McGill's SCRM Stem Cell Regenerative Medicine Network

Fall 2017 Symposium Program

Location: McGill University Health Centre - Research Institute Auditorium
1001 Decarie Blv Montreal H4A 3J1 Room E1.

8:00	**Registration**	
8:30	**Welcome**	Linda Peltier PhD, Chair

CLINICAL RESEARCH (AUDITORIUM)

	Author	Title
	ABBA C. ZUBAIR – KEYNOTE SPEAKER	
8:45	**"MESENCHYMAL STEM CELL (MSC) THERAPY IN PATIENTS WITH CHRONIC LUNG ALLOGRAFT DYSFUNCTION (CLAD)"**	
9:30	**TED TALKS – CLINICAL RESEARCH** (5 min+ 2 questions)	
1	Linda Peltier	*Vaccination of activated autologous dendritic cells from bench to bedside*
2	Rayan Fairag	*3D printed biodegradable scaffolds seeded with mesenchymal stem cells, a portintioal bone graft substitute*
3	Meaghan Boileau	*Leukemic stem cell expression signatures identify novel therapeutics for acute myeloid leukemia*
4	Kashif Khan	*Cytoprotective and proliferative impact of Yes-associated protein 1 (YAP1) to cardiomyocytes after ischemia-reperfusion injury*
5	Georges Makhoul	*Amniotic Stromal Cells in a Chitosan Smart Hydrogel: A Novel Composite that Promotes Cardiac Repair*
6	Seydina Babacar Touré	*Bridging Stem Cell-Based Research and Medicine: An Appraisal of a Learning Health System in Canada*
10:30	**Morning Break**	
10:45	**POSTER PRESENTATIONS (ATRIUM)**	
12:00	**LUNCH BOXES**	

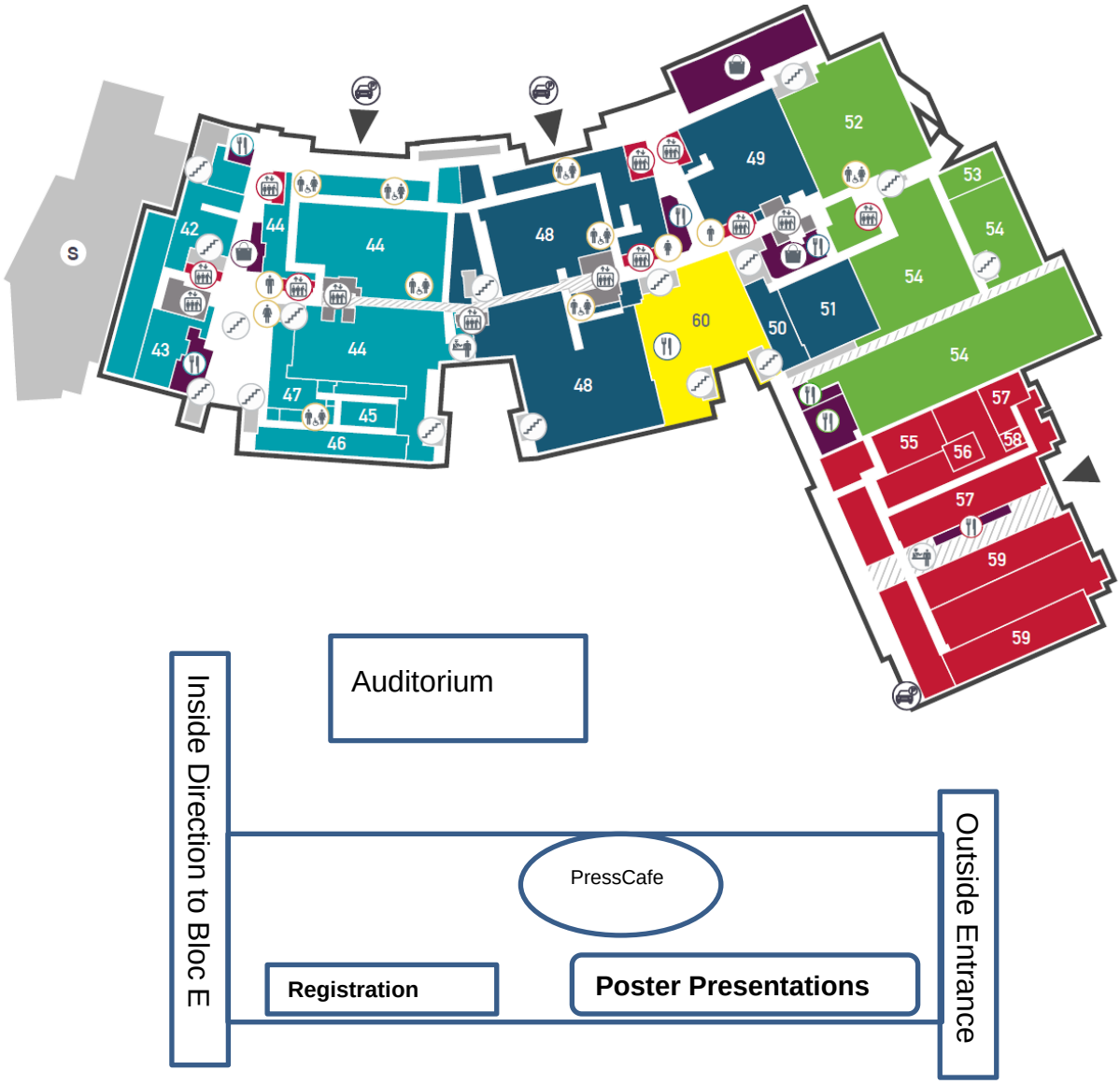
FUNDAMENTAL RESEARCH (AUDITORIUM)

	Julie Fradette - KEYNOTE SPEAKER	
13:00	**"ENGINEERING TISSUES USING ADIPOSE-DERIVED STEM CELLS: FROM 3D MODELS TO IN VIVO WOUND REPAIR"**	
13:45	**TED TALKS – FUNDAMENTAL RESEARCH** (5 min + 2 questions)	
1	Heather Duncan	*Characterization of extracellular vesicle transfer between leukemic stem cell and blast populations*
2	Ildi Troka	*Effect of menin early deletion in the osteoblast lineage in conditional knockout mice*
3	Graham Lean	*Ex vivo expansion of muscle stem cells with small compound inhibitors of eIF2a dephosphorylation.*
4	Ryo Fujita	*Selective mRNA translation mediated by phosphorylation of eIF2 regulates stemness of muscle stem cells*
5	Mathieu Tremblay	*A Gata3/Bmp5 axis regulates prostate stem cells*
6	William Pastor	*TFAP2C is a key regulator of transcription in the human naïve state*
14:45	Short afternoon Break – Jury will meet to discuss the Poster and Oral Presentation winner	
15:00	Poster and Oral Presentation Winner Nominations	
15:30	**PI'S ROUND TABLE ON THE SCRM NETWORK STRATEGIC PLAN**	
16:30	Closing and evaluation	Linda Peltier PhD, Chair

FOOD AND DRINKS ARE NOT ALLOWED IN THE AUDITORIUM – A 50$ FINE WILL BE GIVEN BY MUHC-RI

A B Hôpital de Montréal pour enfants / Montreal Children's Hospital
C D Hôpital Royal Victoria / Royal Victoria Hospital
D Centre du cancer des Cèdres / Cedars Cancer Centre
D Institut thoracique de Montréal / Montreal Chest Institute
E Institut de recherche du CUSM / Research Institute of the MUHC

1001 Décarie Blv Montreal H4A 3J1 Room E1



ORAL ABSTRACTS

Clinical Research #2:

3D PRINTED BIODEGRADABLE SCAFFOLDS SEEDED WITH MESENCHYMAL STEM CELLS, A PORTINTIOAL BONE GRAFT SUBSTITUTE

Authors: Rayan Fairag, Derek Rosenzweig and Lisbet Haglund

Orthopedic Research Laboratory, Department of Surgery, Faculty of Medicine, McGill University, Montreal, QC, Canada.

Abstract:

Background: Critical-sized bone defects represents a major challenge in orthopedic surgeries. Tissue engineering combines cells with biomaterials can play a major role in the concept of filling bone defects. This study aims to evaluate the ability of human mesenchymal stem cells (MSCs) to adhere, proliferate, and differentiate to osteogenic phenotype and form mineralized clusters on 3D-printed scaffolds comparing two deferent types of materials.

