introns of protein-coding genes, and show similar expression patterns to the host transcript. Here, we look earlier in the myogenic lineage than previous studies to investigate the role of miRNA-mediated gene silencing in embryonic myogenesis.

Methods: Generated *Pax3*$^{GFP/+}$;*Myf5*$^{Cre/+}$;*R26*$^{TdTomato/+}$ mouse embryos that fluorescently label the dermomyotome and myotome. Dissected interlimb somites and sorted cells via FACS. Extracted RNA from fluorescent populations for interrogation by qRT-PCR. Computationally predicted the presence of Pax3-regulated miRNAs in the dermomyotome using expression data from Pax3 gain-of-function studies at E10.5. Identified somite-specific candidates by *in situ* hybridization. Tested impact of candidate miRNAs on myogenesis in somite explant and C2C12 cultures using fluorescence microscopy.

Results: miR-378a expression in the somites is restricted to the myotome at 10.5.Overexpression of miR-378a in somite explants delays myogenic differentiation. Overexpression of miR-378a in proliferating C2C12 myoblasts reduces MyoD expression

Conclusions: miR-378a acts in the mouse myotome to modulate progression through the myogenic program. Whether it accomplishes this by direct interactions with myogenic determination factors, by inhibiting differentiation, or by promoting alternative cell fates remains to be tested.

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Title: Senescence alters the stress response in cellular models of aging

Authors: O. Moujaber, M. Kodiha, K. Bednarz, I. Colmegna, U. Stochaj

Laboratory Director: Dr. U. Stochaj

Background: The proper response to stress is crucial for the survival of physiological and environmental insults. Aging impairs the stress response, but the underlying mechanisms are not fully understood. We are addressing these mechanisms in different cellular models. First, we selected kidney cells, because the kidney is particularly prone to aging-related functional decline. Second, we examine mesenchymal stem cells (MSCs), because they have remarkable potential for health-related applications. These include, but are not limited to, regenerative medicine. The potential of MSCs is restricted by their aging-associated decline of cellular homeostasis. As stem cells are continuously exposed to oxidative stress, their ability to respond to oxidants is critical for MSC-based therapies.

Methods: The formation of cytoplasmic stress granules (SGs) is a conserved response to various forms of stress. I have used aging cultured kidney cells (LLC-PK1) to analyze SG formation in a quantitative fashion. Senescence was induced with (a) chemical or (b) pharmacological compounds. Based on my results with LLC-PK1 cells, I have extended these studies to MSCs.

Results: LLC-PK1 cells were aged successfully *in vitro*, and they display specific hallmarks of aging. This is exemplified by irreversible loss of cell proliferation, increased cell size and nuclear dysmorphy. When exposed to oxidative stress, SG formation is compromised during aging. We identified two mechanisms that contribute to these aging-associated changes: (1) depletion of factors required for SG biogenesis, and (2) altered cell signaling.

Conclusions: Our work identified new biomarkers that can be used to score cellular homeostasis during aging. In the long-term, this panel of biomarkers will be applied to monitor the performance of MSCs under stress. The use of MSCs for medical applications is currently limited by their aging-associated functional decline. I expect that my results will provide measurable parameters that evaluate the quality of MSCs for therapeutic intervention.

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Title: Pre-Conditioned Mesenchymal Stem Cells for Kidney Repair

Authors: Malak Ismail, Jing Zhao, Jessica Cuerquis, Kathy-Ann Forner, Julie Fradette and Nicoletta Eliopoulos

Laboratory Director: Dr. Nicoletta Eliopoulos

Background: Mesenchymal stem cells are a potential treatment for various diseases, such as acute kidney injury (AKI). Their ability to home to the injury site and their anti-apoptotic and anti-inflammatory effects on damaged tissue make them valuable tools. However, they are limited by their survival *in vivo*. To increase it, we tested cell pre-conditioning using melatonin, a hormone with anti-oxidative and anti-apoptotic properties. Our *in vitro* study revealed that pre-conditioning of human adipose tissue-derived mesenchymal stem cells (hASCs) improved their *in vitro* survival, as well as their protective and anti-apoptotic effects on human kidney cells *in vitro*. Therefore, we hypothesize that melatonin-preconditioned hASCs will replicate these effects in a mouse model of AKI.

Methods: hASCs will be preconditioned with melatonin 100µM for 3h. AKI will be chemically-induced in male NOD-SCID mice using cisplatin. Each group will receive a unique treatment: unconditioned ASCs, melatonin-conditioned ASCs, or their respective concentrated conditioned media, all injected intraperitoneally. Cell concentration will be optimized. Blood urea nitrogen (BUN) analysis will determine kidney function. Mouse survival will also be assessed. Furthermore, *in vivo* cell imaging will enable post-implantation tracking.

