

## Fall 2018 SCRM Symposium Program

Location: Jewish General Hospital Block B Amphitheater  
3755 Chemin de la Côte-Sainte-Catherine, Montréal

TIME	AGENDA	PRESENTER
8:00	Registration in the Cohen Auditorium, 5750 Chemin de la Côte-des-Neiges	
8:30	Welcome	Linda Peltier PhD, Chair

### FUNDAMENTAL RESEARCH (BLOCK B AMPHITHEATER)

FUNDAMENTAL KEYNOTE SPEAKER		
8:45	CORINNE HOESLI AND JOSEPH MATT KINSELLA "TISSUE FABRICATION USING 3D PRINTING: A BLOODY STORY"	
9:30	TED TALKS – FUNDAMENTAL RESEARCH (5 min+ 2 questions); MODERATOR ROHAN RACINE	
9:35	Mutations in Human Histone H3 are Pre-leukemic Events that Promote Hematopoietic Stem Cell Expansion And Leukemic Aggressiveness	<i>Meaghan Boileau</i>
9:45	Identifying Male Germline Stem Cells: Towards Functional Mapping of the Stem Cell Fate	<i>Joëlle Desmarais</i>
9:55	Regulation of Normal and Cancerous Stemness by a GATA3/BMP Axis in the Prostate	<i>Mathieu Tremblay</i>
10:05	The Role of the Nuclear Deubiquitinase MYSM1 in the Transcriptional Regulation of Hematopoietic Stem Cell Function and Hematopoiesis	<i>Amanda Fiore</i>
10:15	Combinatorial Surface Modification Strategies to Capture Endothelial Progenitor Cells	<i>Omar S Bashth</i>
10: 25	Establishment of a Human Bone Metastases 3D Microenvironment Model	<i>Antone Nour</i>
10:30	Stem Cell: The Genetics Behind the Future	<i>Adam Elkaim &amp; Nathanel Harell</i>
10:45	Morning Break in the Cohen Auditorium, 5750 Chemin de la Côte-des-Neiges	
11:00	<b>POSTER PRESENTATIONS AND EVALUATION ( COHEN AUDITORIUM)</b>	
12:30	LUNCH BOXES in the Cohen Auditorium, 5750 Chemin de la Côte-des-Neiges	

## CLINICAL RESEARCH (BLOCK B AMPHITHEATER)

14:00	<b>CLINICAL KEYNOTE SPEAKER</b> <b>DR PIERRE LANEUVILLE</b> <b>“FROM HUMBLE BEGINNINGS TO THE BRAVE NEW WORLD OF CELLULAR THERAPY”</b>	
14:45	<b>TED TALKS – CLINICAL RESEARCH</b> (5 min + 2 questions); MODERATOR CONNIE YANG	
14:50	Proteomic Characterization of Mesenchymal Stem Cells and Osteoblast-Derived Exosomes of COL1A1 Osteogenesis Imperfecta Patients	<i>Monther Abuhantash</i>
15:00	CD271 a More Precise Marker for Cancer Stem Cell in Head and Neck Squamous Cell Carcinomas than CD44	<i>Osama Elkashty</i>
15:10	Biomechanical cues guide lineage specification of induced pluripotent stem cell derived pancreatic progenitors	<i>Raymond Tran</i>
15:20	First characterization of exosomes derived from amniotic stromal cells and their application for cardiac repair	<i>Kashif Khan</i>
15:30	Mitochondrial Replacement Therapy Continues to Push Ethical and Legal Boundaries: A Litterature Review Analysis	<i>Forough Noohi</i>
15:40	The Effect of Microporosity on the Bioactivity of Scaffolds for Bone Regeneration	<i>Dhanalakshmi Jeyachandran</i>

15:50	Short afternoon Break – Jury will meet to discuss the Poster and Oral Presentation winner	
16:00	Poster and Oral Presentation Winner Nominations	<i>Colin Crist &amp; Vahab Soleimani</i>
16:15	Closing and evaluation	<i>Linda Peltier, Chair</i>
16:30 to 19:00	<b>NETWORKING / POSTER PRESENTATIONS in the Cohen Auditorium, 5750 Chemin de la Côte-des-Neiges</b>	