Methods: Cuboidal 3D scaffolds with square pore size of 750 µm were designed and fabricated using polylactic acid “PLA” and Lactoprene® 7415 using a low-cost desktop 3D printer. Scaffolds were seeded with 750,000 MSCs. Scaffolds were immersed with osteogenic differentiation media for 21 days. Cell adhesion and growth were monitored the whole period of experiment via bright field microscopy. Calcified matrix deposition was assessed histologically by alizarin red dye. Gene expression was analyzed using qPCR in different time points. Structural bone matrix protein was identified by western blot.

Results: Both PLA and Lactoprene scaffolds promoted dense layers of cell ingrowth and matrix-like tissue deposition. MSCs differentiated into osteogenic phenotype as of all scaffolds were positive for Alizarian Red which indicates calcium deposition and mineralization in both materials. Gene expression indicated osteogenic differentiation over various time points for both materials. Osteopontin protein was identified in western blot, indicating the presence of structural bone matrix.

Conclusion: Scaffolds exhibited good biocompatibility and bioactivity in vitro. MSCs adhere, proliferate and differentiate into osteogenic phenotype on both materials. Mechanical stiffness for these scaffolds is currently being tested and compared to bone strength. Furthermore, micro-CT scans can be used to give further morphometric characterization of these scaffolds. These 3D-printed scaffolds might be a promising candidate for bone defect repair and graft substitution.

Clinical Research #3:

Poster #9:

LEUKEMIC STEM CELL EXPRESSION SIGNATURES INDENTIFY NOVEL THERAPEUTICS FOR ACUTE MYLEOID LEUKEMIA

Authors: Meaghan Boileau[1], Isabelle Laverdière[1], Amanda Mitchell[2], Stanley W.K. Ng[3], Jean C.Y. Wang[2], Mark D. Minden[2], John E. Dick[2], Kolja Eppert[1]

[1]Research Institute of the McGill University Health Centre, McGill University, Montreal, QC, Canada; [2]Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada; [3]Department of Chemical Engineering and Applied Chemistry and Institute of Biomaterials and Biomedical Engineering and The Donnelly Centre, University of Toronto, Toronto, ON, Canada

Abstract:

Background: Acute myeloid leukemia (AML) is a hematological malignancy with a poor prognosis due to a high rate of relapse, which is thought to be the result of chemoresistant leukemic stem cells (LSCs). Therefore, a therapy would need to eradicate LSCs to obtain a durable response. We have established an LSC-specific gene expression signature that strongly correlates with low overall survival and poor response to chemotherapy in AML patients.

Hypothesis: We hypothesize that through analysis of LSC and normal hematopoietic stem cell (HSC) gene expression data we can identify bioactive compounds that specifically target LSCs in AML while sparing normal HSCs.

Methods: We probed LSC and HSC gene expression signatures against datasets of drug-gene interactions to identify compounds predicted to target LSCs without harming HSCs. Compounds were screened against a primary AML sample (8227) and viability and phenotype were assessed by flow cytometry. Candidate compounds from the screen were further tested against other primary AML and counter-screened against cord blood, a source of HSCs.

Results: We identified 152 compounds from the *in silico* analysis. 46 of 83 compounds screened decreased the viability of the 8227 AML cells or altered their phenotype. A class of steroids differentiated the 8227 AML cells and a class of ion pump inhibitors preferentially targeted the LSC and progenitor containing populations. We assessed the compounds against multiple primary AML samples and observed that at low nanomolar concentrations the steroids differentiated AML 9642 but did not affect AML 9706. When we tested the ion pump inhibitors, AML 184 was sensitive while AMLs 9642, 9706 and 116 were more resistant compared to AML 8227. The steroids and ion pump inhibitors had a limited toxicity against normal HSCs.

Conclusion: We have identified several possible LSC-specific compounds from our bioinformatic approach which may be repurposed into anti-LSC therapeutics.

Clinical Research #4:

Poster #19:

CYTOPROTECTIVE AND PROLIFERATIVE IMPACT OF Yes-ASSOCIIATED PROTEIN 1 (YAP1) TO CARDIOMYOCYTES AFTER ISCHEMIA-REPERFUSION INJURY

Authors: Kashif Khan, Georges Makhoul, Bin Yu, Adel Schwertani and Renzo Cecere

Abstract:

Introduction: Although adult cardiomyocytes are considered post-mitotic, evidence suggests that the heart displays some regenerative capabilities. In this regard, recent work has investigated the Hippo-signaling pathway, a mechanism implicated in cardiomyocyte cytoprotection and regeneration after a myocardial infarction (MI). Yes-associated protein 1 (YAP1) is its effector protein, binding to transcription factors in the nucleus to activate genes involved in cellular proliferation and survival. The exact mechanisms by which YAP1 protects the heart post-MI are currently unknown. Here, we propose that YAP1 plays a critical role in cardiomyocyte regeneration after simulated ischemia-reperfusion injury.

Methods: The AC16 human cardiomyocyte cell line was used in this study. These cells were infected with lentiviral constructs that contain genes that can over-express YAP1 (YAP1, and YAP1S127A) or silence it (YAP1-TEAD Inhibiting Peptide (YTIP)). To mimic the ischemia-reperfusion injury that occurs during a MI, the AC16 cells were exposed to hypoxia for 24 hours and re-perfused for 5 hours. Cellular viability, cytoprotection, and hypertrophy were assessed via Crystal Violet, TUNEL assay, and actin staining respectively. Changes in reactive oxygen species (ROS) levels were also assessed as well as protein and gene expression.

Results: The AC16 cardiomyocytes were successfully infected with lentiviral constructs containing GFP-fused YAP1, YAPS127A, or YTIP plasmids. Infected cells displayed significant GFP expression. Over-expressing YAP1 in the AC16 cells significantly increased cell viability following ischemia-reperfusion injury. Actin staining revealed significant reductions in hypertrophy in YAP1-over-expressing cells. After injury, the non-phosphorylated YAP1 protein expression was significantly upregulated in the YAP1-infected construct indicating a triggered proliferative phenotype.

Conclusion: Our findings indicate that over-expressing YAP1 in the AC16 cell line is a viable method to protect cardiomyocytes from ischemia following a MI and could be used to repair the distorted myocardium. Hence, pharmacological therapies targeting YAP1 may be a novel approach to reverse cardiomyocytes terminal differentiation and reduce remodeling. Future investigations will attempt to elucidate the underlying mechanisms that contribute to reductions in cellular hypertrophy and ROS generation through YAP1 over-expression.

Clinical Research #5:

Poster #29:

AMNIOTIC STROMAL CELLS IN A CHITOSAN SMART HYDROGEL: A NOVEL COMPOSITE THAT PROMOTES CARDIAC REPAIR

Authors: George Makhoul[1], B. Yu[2], G. Jalani[3], K. Khan[1], M. Cerruti[3], A. Schwertani[2] and R. Cecere[1]

[1]Department of Experimental Surgery; [2] Department of Experimental Medicine; [3]Department of Chemical Engineering, McGill University, Montreal, Canada

Abstract:

Background: Acute myocardial ischemia is frequently irreversible leading to heart failure and death. Experimental therapies using stem cells lack an ideal cell source and suffer from low cellular engraftment rates. Herein, we investigated the cardio-protective potential of a novel composite inserting human amniotic stromal cells (ASCs) in a chitosan/hyaluronic acid (C/HA) scaffold to increase retention rates and reverse heart degeneration.

Methods: A re-designed C/HA scaffold mixed with ASCs was synthesized and characterized. The cardiac impact of ASCs + C/HA composite was then assessed in an induced rat model of myocardial infarction using functional and histological analyses.

Results: Mechanical characterization of the C/HA platform indicated a swift elastic conversion at 40°C and a rapid sol-gel transition time at 37°C. Metabolic analysis assay presented an active and proliferating ASCs after 8 days within the C/HA hydrogel. *In vivo*, ASCs + C/HA injected hearts had significantly increased cardiac function compared to control hearts. Interestingly, the encapsulated ASCs were abundantly detected in the infarcted myocardium 6 weeks post-administration. These cells co-expressed several cardiac proteins and notably proliferative markers. Moreover, significant neo-vessel formation was evident in the myocardia injected with the ASCs + C/HA composite.

Conclusion: Despite a low injected cellular density, the encapsulated ASCs were abundantly retained and increased cardiac functional parameters. Moreover, this optimal C/HA smart hydrogel provided an active milieu for the ASCs to proliferate, co-express cardiac proteins, and induce new vessel formation. Thus, our novel composite of ASCs + C/HA scaffold is a conceivable candidate that could restore cardiac function and reduce remodeling.