Results: In our AKI model, BUN levels increased at day 5 after cisplatin injection, indicating a decrease in kidney function. The treatment with 14 million unconditioned hASCs/animal decreased BUN levels suggesting improved renal function as compared to AKI mice that did not receive hASCs. Next, we will evaluate the preconditioning effect on hASCs *in vivo*. We expect the treatment with melatonin-preconditioned hASCs to be even more beneficial. Moreover, we were able to produce luciferase bioluminescent hASCs (Luc+ hASCs) that will be used for cell tracking studies *in vivo*.

Conclusions: Based on preliminary results, we expect an increase in the beneficial role of hASCs when preconditioned with melatonin, in an AKI mouse model.

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Title: GATA3 Controls Self Renewal in the Prostate

Author: Mathieu Tremblay, Maxwell Shafer, Alana H.T. Nguyen, Sophie Viala, Katharina Haigh, Ismaël Hervé Koumakpayi, Marilène Paquet, Pier Paolo Pandolfi, Anne-Marie Mes-Masson, Fred Saad, Jody J. Haigh, Maxime Bouchard

Laboratory Director: Dr. Maxime Bouchard

Loss of the tumor suppressor PTEN is a common occurrence in prostate cancer. Here, we show that the transcription factor Gata3 is progressively lost in Pten-deficient mouse prostate tumors. Moreover, 75% of the more aggressive hormone-resistant human prostate tumors show loss of active GATA3. Using a genetic approach, we found that the enforced expression of GATA3 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 GATA3 in normal prostate stem cells enhanced their long term self-renewal capacities and is associated with a transcriptional change in expression of self-renewal genes. Together, these data establish Gata3 as an important regulator of prostate cancer progression revealing a role for Gata3 in prostate stem cell homeostasis.

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Title: Phosphatase of Regenerating Liver enzymes in cardiac specification

Author: Tzvetena Hristova[1,2], Teri Hatzihristidis[1,3], Jacinthe Sirois[1], Noriko Uetani[1] and Michel L. Tremblay[1,2,3]

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Laboratory Director: Dr. Michel Tremblay

The three Phosphatase of Regenerative Liver (PRL-1, -2, -3) enzymes represent a group of protein tyrosine phosphatases that has been implicated in a number of diseases, and largely studied in the context of cancer metastasis. However, little is known about their physiological function. We previously showed that PRL-2 plays a key role in cancer progression by modulating intracellular magnesium levels and other groups showed an additional role in the proliferation of hematopoietic stem cells. While PRL-1 and -2 are ubiquitously expressed, PRL-3 is found predominantly in cardiac tissue. Characterization of the PRL-2 knockout mouse indicates that this phosphatase is involved in development. Therefore, it is likely that the other PRLs also play a role in such processes. We are using a CRISPR/Cas9 genome engineering strategy to knockout PRL members in murine embryonic stem cells to study the physiological function of the PRLs in development *in vitro*. Defining the developmental role of this phosphatase family would not only provide a greater understanding of the physiological processes governed by these, but could also contribute insight into potential pathological mechanisms.

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Title: BMP signaling maintains a mesodermal progenitor state in the presomitic mesoderm

Authors: Sharma, R., Shafer M., Bareke E. , Majewski J., Bouchard, M.

Laboratory Director: Dr. Maxime Bouchard

The presomitic mesoderm (PSM) is a mesenchymal tissue made of muscle/bone progenitor cells, that is modulated by signals from the Wnts, Fgfs and Retinoic acid (RA) pathways. These pathways exhibit a graded distribution along the anterior-posterior axis during development of the PSM and the somites. It is unclear how the molecular network is maintained in the PSM progenitor cells. We developed a primary culture system, wherein we isolate PSM cells from E9.5 mouse embyros. These cells differentiate in culture and lose the expression of PSM markers. Using a candidate approach, we found that treatment with Bmp4 can cause them to restore their original PSM fate. Conversely, sustained Bmp4 in newly sorted PSM progenitor cells is able to maintain their undifferentiated state. Expression profiling by RNA sequencing suggests that treatment with Bmp4 modulates most of the known PSM and paraxial mesoderm molecular network. As Bmp4 is not expressed in PSM cells but in the underlying tissues (lateral plate mesoderm), these results suggest that Bmp4 sustains the PSM progenitor fate during development.