# Join Us at the Jewish General Hospital!



**Block B Amphitheater**

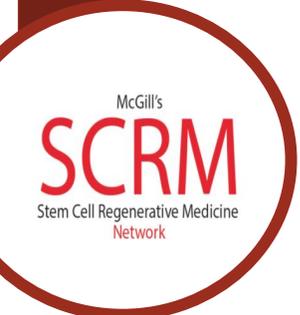
- Keynote speakers
- Oral Presentation

**Cohen Auditorium**

- Registration
- Poster Presentation
- Lunch
- Networking/Poster presentation

**PLEASE BE RESPECTFUL OF THE PATIENTS CIRCULATING IN THIS HOSPITAL**

Fall 2018 SCRM Symposium Program



## ORAL ABSTRACTS - FUNDAMENTAL RESEARCH

### **Title:**

### **MUTATIONS IN HUMAN HISTONE H3 ARE PRE-LEUKEMIC EVENTS THAT PROMOTE HEMATOPOIETIC STEM CELL EXPANSION AND LEUKEMIC AGGRESSIVENESS**

### **Authors:**

**Meaghan Boileau**<sup>1</sup>, Margret Shirinian<sup>2</sup>, Tenzin Gayden<sup>1</sup>, Ashot S. Harutyunyan<sup>1</sup>, Carol C.L. Chen<sup>1</sup>, Leonie G. Mikael<sup>1</sup>, Heather M. Duncan<sup>1</sup>, Andrea L. Neumann<sup>1</sup>, Patricia Arreba-Tutusa<sup>1</sup>, Nicolas De Jay<sup>1</sup>, Michele Zeinieh<sup>1</sup>, Katya Rossokhata<sup>1</sup>, Yelu Zhang<sup>1</sup>, Hamid Nikbakht<sup>1</sup>, Carine Mouawad<sup>2</sup>, Radwan Massoud<sup>2</sup>, Felice Frey<sup>2</sup>, Rihab Nasr<sup>2</sup>, Jean El Cheikh<sup>2</sup>, Marwan El Sabban<sup>2</sup>, Claudia L. Kleinman<sup>1</sup>, Rami Mahfouz<sup>2</sup>, Mark D. Minden<sup>3</sup>, Nada Jabado<sup>1</sup>, Ali Bazarbachi<sup>2</sup>, and Kolja Eppert<sup>1</sup>

<sup>1</sup> McGill University, Montreal, Quebec, Canada;

<sup>2</sup> American University of Beirut, Beirut, Lebanon;

<sup>3</sup> Princess Margaret Cancer Centre, University Health Network, University of Toronto, Toronto, Ontario, Canada

### **Abstract:**

Aberrant epigenetic regulation is often an early step in the development of acute myeloid leukemia (AML). Our ability to prevent or treat AML is limited by our incomplete understanding of this epigenetic disruption, including improper histone methylation. Rare K27 mutations in histone H3 have been identified in T-acute lymphoid leukemia, myelodysplastic syndrome and AML. Here, in a comprehensive analysis of all histone H3 genes, we demonstrate that H3 mutations are early events in AML leukemogenesis.

We sequenced 16 H3 genes in 434 primary AML samples and identified 7 mutations (1.6%) (HIST1H3F-K27I, HIST1H3H-K27M, H3F3A-K27M/A26P, HIST1H3A-Q69H, H3F3C-R2Q/R8H), with an enrichment in secondary AML (9%). The mutations were present in the founding clones, indicated by high variant allele frequencies. In two cases with corresponding normal samples, we identified Q69H and K27M mutations in pre-leukemic hematopoietic stem cells (HSCs).

Consistent with a role in pre-leukemic HSC clonal expansion, primary and secondary xenotransplantation confirmed that K27M/I mutant histones increased normal human HSC frequency. Furthermore, K27 and Q69 mutations altered myeloid and erythroid differentiation. In established AML, K27M/I mutations increased proliferation and progenitor frequency in vitro demonstrated by colony formation unit assay and drastically increased leukemic aggressiveness in vivo. We performed RNA-seq and ChIP-seq analyses and observed changes in gene expression, with a global decrease in H3K27 tri-methylation in AML cells with the K27M/I mutations. Furthermore, there was a more drastic loss of H3K27 tri-methylation at promoters of upregulated genes and a concurrent increase in H3K27 acetylation. Gene ontology analysis indicated alterations in regulators of biological processes involved in erythrocyte and myeloid differentiation. These findings place H3 mutations alongside ASXL1 and EZH2 mutations as drivers of an altered histone epigenome in pre-leukemia and secondary AML.