Clinical Research #6:

BRIDGING STEM CELL-BASED RESEARCH AND MEDICINE: AN APPRAISAL OF A LEARNING HEALTH SYSTEM IN CANADA

Authors: Seydina Babacar Touré[1], E. Kleiderman[1], BM Knoppers[1]

[1] Centre of Genomics and Policy, McGill University Faculty of Law

Abstract:

As the stem cell tourism market continues to expand, more Canadians are willing to undergo unproven and unsafe stem cell-based therapies abroad. Stem cells do not systematically obey traditional phase I-IV clinical translation models. In response, various actors have suggested that progressive stem cell-based medical innovation policies instead could catalyze translation. Accordingly, calls were made to adopt more permissive approaches to stem cell translation. Yet, there remains within the scientific community an insistence on the phase I-IV paradigm. We argue that a learning health system may be able to reconcile these philosophies. Centered around patient-focused innovation, it will maximize patient retention and data follow-up in Canada, thereby promoting safe and efficient system learning and improvement.

Fundamental Research #1:

Poster #8:

CHARACTERIZATION OF EXTRACELLULAR VESICLE TRANSFER BETWEEN LEUKEMIC STEM CELL AND BLAST POPULATIONS

Authors: Heather M. Duncan[1,2], I. Laverdière[3], H. Frison and K. Eppert[2,4]

[1]Division of Experimental Medicine, McGill University, Montreal, Canada ; [2] Child Health and Human Development Program, Research Institute of the McGill University Health Center, Montreal, Canada; [3]Faculty of Pharmacy, Laval University, Québec City, Canada; [4]Department of Pediatrics, McGill University, Montreal, Canada

Abstract:

Background: In acute myeloid leukemia (AML), leukemic stem cells (LSCs) are resistant to therapy and lead to relapse. While evolving evidence demonstrates that extracellular vesicles (EVs) from leukemic cells promote disease progression and therapy resistance in AML, little is known about EV trafficking between populations of the AML hierarchy.

Methods: AML sample 8227 was used as a source of EVs. Previously functionally validated LSC and blast populations were sorted from 8227 by flow cytometry. Exosome-enriched EVs were isolated by ultracentrifugation. EVs were quantified by nanoparticle tracking analysis. Uptake of EVs labeled with PKH67 dye was confirmed by microscopy and quantified by flow cytometry. Mass spectrometry data was analyzed using FunRich software.

Results: LSC (CD34+/CD38-) and blast cells (CD34-) were sorted from AML sample 8227 following *in vitro* expansion. Blast cells were found to produce a greater concentration of EVs overall, particularly within the <200nm range characteristic of exosomes. 8227 cells preferentially took up exosome-enriched EVs isolated from 8227 cells versus control particles. Proteomic analysis revealed that compared to the cells of origin, 8227 EVs showed enrichment for proteins associated with extracellular, lysosome, and exosome cellular compartments, and for proteins involved in cell growth, maintenance, communication and signaling. We detected Kit ligand, not previously reported in EVs, which plays an essential role in regulation of cell proliferation, hematopoiesis, and stem cell maintenance.

Conclusion: 8227 cells exhibit selective uptake of exosome-enriched EVs of autologous origin, which are predominantly produced by the leukemic blast population. 8227 EVs are enriched for a number of proteins with known roles in directing cell growth and maintenance. Blast cells could support proliferation and self-renewal of LSC populations to maintain hierarchical organization in AML. Future studies will aim to determine the functional impact of EV uptake in various 8227 cell populations, and investigate EV transfer in primary AML.

Fundamental Research #2:

Poster #15:

EFFECT OF MENIN EARLY DELETION IN THE OSTEOBLAST LINEAGE IN CONDITIONAL KNOCKOUT MICE

Authors: Ildi Troka[1,2], Jad Abi-Rafeh[1,2], Lucie Canaff[2], and Geoffrey N. Hendy[1,2]

[1]Faculty of Medicine, Division of Experimental Medicine, McGill University, Montreal, Canada ; [2]Metabolic Disorders and Complications Program RI-MUHC, Montreal, Canada

Abstract:

Background: In humans, mutations in the MEN1 tumour suppressor gene cause the Multiple Endocrine Neoplasia Type 1 disorder. Menin, the product of the MEN1 gene, is predominantly a nuclear protein that also facilitates cell proliferation and differentiation control. Our previous *in vivo* study illustrated the importance of menin for proper functioning of mature osteoblasts and maintenance of bone mass in adult mice. In the present study, we examined the *in vivo* role of menin at earlier stages of the osteoblast lineage through 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 (MSC) and the preosteoblast, respectively.

Results: Body weight and femur length are reduced in knockout animals. Trabecular and cortical bone formation are also decreased in the earlier menin knockout mice models. 3-point bending test showed a significant reduction in femur stiffness and maximum load in adult *Prx1-Cre; Men1f/f* and *Osx-Cre; Men1f/f* mice. Proliferative capabilities of bone marrow derived MSCs from *Prx1-Cre; Men1f/f* animals are altered. Mineralization and differentiation of the primary calvarial osteoblasts in the knockout mice were deficient relative to those of wild-type mice as assessed by Alizarin red. Gene expression profiling of RNA extracted from the primary calvarial osteoblast revealed an increase in the RANKL/OPG ratio that would favor osteoclastogenesis in the knockout animals. This is consistent with ongoing *in vivo* histomorphometric analysis that also demonstrates an increase in osteoclast number and activity in the *Prx1-Cre; Men1f/f* and *Osx-Cre; Men1f/f* animals.

Conclusion: Osteoblast menin plays a crucial role in the development as well as maintenance of bone mass, and may serve as a potential gain-of-function therapeutic target for low bone mass disorders, such as osteoporosis.

Fundamental Research #3:

Poster #17:

EX VIVO EXPANSION OF MUSCLE STEM CELLS WITH SMALL COMPOUND INHIBITORS OF eIF2α DEPHOSPHORYLATION

Authors: Graham Lean, Solene Jamet, Ryo Fujita, Matt Halloran, Jean-Philip Lumb and Colin Crist

Abstract:

Regeneration of adult skeletal muscle depends on rare skeletal muscle stem cells (MuSCs) that normally reside in a quiescent state underneath the basal lamina of the myofibre. The study, manipulation and use of MuSCs for cell-based therapies is hindered by their scarcity and the inability to expand them *ex vivo* under normal culture conditions. We have shown that a general repression of translation, mediated by the phosphorylation of translation initiation factor eIF2 at serine 51 (P-eIF2α), is essential for maintenance of MuSC quiescence and self-renewal. MuSCs unable to phosphorylate eIF2 exit quiescence, activate the myogenic program and contribute to muscle differentiation, but do not self-renew or return to their quiescent state underneath the basal lamina of the myofibre. Pharmacological inhibition of eIF2 dephosphorylation by the small compound sal003 permits the expansion of MuSCs retaining capacity to regenerate muscle and self-renew after engraftment into a preclinical mouse model of Duchenne muscular dystrophy. Here we optimize sal003 in culture conditions to facilitate a) passaging of MuSCs retaining regenerative capacity and b) genome editing of MuSCs with CRISPR/Cas9 prior to their engraftment.

Fundamental Research #4:

Poster #18:

SELECTIVE mRNA TRANSLATION MEDIATED BY PHOSPHORYLATION OF eIF2α REGULATES STEMNESS OF MUSCLE STEM CELLS

Authors: Ryo Fujita, Graham Lean, Solene Jamet and Colin Crist

Department of Human Genetics, McGill University, and the Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, H3A 1B1, Canada

Abstract:

Muscle stem cells, also called satellite cells, are crucial for skeletal muscle regeneration after injury. Satellite cells reside as quiescent cells and quickly respond to amplify their progenitors that differentiate to myofibers, or self-renew to preserve their stem cell pool. Previously, we demonstrated that phosphorylation of eIF2α mediates the quiescence and self-renewal of satellite cells, however the mechanisms by which eIF2α phosphorylation regulates these stemness properties of satellite cells are not clear. Although eIF2α phosphorylation globally suppresses protein synthesis, some mRNAs are selectively translated by virtue of their upstream open reading frames (uORFs) in their 5' untranslated regions (UTRs). We therefore hypothesized that selectively translated mRNAs play a role in regulating quiescence and self-renewal of satellite cells when eIF2α is phosphorylated. By combining RNA-seq with iTRAQ proteomics, we identify several genes regulated at the level of protein synthesis in satellite cells cultured in the presence of sal003, a small molecule inhibitor of the eIF2α phosphatase GADD34/PP1, which permits the ex vivo expansion of satellite cells retaining regenerative capacity. We further investigated whether the mRNAs for these proteins are selectively translated by eIF2α, and whether their inhibition affects satellite cell quiescence, self-renewal or entry into the myogenic program. We show that the inhibition of one selectively translated candidate, transforming acidic coiled coil-containing protein 3 (TACC3) decreased Pax7+ and Myogenin+ number in the presence of sal003. We propose a model in which selectively translated mRNAs, mediated by phosphorylation of eIF2α, maintains the quiescence and self-renew of muscle stem cells.

Fundamental Research #5:

A Gata2/Bmp5 AXIS REGULATES PROSTATE STEM CELLS

Authors: Mathieu Tremblay, Maxwell Shafer, Alana H.T. Nguyen, Sophie Viala and Maxime Bouchard

Goodman Cancer Research Centre and Department of Biochemistry, McGill University, Montreal, Canada

Abstract:

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 both in vitro as well as stem cell frequency in vivo. Using RNAseq, we found that TGFbeta signaling is important for prostate self-renewal and loss of GATA3 is specifically associated with the transcriptional up-regulation of Bmp5 in an autocrine fashion. In addition, we showed that treatment of normal cells with Bmp5 increased their stem cell potential. Together, these data establish Gata3 as an important regulator of prostate cancer progression revealing a role for Gata3 in prostate stem cell homeostasis through a Bmp5 dependent mechanism.

Fundamental Research #6:

TFAP2C IS A KEY REGULATOR IF TRANSCRIPTION IN THE HUMAN NAÏVE STATE

Authors: William Pastor

Abstract:

Naïve human embryonic stem cells (hESCs) largely recapitulate the transcriptional state of pre-implantation epiblast. In contrast, primed hESCs more closely resemble post-implantation epiblast. Therefore, naïve and primed hESCs constitute a developmental model for understanding the earliest pluripotent stages in human embryo development. To identify new transcription factors that differentially regulate the human naïve and primed pluripotent states, we mapped open chromatin using ATAC-Seq and found enrichment of the AP2 transcription factor binding motif at naïve-specific open chromatin. We determined that the AP2 family member TFAP2C is upregulated in naïve state and is widespread at naïve-specific enhancers. Use CRISPR/Cas9, we found that TFAP2C is essential for establishment of the naïve state in hESCs. TFAP2C specifically functions by facilitating the opening of enhancers proximal to key pluripotency factors, including a previously undiscovered human naïve-specific OCT4 enhancer. Finally, we confirmed that the vast majority of TFAP2C^{+} naïve-state enhancers are present in human pre-implantation epiblast, supporting the relevance of this finding to human development. Taken together, our work supports a role for TFAP2C in establishing the naïve pluripotent state *in vitro* and *in vivo*.

POSTER ABSTRACTS

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8	Heather M. Duncan	Characterization of extracellular vesicle transfer between leukemic stem cell and blast populations	p. 8
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Poster #1:

HUMAN IPSC NEURONS AS A TOOL TO STUDY PARKINSON'S DISEASE

Authors: Fréderique Larroquette, E. A. Fon

Abstract:

The characteristic hallmarks of Parkinson's disease (PD) are a loss of dopaminergic neurons (DNs) in the substantia nigra pars compacta, accompanied by the presence of inclusions within neurons termed "Lewy bodies". The primary component of these inclusions is α-synuclein (α-syn), a PD-associated protein with a propensity to misfold and form fibrillar aggregates. The cellular consequences of α-syn mutation or aggregation are still unclear. The degeneration of DNs in PD has been related to mitochondrial dysfunction, and these neurons were also shown to have a specific physiological phenotype, which could render them more vulnerable to PD stressors. However, whether and how these distinct cellular pathways come together in a unifying selective degenerating process is still very much unknown. In our study, we generate human dopaminergic neurons from induced pluripotent stem cells (iPSC), either obtained from healthy individuals or PD patients bearing a mutation in the gene coding for α-syn. We then study both the mitochondrial phenotype and electrical activity of these neurons, and we take advantage of the use of microfluidics devices to facilitate our analysis.

Poster #2:

GENERATION OF HUMAN MIDBRAIN-LIKE ORGANOIDS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS TO STUDY PARKINSON'S DISEASE

Authors: Meghna Mathur, N. V. Mohamed, T. M. Durcan and E. A. Fon

Neurodegenerative Diseases Group and iPSC-CRISPR Core Facility, Montreal Neurological Institute, McGill University, Montreal, Canada

Abstract:

Parkinson's disease (PD) is characterised by progressive degeneration of dopaminergic neurons of substantia nigra in the midbrain region. Genetic factors like triplication in the alpha-synuclein (α-syn) gene SNCA is one of the greatest risk factors in the development of PD. However, the complex progression of PD still lacks sufficient research. It has been reported that more than 90% of drugs developed in mouse models that pass through preclinical research and development stage, fail to achieve the desired efficacy required in subsequent clinical trials that might be due to differences that exist between mice and humans. Therefore, our working hypothesis is that having controlled physical and chemical parameters can help generate the closest system analogue of human midbrain, called midbrain organoids, from induced pluripotent stem cells (iPSCs). In this study, organoids were generated from healthy individuals (NCRM-1) iPSCs and PD patients' (SNCA-PD) iPSCs- carrying SNCA triplication. The identity of organoids was identified as midbrain by treating 35 days organoids with 100µM L-dopa and dopamine for 10 days, to induce neuromelanin granules formation, which are characteristic pigments of human midbrain. Fontana-Masson staining confirmed the presence of neuromelanin pigments in the organoids. The organoids undergo differentiation and contain distinct layers of neuronal cells expressing different midbrain markers at specific maturation stages. We assessed neuronal differentiation into midbrain dopaminergic neurons by immunofluorescence and immunohistochemistry. MAP2+ (mature neurons), TH (tyrosine hydrolase for dopaminergic neurons) and DAT (dopamine transporter) positive cells showed that the organoids self-organize into a complex, spatially patterned neuronal tissue of midbrain region. In conclusion, this study has led to a successful generation of midbrain organoids from control and SNCA triplication iPSC lines, which will contribute to investigate PD pathogenesis and therefore help develop personalized drugs.

Poster #3:

MITOCHONDRIAL-DERIVED VESICLES IN NEURONS: IMPLICATIONS FOR MITOCHONDRIAL QUALITY-CONTROL AND PARKINSON'S DISEASE

Authors: Rosalind F. Roberts, T. M. Durcan and E. A. Fon

Centre for Neurodegenerative Disease, Montreal Neurological Institute, McGill University, Montreal, Canada

Abstract:

Background: Mitochondrial quality control (QC) mechanisms have evolved to ensure the maintenance of a healthy mitochondrial population, which is essential for normal neuronal function. PINK1 and Parkin, which are mutated in autosomal recessive forms of Parkinson's disease (PD), mediate the removal of damaged mitochondria by autophagy (mitophagy). Our lab recently demonstrated that PINK1 and Parkin regulate a distinct mitochondrial QC pathway, the generation of a subtype of mitochondrial derived vesicles (MDVs) in response to oxidative stress. PINK1/Parkin-dependent MDVs shuttle damaged mitochondrial cargo to the lysosome for degradation. We hypothesize that MDVs are crucial for neuronal health and are the first line of mitochondrial QC, eliminating damaged mitochondrial components before sufficient damage accrues to induce mitophagy. However, MDVs have not yet been studied in neurons. Utilizing human induced pluripotent stem cells (iPSCs) to differentiate neurons, we undertook to study MDVs for the first time in a neuronal system.

Methods: Neurons of different lineages were differentiated from iPSCs using small molecules or the over-expression of a neuronal transcription factor (NGN2) by lentiviral delivery. Neurons were treated with antimycin A to induce MDV formation and stained for mitochondrial markers to allow MDV identification by microscopy.

Results: Preliminary data indicate that MDVs are formed basally in cultures of different neuronal types (dopaminergic, cortical and motor neuronal cultures). In line with previous data from immortal cell lines, MDV production is stimulated by oxidative stress and increased mitochondrial respiration.

Conclusion: We have demonstrated for the first time the presence of PINK1-Parkin dependent MDVs in neurons. Future investigations will focus on dissecting the MDV pathway in neurons and using iPSCs derived from patients with PD to study whether the pathway is abrogated in diseased neurons. This work will provide new insights into the role of MDVs in mitochondrial QC in neurons and in PD.