**Title:**

**IDENTIFYING MALE GERMLINE STEM CELLS: TOWARDS FUNCTIONAL MAPPING OF THE STEM CELL FATE**

**Authors:**

Joëlle Desmarais<sup>1</sup>, Xiangfan Zhang<sup>1</sup>, Makoto Nagano<sup>1</sup>

1. Research Institute of the McGill University Health Center and McGill University, Department of Obstetrics and Gynecology, 1001 Decarie Blvd., Montreal, QC, H4A 3J1.

**Abstract:**

Spermatogonial stem cells (SSCs) are the foundation of high-throughput, life-long production of sperm. Clinically, SSCs are expected to become an important resource to restore male fertility, particularly for childhood cancer patients whose fertility is at risk. Yet, fundamental characteristics of SSCs remain elusive, making mechanistic studies difficult to control SSC fate for preserving stem cells or encouraging sperm production. Our ultimate goal is to determine a fate map of SSCs and to visualize them. Using a functional transplantation assay and multi-parameter flow cytometry, we found that loss of THY1 or ITGA6 indicates the exit from the SSC state, while the expression of widely-used SSC markers (GFRA1 and CDH1), as well as KIT, denotes the transition to commitment. To enrich the SSC population from “adult intact” mouse testes, which has been hard to achieve, we fractionated primitive germ cells using five surface antigens simultaneously (THY1, CDH1, GFRA1, KIT, and ITGA6). SSC transplantations showed that two cell fractions, the THY1+ / ITGA6HiMed / GFRA1- / CDH1- / KIT- and the THY1+ / ITGA6Hi, exhibit the degrees of SSC enrichment that exceed any of those reported in the past two decades. Further characterization of these cell fractions is underway (e.g., cell cycle, protein/mRNA expression). We are also in preparation for single-cell RNA sequencing to understand the SSC fate commitment process at the transcriptome level. Our data should establish a powerful platform to dissect SSC fate decision mechanisms and to identify SSC niches, leading to clinical SSC applications for male fertility preservation and restoration. (Supported by CIHR and FRSQ)

**Title:**

**REGULATION OF NORMAL AND CANCEROUS STEMNESS BY A GATA3/BMP AXIS IN THE PROSTATE**

**Authors:**

**Mathieu Tremblay**, Maxwell Shafer, Sophie Viala, Alana H.T. Nguyen, Adda Lee-Graham, Maxime Bouchard

**Abstract:**

Adult stem cells are found in prostate tissue and can act as the cells-of-origin for prostate cancer. Loss of the tumor suppressor PTEN is a common occurrence in prostate cancer. We showed 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 primary prostate stem cells enhanced their long term self-renewal capacities both in vitro (serial sphere formation assay) and stem cell frequency in vivo (limiting dilution transplantation assay). Using RNAseq analysis, we found that BMP signaling is important for stem cell maintenance and loss of GATA3 is associated with an autocrine up-regulation of Bmp5. In addition, BMP5 treatment increased normal stem cell potential and numbers in vitro and in vivo whereas its inhibition (Noggin) corrects GATA3-deficient sphere forming potential to normal levels as well as the aberrant Pten-deficient cancerous sphere forming potential. Finally, loss of Bmp5 in the mouse affect prostate tissue homeostasis which is associated with a reduction in stem cell potential. Together, these data establish GATA3 as an important regulator of normal and cancerous prostate stem cells homeostasis through a Bmp5 dependent mechanism.

**Title:**

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

**Authors:**

**Amanda Fiore**<sup>1,3</sup>, HanChen Wang<sup>1,3</sup>, Jad I. Belle<sup>1,3</sup>, Philippe Gros<sup>2,3</sup>, David Langlais<sup>4,5</sup>, Anastasia Nijnik<sup>1,3</sup>

<sup>1</sup> Department of Physiology, McGill University

<sup>2</sup> Department of Biochemistry, McGill University

<sup>3</sup> McGill University Research Centre on Complex Traits, McGill University

<sup>4</sup> Department of Human Genetics, McGill University

<sup>5</sup> McGill University and Génome Québec Innovation Centre, McGill University

**Abstract:**

Myb-like SWIRM and MPN Domains 1 (MYSM1) is a chromatin-binding histone H2A lysine 119 deubiquitinase that is essential for normal hematopoiesis in both mice and humans. Mym1 - 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 Mym1-deficient hematopoietic stem cells. Additionally, our ChIP-seq analyses performed in the Ba/F3 hematopoietic progenitor cell line revealed that a significant portion of MYSM1-binding sites across the genome are associated with these genes. My current aims are to validate and establish the role of MYSM1 in the regulation of the expression of these genes, understand whether this regulation operates through MYSM1's catalytic domain, and investigate the crosstalk between MYSM1 and other transcription factors in their regulation. This work will expand our current understanding of the role of MYSM1 in hematopoiesis and stem cell biology and the mechanisms driving bone marrow failure in human MYSM1-deficiency.

**Title:**

**COMBINATORIAL SURFACE MODIFICATION STRATEGIES TO CAPTURE ENDOTHELIAL PROGENITOR CELLS**

**Authors:**

**Omar S. Bashth**<sup>1</sup>, Marieve D. Boulanger<sup>1</sup>, Mohamed A. Elkhodiry<sup>1</sup>, Gaétan Laroche<sup>2,3</sup>, Corinne A. Hoesli<sup>1</sup>

1. Department of Chemical Engineering, McGill University, Montréal, Québec, Canada.
2. Centre de Recherche du Centre Hospitalier Universitaire de Québec, Québec, Canada.
3. Département de génie des mines, de la métallurgie et des matériaux, Université Laval, Québec, Canada.

**Abstract:**

To treat heart disease, the most common intervention is the implantation of a stent, which can lead to restenosis – a renewed narrowing of the blood vessel. The aim of this project is to design a stent that would capture endothelial progenitor cells (EPCs) and facilitate reendothelialization on the stent's surface to avoid restenosis and other complications. Our strategy is to functionalize surfaces with antibodies that would capture EPCs, as well as extracellular matrix (ECM) derived peptides to favour EPCs attachment, proliferation and differentiation. Endothelial colony forming cells (ECFCs, which contain EPCs) were successfully isolated from peripheral blood. In this study, different antibody grafting methods were compared to identify a reaction scheme allowing EPC capture from the circulation. Different reaction schemes were investigated to immobilize antibodies that bind EPC surface antigens such as anti-CD144. First, aminated polystyrene surfaces were reacted with the linking arm sulfo-SMPB, which reacts with primary amines as well as thiol groups. To immobilize the antibodies through the fragment crystallisable region (Fc region), Cys-protein G or RRGW(mini-PEG3)Cys peptide were spotted onto the SMPB-treated polystyrene surfaces to achieve covalent conjugation via the thiol group of the cysteine residue. Next, antibodies were incubated with the Protein G or RRGW surfaces, leading to specific attachment of antibodies to regions functionalized with Protein G or RRGW. Future work will also investigate direct conjugation of antibody fragments via free thiol groups obtained after partial reduction of disulfide bonds. This project should lead to new EPC capture stent technologies with reduced incidence of long-term complications such as restenosis.

**Title:**

**ESTABLISHMENT OF A HUMAN BONE METASTASES 3D MICROENVIRONMENT MODEL**

**Authors:**

**Antone Nour<sup>1</sup>** and Derek Rosenzweig<sup>1</sup>

<sup>1</sup>Research Institute of the McGill University Health Centre, Injury Repair Recovery Program, Department of Surgery, Division of Orthopaedics, Montreal, Quebec, Canada.

**Abstract:**

**BACKGROUND:** When various cancers invade the bone, they can disrupt the physiological balance leading to various deleterious changes in bone structure and function. Local delivery of chemotherapeutics during surgical tumor resection may provide advantages over systemic delivery. However, there is currently no relevant physiological in vitro models of human bone metastases. We set out to generate a 3D bone microenvironment model containing human bone cells, bone marrow stromal cells, bone mineral with patient derived tumor cell core. This in vitro bone metastasis model will serve to establish effective chemotherapeutic doses for local delivery applications.

**METHODS:** Human osteoblasts (1 x 10<sup>6</sup> cells/mL) were mixed into 3% alginate/7% gelatin with or without 0.5mg/mL nanohydroxyapatite (HA). The constructs were cultured for 28 days in either normal growth medium (DMEM) or osteogenic medium (OM). Live/Dead assays were performed, and fixed samples were cryosectioned and stained with Alizarin red for calcified matrix deposition, and hematoxylin and eosin to observe cells within the matrix.