Poster #4:

AN ISOGENIC SERIES OF METHYLTRANSERASE MUTATIONS FOR THE STUDY OF HUMAN NEURODEVELOPMENTAL DISEASES

Authors: Malvin Jefri, Huashan Peng and Carl Ernst

Abstract:

Gene expression is explicitly programmed in specific spatiotemporal patterns and is dynamically modulated throughout neuron differentiation from a progenitor cell state. While much is known about some important genes in neurodevelopment, the integration of how, when, and where gene expression is tuned during human brain development has yet to be elucidated. To address this question, we are deleting eight genes that code for histone demethylases or histone methyl transferases that are associated with intellectual disability, because genes regulated by these factors are likely critical in normal human brain development. Mutations in histone modifier genes are known to cause robust modifications in global gene expression patterns and may lead to similar transcriptional effects, and similarly dysregulated genes common across two or more histone modifies may point to gene expression levels necessary for normal development. For instance, a mutation in either KMT2D (lysine methyltransferase) or KDM6A (lysine demethylase) causes Kabuki Syndrome - a very particular clustering of clinical features on the autism spectrum. This supports the idea that histone modifiers work together to regulate gene expression and some nodes of this network may be common mutations in different genes. We will study this convergence using our previously developed method to rapidly produce induced pluripotent stem cells through simultaneous reprogramming and CRISPR/Cas9 gene editing to create isogenic heterozygous and homozygous knock-out models of histone modifier deficiency disorders and investigate their gene expression patterns using RNAseq at four different developmental stages (iPSC, NPC, and mature neuron). Results from this study may identify a neurodevelopmental program that controls fundamental genes required for neurodevelopment in humans.

Poster #5:

INVESTIGATING EPIGENETIC MEMORY AS A PROOF-OF-PRINCIPLE FOR URINE-DERIVED NEURAL STEM CELLS

Authors: Nuwan Hettige[1,2], Huashan Peng[2], Gary Chen[2], Hanrong Wu[2], Carl Ernst[1,2]

1. Department of Human Genetics, McGill University, Montreal, QC, Canada.
2. Psychiatric Genetics Group, Douglas Hospital Research Institute, Montreal, QC, Canada.

Abstract:

Background: Cells in mature organisms are highly specialized with a unique molecular pattern. Cell commitment and differentiation are largely determined by stable epigenetic marks such as DNA methylation. Stem cell reprogramming requires the erasure of existing epigenetic marks and the establishment of new cell type-specific marks. Previous evidence, however, suggests that these original epigenetic marks may persist as an 'epigenetic memory' in the new cell type. Here, we compare urine- and skin-derived iPSCs and neural stem cells (NSCs) to assess whether their epigenetic profiles are indistinguishable and therefore interchangeable as a source for iPSCs.

Methods: Reduced Representation Bisulfite Sequencing data were accessed from the ENCODE project for fibroblast (n=3) and renal epithelial (n=3) cells. Differentially methylated regions (DMRs) were identified between the cell types to indicate epigenetic marks for skin and kidney cells. Genes with promoters that overlapped highly significant DMRs were assessed for their expected expression between the two cell types (i.e. GTEx) according to their epigenetic profiles and validated using qPCR. Furthermore, we reprogrammed urine-derived iPSCs to NSCs from a patient with FOXG1 syndrome and a healthy control to measure FOXG1 expression to demonstrate efficient reprogramming.

Results: The expression of genes overlapping with significant DMRs conformed to their expected methylation pattern. A gene overlapping a DMR that was more hyper-methylated in skin, for example, showed greater gene expression in kidney than skin. FOXG1 expression was drastically more expressed in NSCs compared to iPSCs and renal epithelial cells in the healthy control, and expectedly reduced in the patient NSCs – demonstrating an expression pattern consistent with cell type and disease state.

Conclusion: Our results suggest that renal epithelial cells derived from urine act as a valid approach for deriving and pooling NSCs, particularly when dealing with limited sample sizes (i.e. rare neurodevelopmental disorders) where fibroblasts may not be readily available.

Poster #7:

LINEAGE SPECIFICATION FROM PROSTATE PROGENITOR CELLS REQUIRES GATA3-DEPENDENT MITOTIC SPINDLE ORIENTATION

Authors: M.E.R. Shafer[1,2], A.H.T. Nguyen[1], Mathieu Tremblay[1,2], Sophie Viala[1,2], M. Béland[1], N.R. Bertos[1], M. Park[1,2,3], Maxime Bouchard[1,2]

[1]Rosalind and Morris Goodman Cancer Research Centre, McGill University; [2]Department of Biochemistry, McGill University; [3]Departments of Medicine and Oncology, McGill University, Montreal, Canada

Abstract:

During prostate development, basal and luminal cell lineages are generated through symmetric and asymmetric divisions of bipotent basal cells. However, the extent to which spindle orientation controls division symmetry or cell fate, and the upstream factors regulating this process, are still elusive. We report that GATA3 is expressed in both prostate basal progenitor and luminal cells and that loss of GATA3 leads to a mis-localization of PRKCZ, resulting in mitotic spindle randomization during progenitor cell division. Inherently proliferative intermediate progenitor cells accumulate, leading to an expansion of the luminal compartment. These defects ultimately result in a loss of tissue polarity and defective branching morphogenesis. We further show that disrupting the interaction between PRKCZ and PARD6B is sufficient to recapitulate the spindle and cell lineage phenotypes. Collectively, these results identify a critical role for GATA3 in prostate lineage specification, and further highlight the importance of regulating spindle orientation for hierarchical cell lineage organization.

Poster #10:

IDENTIFICATION OF PAX3 PROXIMITY INTERACTORS DURING THE DIFFERENTIATION OF PLURIPOTENT STEM CELLS INTO THE MYOGENIC PROGRAM

Authors: Solene Jamet[1], V Tran[2], J-F Côté[2], and C Crist[1]

[1.] Department of Human Genetics, McGill University, Lady Davis Institute for Medical Research, Montréal; [2.] Institut de recherches cliniques de Montréal (IRCM), Montréal

Abstract:

Pax3 is a key factor regulating myogenic progenitor cells in the embryo and the adult. Pax3 is critical for maintaining and proliferating a population of myogenic precursor cells that migrate from the hypaxial domain of the dermomyotome, as well as a second population, coexpressing Pax7, that arise from the central dermomyotome and migrate into the myotome. Pax3 is a transcriptional activator in the context of myogenesis and a coordinated action of Pax3 with cofactors to activate transcription is likely. To identify Pax3 cofactors, we are taking advantage of the E. coli biotin ligase BirA, which permits purification of protein complexes based on the affinity of biotin to streptavidin. We propose to identify Pax3 interactors within the context of driving mouse embryonic stem (mES) cells into the myogenic program. We developed mES cells that express in a doxycycline (Dox) inducible manner a Pax3 fusion to a 'promiscuous' BirA (BirA-Pax3), designed to biotinylate interactors in a proximity-dependent manner. After driving mES cells into the myogenic program, expression of the BirA-Pax3 protein is synchronised with the expression of the endogenous Pax3. Then a streptavidin pull-down followed by an on beads trypsin digestion is performed and peptides are analysed by mass spectrometry. JMJD1C was identified as one of PAX3 proximity interactor by SAINT analysis and we are currently validating its expression at sites of myogenesis in the embryo.

Poster #11:

IDENTIFICATION OF MOLECULAR CHANGES DURING COMPACTION IN THE EARLY PREIMPLANTATION MOUSE EMBRYO

Authors: Deepak Saini[1,2], and Yojiro Yamanaka[1,2]

[1]Department of human Genetics, McGill University; [2]Goodman Cancer Research Center, McGill University, Montreal, Canada

Abstract:

Compaction is the first morphogenetic event at the 8-cell stage of the preimplantation embryo. Compaction is recognized as a morphological change, which increases the amount of cell-cell contact to minimize the cell surface area of an embryo. no molecular mechanisms have been elucidated to drive compaction.

To identify molecular changes occurring before and after compaction, we investigated the distribution pattern of cortical F-actin, a regulator of cells shape. Before compaction, we observed an even F-actin signal at both cell-cell contacts and non-contact surfaces, suggesting uniform distribution across the cell surface. However, after compaction, we observed the reduction of F-actin at cell-cell contacts, suggesting the generation of two cortical subdomains within a cell. We speculate that the F-actin amount on the cortex reflect cortical contractility and the reduction of F-actin at cell-cell contacts facilitates the flattening of cells.