**RESULTS:** Live/Dead analysis revealed >85% primary human osteoblast viability in all conditions after 28 days of culture. Similar viability was observed in primary human MSCs. Alizarin red staining showed that cells grown in DMEM without HA had the least amount of bone mineralized matrix. The combination found to have the most amount of bone mineralized matrix was OM/HA+.

**CONCLUSION:** Human osteoblasts cultured in presence of HA and osteogenic medium generates the most bone-like microenvironment compared to other conditions. This may present an optimal strategy for studying bone metastases in vitro.

**Title:**

**THE STEM CELL – THE GENETICS BEHIND THE FUTURE**

**Authors:**

Adam Elkaim & Nathanel Harell

**Abstract:**

After months of research, we have found a hypothetical way to take adult human stem cells to generate or regenerate parts of the human brain. It is believed that with the properties of induced pluripotent stem cells (iPSC) derived from dermal fibroblast cells. These cells are autonomous and can create other types of rare cells that have helped the world of medicine in a revolutionary way. They can repair human organs and tissues. These types of cells have been used to treat many kinds of diseases. Therefore, using the Yamanaka Factors and tweaking them with the help of a retroviral virus we want to take this to the next level and try to develop new neurons in the human brain, and our final big goal is to make a fully functional human brain.

## ORAL ABSTRACTS - CLINICAL RESEARCH

### **Title:**

## **PROTEOMIC CHARACTERIZATION OF MESENCHYMAL STEM CELLS AND OSTEOBLAST-DERIVED EXOSOMES OF COL1A1 OSTEOGENESIS IMPERFECTA PATIENTS**

### **Authors:**

Monther Abuhantash<sup>1,3</sup>, Frank Rauch<sup>1,4</sup>, Janusz Rak<sup>4</sup>, Reggie Hamdy<sup>1,5</sup>, Hadil Al-Jallad<sup>1,2</sup>

1. Shriners Hospital for Children, Montreal, Québec, Canada.

2. Department of Surgery, Division of Orthopedic Surgery, Faculty of Medicine, McGill University, Montreal, Québec, Canada

3. Department of Experimental Surgery, Faculty of Medicine, McGill University, Montreal, Québec, Canada

4. Department of Pediatrics, Faculty of Medicine, McGill University, Montreal, Québec, Canada

5. Department of Surgery, Division of Pediatric Orthopedic Surgery, McGill University, Montreal, QC, Canada

### **Abstract:**

**INTRODUCTION:** Osteogenesis Imperfecta (OI) is a bone disorder characterized by skeletal deformities often caused by mutations in the Type I collagen-encoding genes COL1A1 and COL1A2. We have shown that around 50% of patients who underwent corrective surgery by osteotomy, either had delayed healing or non-union. This prompted us to study extracellular vesicles (EVs) as a tool for targeted localized therapy of these complications.

Mesenchymal stem cells (MSCs) and osteoblasts (Obs) communicate among each other and with the surrounding environment through multiple pathways including EVs. EV is a term that refers to a heterogeneous group of membrane-bound nanoparticles secreted by cells for long-distance communications. EVs carry a cargo of proteins and RNAs, with the specific content depending on the secreting cell and its metabolic status at the time of secretion.

The aim of this study is to characterize the proteomic profile of EVs secreted by MSCs and their derived Obs in OI and human control (HC) patients.

**METHODS:** MSCs were derived from 6 COL1A1-OI and 2 HC patients. They were then proliferated and differentiated into Obs in EV-depleted growth media. EVs were then purified from conditioned media before and after osteogenic induction.

**RESULTS:** Proteomic profiling has identified 1011 proteins, out of which 938 proteins were found in Vesiclepedia. The remaining 44 proteins are likely to be specific to the biological samples under investigation. The EV samples contained many expected surface protein markers confirming the identity of exosome-like EVs. We found significant differences in both protein content and levels of EVs secreted by differentiating and mineralizing OI- and HC-osteoblasts

**CONCLUSIONS:** The genetic mutation affected the protein content of OI-EV during osteoblast differentiation and mineralization of the extracellular matrix with no detected changes at the  $\alpha$ MSC proliferative phase.

**Title:**

**CD271 A MORE PRECISE MARKER FOR CANCER STEM CELL IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS THAN CD44**

**Authors:**

OSAMA ELKASHTY, SIMON TRAN.

**Abstract:**

Head and neck squamous cell carcinoma (HNSCC) is the seventh most common cancer with over 500,000 new cases diagnosed yearly, and 4.6% of cancer cases. Despite the improvements in treatment modalities, the five-year survival rate for SCCHN has remained unchanged at ~50% over the past 30 years. One reason for HNSCC treatment failure is related to a subpopulation of cells in the tumor called cancer stem cells (CSCs) which are suggested to have tumor initiating potential, combined with the self-renewal ability and multilineage differentiation. According to many studies, CD44 surface marker can be used to identify CSCs. The purified CD44+ cells from the primary tumors can give rise to tumors faster and by injecting less cell number in xenograft model compared to CD44- cells, and these xenograft tumors subsequently reproduce the original tumor heterogeneity observed in the primary tumor. Recently, CD271 was identified as a marker of CSCs in many tumors, such as human melanoma and hypopharyngeal carcinoma. In our study, we investigated if CD271 is a more precise marker for CSCs than the widely used CD44. Our results revealed that CD271+ cells are a subpopulation from the CD44+ cells. The CD44+/CD271+ cells have a faster dividing rate, higher proliferation rate, higher self-renewal ability and chemo/radio-resistance when compared to CD44+/CD271- and the total population. CD44+/CD271+ cells showed higher expression of stemness genes such as BMI1, SOX2, OCT4 and GLI1 when compared to CD44+/CD271-. Our results suggest that CD271+ is a more accurate marker to purify the CSCs from HNSCC.

**Title:**

**BIOMECHANICAL CUES GUIDE LINEAGE SPECIFICATION OF INDUCED PLURIPOTENT STEM CELL DERIVED PANCREATIC PROGENITORS**

**Authors:**

Raymond Tran<sup>1</sup>, Christopher Moraes<sup>1,2</sup>, Corinne A. Hoesli<sup>1</sup>

<sup>1</sup>Department of Chemical Engineering, McGill University

<sup>2</sup>Department of Biomedical Engineering, McGill University

**Abstract:**

**Background:** The differentiation of induced pluripotent stem cells (iPSCs) into beta cells could provide an unlimited cell source to treat type 1 diabetes. However, existing in vitro protocols results are inefficient and produce cells with impaired insulin-secretion. Pancreas development is accompanied by structural transformation in which PDX1+ pancreatic progenitors bud off from the primitive gut tube, and NKX6.1+ precursors aggregate and delaminate to form islets. We hypothesized that biomechanics arising during pancreas development support pancreatic lineage specification. By leveraging tensional gradients present in micropatterned cultures, we demonstrate that pancreatic differentiation can be influenced by biomechanical cues.

**Methods:** Human iPSC-derived pancreatic progenitor cells (PPCs) were spatially confined with circular cell-adhesive islands for 3 days. The expression of PDX1 and NKX6.1 were then quantified by fluorescent immunostaining. Finite element models (FEMs) were constructed to determine the tensional gradients present in these patterns.

**Results:** PPCs confined within micropatterns showed increased PDX1 and NKX6.1 expression in 150 and 300 $\mu$ m patterns but this effect was not present in larger diameters and was also abrogated upon inhibition of the mechanosensitive ROCK pathway. Increases in PDX1 expression were correlated with increases in cell density and changes in actin cytoskeletal conformation, suggesting morphogenetic patterns similar to pancreatic budding during differentiation. The FEMs revealed high tension gradients throughout smaller microwells further suggesting a role of mechanics in this process.

**Conclusions:** PDX1 and NKX6.1 expression was increased in PPCs when cultured in confining micropatterns. These patterns matched the diameters of the developing pancreatic bud, suggesting gradients arising from geometry can guide endocrine differentiation. Our ongoing work is focused on determining the temporal effects of geometry on pancreatic specification. These results demonstrate the importance of biomechanics and suggests novel strategies to improve beta cell production for transplantation.

**Title:**

**FIRST CHARACTERIZATION OF EXOSOMES DERIVED FROM AMNIOTIC STROMAL CELLS AND THEIR APPLICATION FOR CARDIAC REPAIR.**

**Authors:**

Khan K, Yu B, Schwertani A, Cecere R

**Abstract:**

**INTRODUCTION:** The use of stem cells for cardiac repair after myocardial infarction (MI) is promising, yet clinical trials suggest that these cells fail to integrate into the native tissue, resulting in limited improvements in cardiac function and repair. Some reports suggest that stem cells are not necessarily needed to induce cardiac repair, but rather it's their secretions that have regenerative ability. Here, we have investigated the use of exosomes derived from amniotic stromal stem cells (ASCs) and their protective and regenerative potential in cardiac endothelial cells and cardiomyocytes.