To determine what regulates the reduction of F-actin, we investigated the distribution of E-cadherin, a calcium dependent adhesion molecule, and its associated catenins. Although E-cadherin and b-catenin were uniformly distributed on both cell-cell contacts and non-contact surfaces. We observed an increase of α-catenin at cell-cell contacts during compaction. Since homodimer forms of α-catenin can compete with ARP2/3, suppressing actin polymerization to reduce levels of F-actin at cell-cell contacts, we speculate that the increased signal of α-catenin reflects its homodimer conformation

In addition, we observed that the cadherin-catenin complex was localized on non-contact surfaces before compaction. To investigate if it has any role on non-contact surface, we analyzed E-cadherin null embryos. we found two phenotypes not related to cell adhesion functions of E-cadherin ;(1) precocious polarization at the 4-cell stage, (2) polarized 8-cell embryos without F-actin rings. These results suggest that E-cadherin has adhesion-independent roles on non-contact surface to negatively regulate polarization as well as regulating the actocytoskeletion.

Poster #12:

DETERMINATION OF MOUSE OVIDUCT EPITHELIAL HETEROGENEITY AND HOMEOSTASIS TO UNDERSTAND THE INITIATION OF HIGH-GRADE SEROUS OVARIAN CANCER

Authors: Matthew J. Ford[1], Dunarel Badescu[2], Yu Chang Wang[2], Nobuko Yamanaka1, Ioannis Ragoussi[2] and Yojiro Yamanaka[1]

[1]Goodman Cancer Research Center, Human Generics Department, McGill University; [2]Genome Quebec Innovation Centre, McGill University, Montreal, Canada

Abstract:

The oviduct or fallopian tube is a tube connecting the ovary to the uterus and is the site of fertilisation and early embryonic development. The luminal epithelium is currently considered to consist of terminally differentiated ciliated cells and a pluripotent Pax8+ secretory cell population capable of generating both secretory and ciliated cells. The presence of an adult stem cell population in the mouse oviduct has been implicated by the identification of label retaining and Lgr5+ cells but the generative capacity of these populations has not been confirmed. Recently the epithelium of the distal portion of the oviduct, also known as the fimbria, has been identified as the site of origin of high-grade serous ovarian cancer (HGSOC). However, currently our limited understanding of the cellular composition and homeostasis of the oviduct hinders our understanding of the early stages in HGSOC development. Preliminary data from our lab has identified uncharacterised heterogeneity within the two known cell populations. Specifically, we have identified a minor cell population labelled with the known stem cell marker Prom1. This cell population is located at the base of epithelial folds, is proliferative and has enriched organoid forming potential *in-vitro*. Taking an unbiased single cell RNA-sequencing approach we have also identified heterogeneity in the adult mouse oviduct epithelial cell population biased on their molecular signatures.

Poster #13:

DETERMINANTS OF RENAL PROGENITOR CELL RESPONSIVENESS TO THE INDUCTIVE WNT9B SIGNAL FROM URETERIC BUD

Authors: Kyle Dickinson[1], L. Hammond[1], T. Carroll[2], P. Goodyer[1]

[1]McGill University, Montreal, Canada; [2]UT Southwestern

Abstract:

Background: The Wnt-signalling pathway is essential for kidney development as fully primed renal progenitor cells (RPC) appear in the metanephric mesenchyme by E11.5 and initiate responsiveness to Wnt9b. However, the specific molecular components conferring responsiveness to RPCs have yet to be identified. To address this issue, we obtained M15 cells, derived from E10.5 metanephric mesenchyme and systematically analyzed Wnt receptor/signalling components required for a canonical Wnt response.

Methods: To measure activation of the canonical Wnt pathway, we transfected M15 cells with reporter plasmid, 8X TOPFlash, and measured luciferase activity using a GloMax luminometer. Exposing M15 cells to external Wnt9b resulted in minimal luciferase activity suggesting a signalling component is missing.

Results: Specificity of Wnt ligand binding is determined by the co-receptor complex, consisting of a Frizzled (Fzd) and Lipoprotein related receptor protein (Lrp). The receptor complex is stabilized by R-spondin1 and R-spondin3 (Rspo1 and Rspo3), thus, amplifying the Wnt signal. We analyzed M15 cells for components of the pathway and found mRNA expression of Fzd1-6, Lrp6 but neither Rspo1/3. To ascertain whether absence of R-spondin accounts for the lack of response, we transfected M15/TOPFlash cells with Wnt9b and added recombinant Rspo1 or Rspo3 and observed a 4.77-fold and 7.77-fold increase in luciferase activity, respectively. In the presence of Rspo1, we transfected the cells with Fzd1-10 and observed an additional 5-fold increase in the presence of Fzd5. Knockdown of Lrp6 with siRNA resulted in a 60% reduction in mRNA levels which was mirrored by a 60% reduction in luciferase activation.

Conclusion: These data suggest that early RPCs must acquire a specific receptor complex consisting of Fzd5, Lrp6 and Rspo1/3 to undergo an optimal B-catenin/TCF response to the inductive Wnt9b signal during nephrogenesis. We speculate that putative RPCs lacking these components are incompetent for primary nephrogenesis and/or regeneration of damaged adult kidneys.

Poster #14:

LESSONS LEARNED FROM MODELING DICER1 SYNDROME IN CELLS

Authors: Mona Wu, William Foules and Marc Fabian

Abstract:

Background: DICER1, an endoribonuclease is central to generating microRNAs (miRNAs), small RNA molecules that downregulate the expression of approximately 30% of protein-coding genes. DICER1 utilizes its RNase IIIa and IIIb endonuclease domains to cleave pre-miRNA stemloops to release the mature single-strand miRNA, coded within either the 5' (5p) or 3' (3p) arms. Germ-line mutations in *DICER1* have been identified in patients afflicted with a pleiotropic tumour predisposition syndrome, usually referred to as DICER1 syndrome (OMIM 606241). Tumours and lesions that comprise *DICER1* syndrome are observed primarily in children and arise from mesenchymal tissue. While defects in DICER1 function have been observed in *DICER1* syndrome, we still do not understand how DICER1 defects promote tumorigenesis. Most DICER1 studies involve total knockout of the gene and to date there are no models (either cell culture or animal) with which to study DICER1-mutated diseases. We hypothesize that defective DICER1 protein production in a mesenchymal stem cell at a critical time in development leads to altered miRNA populations that, in turn, initiate or prime cells for tumorigenesis.

Methods: SV-40 immortalized mouse mesenchymal stromal cells (MSCs) containing a homozygous floxed exon for DICER1 were *ex vivo* cre inactivated to produce Dicer-/- MSCs. Into these Dicer-/- MSCs, a FLAG-tagged human DICER1, a FLAG-tagged RNAse IIIa mutant DICER1, and a FLAG-tagged RNAse IIIb mutant DICER1 were stably introduced. Cells were characterized by Western Blot in response to serum starvation and their transcriptomes interrogated by microarray. Oncogenes were introduced into the cell lines and their ability to form anchorage-independent colonies was assessed by soft agar assay.

Results: Alterations in P-ERK were observed in cells in response to starvation and stimulation. Certain cell lines were able to produce anchorage-independent colonies.

Conclusion: We have created MSCs bearing RNase IIIb-mutated DICER1 to model DICER1 syndrome. We observe a defect in P-ERK. We suggest that the ability for an MSC to produce only 3p miRNAs in the presence of an oncogene can allow for anchorage-independent growth.

Poster #16:

REGULATION OF mESCs and DIFFERENTIATION BY eIF2α PHOSPHORYLATION

Authors: Mehdi Amiri, WiSaroush Tahmasebi, Aaron Kwong, Yojiro Yamanaka and Nahum Sonenberg

Abstract:

Translation of mRNA plays a vital role in controlling the gene expression. A coordinated action of several translation factors, mRNA, tRNAs and ribosomes undertakes the translation process. Initiation is the rate limiting step of translation and subjected to tight regulation. Embryonic stem cells (ESCs) maintain a low translation rate and a small change in the expression of stem cell factors can greatly influence ESCs self-renewal and differentiation. Translation initiation controlled by two multi-subunit complexes, eIF4F complex (eIF4E, eIF4A and eIF4G) and ternary complex (eIF2 alpha–GTP–MettRNA Meti). 4E-BPs are small proteins that inhibits translation initiation through interaction and sequestering of eIF4E. We have shown that the activity of 4E-BPs plays an important role in regulating ESCs partly through translational control of Yin-Yang 2 (YY2). In current study, we examine the effect of eIF2α phosphorylation on mouse ESCs self-renewal and differentiation. Phosphorylation of eIF2α at serine 51 interferes with ternary complex formation and as a result inhibits global translation. However, p-eIF2α promotes the translation of a subset of mRNAs containing upstream open-reading frames. Substitution of serine to alanine at residue 51 blocks the phosphorylation of eIF2α and the mice carrying this mutation (Eif2αS51A/S51A mice) die 18 hours after birth indicating the importance of this phosphorylation in development. In order to study the effect of eIF2α phosphorylation in self-renewal and differentiation, we derived mESCs from Eif2αS51A/S51A and wild type (WT) mice. Preliminary analysis revealed that the proliferation rate of Eif2αS51A/S51A mESCs is significantly reduced compared to WT cells. When mESC cultured in absence of GSK3 and MEK inhibitors (2i), two factors that protect an undifferentiated state, eIF2αS51A/S51A cells display an accelerated differentiation phenotype. To identify the translational targets of eIF2α in mESC we will employ ribosome footprinting. We will validate the results obtained from genome-wide analysis using cellular and molecular techniques.

Poster #20:

THE ROLE OF THE NUCLEAR DEUBIQUITINASE MYSM1 IN THE TRANSCRIPTIONAL REGULATION OF HEMATOPOIETIC STEM CELL FUNCTION AND HEMATOPOIESIS

Authors: Amanda Fiore [1,3], H.C. Wang[1,3], J.I. Belle[1,3], D. Langlais [2,3], P. Gros [2,3], A. Nijnik [1,3]

[1]Department of Physiology, McGill University; [2]Department of Biochemistry, McGill University; [3]McGill University Research Centre on Complex Traits, McGill University, Montreal, Canada

Abstract:

Myb-like SWIRM and MPN Domain 1 (MYSM1) is a chromatin-binding histone H2A lysine 119 deubiquitinase that is essential for normal hematopoiesis in both mice and humans. Mysm1-knockout mice exhibit severe hematopoietic defects, including loss of function of hematopoietic stem cells, apoptosis of hematopoietic progenitors, and impaired production of multiple downstream hematopoietic cell types. Although MYSM1 was reported to regulate the expression of some genes required for normal progression of hematopoiesis, no genome-wide analyses of MYSM1-regulated genes have been published to date. RNA-seq analyses conducted in our lab revealed an important category of genes that exhibit significantly altered expression in primary Mysm1-deficient hematopoietic stem cells. Additionally, our ChIP-seq analyses performed in the Ba/F3 hematopoietic progenitor cell line mapped the MYSM1 DNA-binding sites across the genome. I will discuss the results of these studies, the molecular and functional work I conducted in my graduate research project to validate these data, and its significance to hematopoietic stem cell biology. The work expands the current understanding of the role of MYSM1 in hematopoiesis and stem cell biology, and the mechanisms driving bone marrow failure in human MYSM1-deficiency.

Poster #21:

EXPLORING THE EFFECTS OF MYSM1-DEFICIENCY ON SENSITIVITY TO CELLULAR STRESS IN HEMATOPOIETIC STEM AND PROGENITOR CELLS

Authors: Chloe Feng[1,2,3], Yun Hsiao Lin[2,3], Anastasia Nijnik[2,3]

[1]Department of Microbiology and Immunology, McGill University; [2]Department of Physiology, McGill University; [3]McGill University Research Centre on Complex Traits, McGill University, Montreal, Canada

Abstract:

MYSM1 is a chromatin-binding deubiquitinase protein, reported to deubiquitinate histone H2A-K119ub and promote gene expressions. MYSM1 acts as an essential transcriptional regulator in hematopoietic stem cells, hematopoiesis, and lymphocyte development in both mouse and human. To understand the molecular mechanisms of MYSM1 activity, our research team performed RNA-Seq and ChIP-Seq analysis to identify MYSM1 regulated genes on a genome-wide scale in mouse hematopoietic stem cells, and this represents the first such genome-wide study of MYSM1 regulated genes to date. This unpublished data identified a large number of potential MYSM1-regulated genes, with essential roles in the maintenance of hematopoietic homeostasis. The aims of my project are to validate the altered activity of these genes in Mysm1-deficient hematopoietic progenitor cells through functional assays. To date, I used Mysm1-shRNA knockdown and control hematopoietic progenitor cell lines Ba/F3 to assess the effects of MYSM1-deficiency on the sensitivity of hematopoietic cells to different types of cellular stress. I will present the results of these studies, and discuss their implications for the understanding of the role of MYSM1 in hematopoiesis and stem cell biology. Overall, our data supports the dysregulated of specific pathways essential for hematopoietic homeostasis in MYSM1-deficient cells, and provides novel insights into transcriptional regulation of hematopoiesis by MYSM1.

Poster #22:

THE ROLE OF BAP1 IN B CELL PHYSIOLOGY

Authors: Yun Hsiao Lin[1,2], Anastasia Nijnik[1,2]

[1]Department of Physiology, McGill University; [2]McGill University Research Centre on Complex Traits, McGill University, Montreal, Canada

Abstract:

Introduction: Rare hematopoietic stem cells (HSCs) residing in our bone marrow perform the vital process of hematopoiesis, to continually replenish our blood and immune cells. BRCA1 associated protein-1 (BAP1) is a protein important for the biology of HSCs and hematopoiesis. At the molecular level, BAP1 acts as a nuclear deubiquitinase that interacts with various protein partners to regulate transcription, cell cycle, and DNA repair. As BAP1 mutations are associated with uveal melanoma, mesothelioma, and other cancers, BAP1 also functions as a tumour suppressor protein. Previous studies have reported *Bap1*-knockout mice to be embryonic lethal, whereas inducible *Bap1*-knockout mice have dysfunctional HSCs, along with ineffective hematopoiesis and myeloid malignancies resembling human myelodysplastic syndrome (MDS). However, the role of BAP1 in the lymphoid lineage remains unknown.

Objective: In this study, we investigate the role of BAP1 nuclear deubiquitinase in HSC differentiation to the lymphoid lineage.

Methods: Using the conditional *Bap1*-knockout mouse model, we focus on the involvement of BAP1 specifically in B cell lineage development and B cell-mediated immune responses.

Significance: This work will elucidate the contribution of BAP1 to lymphopoiesis and B cell development. These findings will help deepen our knowledge of hematopoiesis.

Poster #23:

CHARACTERIZING THE MYSM1-REGULATED TRANSCRIPTIONAL NETWORK IN HEMATOPOIETIC STEM AND PROGENITOR CELLS

Authors: H.C. Wang[1,3], J.I. Belle[1,3], J.C. Petrov[1,3], D. Langlais[2,3] and Anastasia Nijnik[1,3]

[1]Department of Physiology, McGill University; [2]Department of Biochemistry, McGill University; [3]McGill University Research Centre on Complex Traits, McGill University, Montreal, Canada

Abstract:

MYSM1 is a chromatin-binding protein that deubiquitinates histone H2A and is a regulator of gene expression. In both mouse and human, MYSM1 is an essential factor to the normal function, maintenance, and differentiation of hematopoietic stem cells (HSCs). Our previous study has shown that, in Mysm1-/- mouse, an elevation in the p53-regulated stress response is induced, leading to loss of HSC quiescence, depletion of downstream cells such as multipotent progenitors (MPPs), B cells, and T cells, and induction of bone marrow failure phenotypes. In Mysm1-/-p53-/- mouse, these phenotypes revert to normal, suggesting an antagonistic role of MYSM1 to the tumor suppressor and transcription factor p53. It is known that MYSM1 can co-localize to the promoters of some p53-regulated stress response genes. However, the mechanisms of MYSM1 in the regulation of these genes and in transcription remain unknown.

In recent unpublished work in our lab, the genome-wide DNA-binding sites of MYSM1 were mapped in hematopoietic progenitor cell-lines with ChIP-Seq. Furthermore, the transcriptional signatures of Mysm1-deficient HSCs were acquired and compared to control cells with RNA-Seq. I will present my analyses of these ChIP-Seq and RNA-Seq datasets, identify a set of MYSM1 regulated genes essential to HSC biology and normal progression of hematopoiesis, and suggest possible mechanisms leading to p53-stress response activation in Mysm1-deficient HSCs. Overall, our study provides novel insights into the MYSM1-regulated transcriptome in HSCs and facilitates future studies on the molecular mechanisms of MYSM1 in the regulation of gene expression. In long term, our finding contributes to the better understanding of human MYSM1-deficiency and other bone marrow failure syndromes.

Poster #24:

CELLULAR MODELS OF AGING UNCOVER IMPAIRED STRESS RESPONSES IN SENESCENT CELLS

Authors: Ossama Moujaber, Dana samhadaneh, Jeffrey Liang, Ines Colmegna and Ursula Stochaj

Abstract:

Background: The proper response to stress is crucial for the survival of physiological and environmental insults. Aging impairs stress responses, but the underlying mechanisms are not fully understood. We are addressing these mechanisms in different cellular models. First, we selected kidney cells, as the kidney is particularly prone to aging-related functional decline. Second, we examine mesenchymal stromal 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 MSCs are exposed to oxidative stress in the context of many diseases, 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 *in vitro*; 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 exogenous and disease-associated 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 MSC quality for therapeutic intervention.

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Poster #25:

RESPONSE TO SHEAR STRESS OF PROGEROID ENDOTHELIAL CELLS

Authors: Ariane Beland[1], Jonathan Brassard[2] and Corinne A. Hoesli[1]

[1]Department of Chemical Engineering, McGill University, Montreal, Canada; [2]École polytechnique fédérale de Lausanne, Lausanne, Switzerland

Abstract:

Hutchinson Gilford progeria syndrome (HGPS) is a premature aging disorder caused by a mutation of the LMNA gene leading to the accumulation of progerin. Since progerin also accumulates in an age-dependent manner in adults who do not carry the mutation, HGPS has been used as a model of vascular aging. While endothelial cell dysfunction is thought to play a leading role in vascular aging and the initiation of atherosclerosis in physiological aging, the role of these cells in the development of HGPS is not well understood. The reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) offers a powerful platform to study rare diseases like HGPS. IPSCs derived from HGPS patients' fibroblasts were differentiated into endothelial cells. The differentiation protocol yielded approximatively 20% VE-Cadherin-positive cells. The differentiation efficiency and expression of endothelial cell surface markers VEGFR, CD31, CD34, CD105 and TRA-1-60 were not statistically different between HGPS and control samples. Following the selection via magnetic-activated cell sorting of VE-Cadherin-positive cells, the endothelial cells were expanded and introduced into a multi-well laminar flow chamber laminar flow. The cells were exposed to 20 dyn/cm^2 wall shear stress for 6 hours. Fluorescence microscopy images suggest that progerin accumulation is associated to abnormal nuclear shape and alignment under flow. Based on preliminary experiments quantifying cytoskeletal alignment and cell shape, this phenomenon could cause hindered mechanotransduction. Characterizing endothelial dysfunction in response to shear stress in HGPS could lead to the development of more effective therapies for cardiovascular diseases.

Poster #26:

DEVELOPMENT OF A STIRRED MICROCHANNEL EMULSIFICATION DEVICE FOR TYPE 1 DIABETES TREATMENT

Authors: Chloé Selerier, Christina Bitar and Corinne A. Hoesli

Department of Chemical Engineering, McGill University, Montreal, Canada

Abstract:

Type 1 diabetes treatments include periodic insulin injections and pancreatic β-cell transplantation; the latter of which requires immunosuppressive drugs. Alternatively, islet cells can be encapsulated in alginate beads, bypassing the need for immunosuppressants since the pores of the hydrogel is impermeable to antibodies. The optimization of a novel Stirred Microchannel Emulsification (SME) device to produce uniform alginate beads of a controlled size of approximately 600 µm suitable for islet encapsulation will be presented.

The SME device consists of a batch stirred system with a polytetrafluoroethylene microchannel plate placed between an oil phase (3M™ Novec™ 7500 Engineered fluid) and an alginate aqueous phase (FMC Manugel® GHB alginic acid, FMC BioPolymer) containing the islets. The dispersed 1.5% alginate phase forms droplets as the fluid flows through the hydrophobic microchannels. The beads gel upon contact with the stirred acidified continuous phase.

The stirring speed and alginate flow rates were optimized to produce beads with the lowest bead diameter and most uniform size distribution. The average bead diameter ranged from 600 to 1200 µm with a coefficient of variation between 12 to 24%. Mouse Insulinoma cell line (MIN6) aggregates were harvested and encapsulated using this novel approach. Continuous phase recycle was determined to be beneficial through process cost reduction and increased sustainability.

Compared with current techniques, SME is a promising technique to produce alginate encapsulated β-cell islets propitious to transplantation and to restore the normal function of the pancreas.

Poster #27:

IS OXYGEN A REQUIREMENT FOR MESENCHYMAL STEM CELL FUNCTION; IMPLICATIONS FOR SCAFFOLD DESIGN

Authors: Fiona Lau[1], Nicoletta Eliopoulos[1,2] and Jake Barralet[1]

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Abstract:

Background: A key limitation for development of bulk tissue engineered scaffolds is the inability to deliver oxygen to cells found further within scaffolds – which can result in hypoxia, and tissue necrosis. Mesenchymal stem cells (MSCs) have various regenerative medicine applications due to their ability to differentiate into various cell types, and to their paracrine actions. Therefore, these cells are desired cellular vehicles for the continuous delivery of beneficial gene products. We hypothesize that in the absence of oxygen, MSCs will alter their metabolic pathway to survive without oxygen.

Methods: $1x10^4$ unmodified and Erythropoietin (Epo)-gene modified mouse marrow-derived MSCs were cultured in 35mm tissue culture plates in 2mL α-MEM media (supplemented with 10% FBS, 1% Penicillin/Streptomycin, and 1% L-Glutamine) for 7 days in normoxic and anoxic (21% and <1% oxygen, respectively) conditions. Anoxia was created by placing the culture plates inside glass jars and flushing with a mixture of carbon dioxide and nitrogen for a total of 10 minutes per sample. The conditioned medias were collected and used to measure Epo levels by ELISA. The cells from each plate were trypsinized and cell viabilities determined using Trypan Blue.

Results (preliminary): Anoxia showed significant decrease in the total cell number of unmodified MSCs compared to normoxic control ($P<0.01$), while no significant decrease was found for Epo-MSCs. However, within Anoxic conditions, unmodified MSCs are significantly reduced compared to Epo-MSCs ($P<0.01$), whereas compared to normoxic conditions no difference can be found. Epo production was not eliminated by absence of oxygen, as no significant difference was detected between normoxic and anoxic conditions.

Conclusion: Our results indicate that MSCs could survive for up to 7 days in the absence of oxygen. Epo production is unaffected by the absence of oxygen compared to normoxic controls. This indicates that gene modified MSCs could sustain protein production in anoxia.

Poster #28:

A BI-PHASE MATERIAL DESIGN WITH BIOGLASS-PLGA SCAFFOLDS AND FIBRIN GEL FOR BONE REGENERATION THROUGH ENDOCHONDRAL OSSIFICATION

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Abstract:

Background: Traditional bone regeneration strategies follow intramembranous ossification by promoting bone growth through direct differentiation of stem cells into osteoblasts. This approach often fails due to poor angiogenesis and hypoxia, leading to apoptosis. Thus, mimicking the process of endochondral ossification, wherein the bone is formed through a cartilage intermediate, would be a more appropriate approach for bone regeneration than direct ossification as it prevents hypoxia induced cell death. As cartilage and bone tissue and requirements are very different from each other, a bi-phase design could be beneficial to support both cartilage and bone.

Methods: We propose a bi-phase design including a fibrin hydrogel encapsulating and infiltrating a porous Bioglass-PLGA scaffold to support cartilage and bone respectively. The Bioglass-PLGA scaffold is prepared by solvent casting/particulate leaching using paraffin microspheres as a template. Fibrinogen with mesenchymal stem cells (MSCs) is casted onto the porous composite template and subjected to gelation ensuring even cell distribution.

Results: We prepared a bi-phase material including a fibrin hydrogel to support cartilage and a composite scaffold to support bone formation. Bioglass-PLGA composite layer has evenly distributed and well interconnected spherical pores. Fibrin gel supported the chondrogenic differentiation of the MSCs and the cells were found to be well distributed throughout the gel layer.

Conclusion: Our preliminary results show that a bi-phase material design for endochondral ossification is feasible. We expect that the cells in the gel layer will degrade the gel and reach the composite layer, allowing enough time for the vasculature formation prior to bone growth. As stiffer scaffolds promote hypertrophy, which is the terminal differentiation of chondrocytes before undergoing apoptosis, the stiff BG-PLGA composite layer should promote the hypertrophic differentiation of the chondrocytes. When the hypertrophic chondrocytes die, they will be eventually replaced by osteoblasts brought in by the vasculature, leading to new bone formation.