**METHODS:** Sequential ultracentrifugation was used to isolate all extracellular vesicles from the human ASCs secretome. Characterization and analysis of nanoparticles was done using Nanosight, transmission electron microscopy (TEM), Bradford assay and Western blotting. Human cardiac microvascular endothelial cells and cardiomyocytes were treated with ASC-derived exosomes and assessed for changes in phenotype through proliferation, metabolic activity and cell viability assays.

**RESULTS:** Extracellular vesicles derived from ASCs were found to be solely exosomes, as determined by Nanosight tracking analysis, Western blotting for exosome markers and TEM. Human cardiac endothelial cells and cardiomyocytes treated with ASC-derived exosomes displayed significantly increased cellular proliferation, metabolic activity and cytoprotection after hypoxia-induced injury. Endothelial cells treated with exosomes also increased expression of vascular endothelial growth factor, a marker of angiogenesis.

**CONCLUSIONS:** Here, we provide the ground work for the use of ASC-derived exosomes for cardiac repair after MI. Future work will continue characterize these vesicles through proteomics analysis and how they promote cardiac repair in a rat MI model.

**Title:**

**MITOCHONDRIAL REPLACEMENT THERAPY CONTINUES TO PUSH ETHICAL AND LEGAL BOUNDRIES: A LITERATURE REVIEW ANALYSIS**

**Authors:**

Noohi, F.1,2, Caulfield A.2, Joly, Y.1,2

1. Department of Human Genetics, McGill, Montreal, QC, Canada
2. Centre of Genomics and Policy, Montreal, QC, Canada

**Abstract:**

Mitochondrial Replacement Therapy (MRT) is a new type of in vitro fertilization that aims to prevent the transmission of mitochondrial diseases (matrilineal transmission) by replacing mutated mitochondrial DNA in unfertilized oocytes or zygotes with normal mitochondria from a healthy donor. Since as a result of MRT, permanent changes are made to the germline that would be transmitted through generations, this controversially so-called “three-parent IVF” is considered as a means of genetic modification. Besides the UK, which became the first country to approve MRT in 2015, only a few countries have addressed this controversial technique through public policy.

In order to explore how the interdependence of ethics, policy, and public views can address questions that arise from new reproductive technologies and analyze the influence of socio-cultural factors upon stakeholders’ motives for using MRT, we conducted a systematic literature review of relevant academic sources which characterized the socio-ethical issues and scientific advancements of MRT according to Oxford’s Bodleian Libraries review guidelines. Next, we reviewed and assessed pertinent legislation, policies, and ethical guidelines, in both the research and clinical contexts, in Canada, the US, the UK, and Mexico whose approaches in governing the controversial technique ranged from strict bans with serious consequences to an absence of policy. The major themes identified in our review included, concerns over safety and efficacy, slippery slope of germline modification, issues of identity, risk for future generations, equity of access, available resources, and beneficence.

Next, the interdisciplinary knowledge produced as a result of this research will be used to analyze perceptions and expectations of relevant stakeholders (clinicians, policymakers, couples with affected children, at risk couples who wish to have biological children, and egg donors–potential so-called third parents–with regard to MRT in the aforementioned countries.

**Title:**

**THE EFFECT OF MICROPOROSITY ON THE BIOACTIVITY OF SCAFFOLDS FOR BONE REGENERATION**

**Authors:**

Dhanalakshmi Jeyachandran<sup>1</sup>, Rayan Fairag<sup>2</sup>, Lisbet Haglund<sup>2</sup> and Marta Cerruti<sup>1</sup>

<sup>1</sup>Department of Mining and Materials Engineering, McGill University, Montreal, Canada;

<sup>2</sup>Experimental Surgery, Montreal General Hospital, Montreal, Canada

**Abstract:**

Background: Scaffold microporosity plays an important role in promoting osteogenesis, by improving protein adsorption and cell adhesion. A conventional technique to impart microporosity in materials is thermally induced phase separation. This technique generates tubular pores in the size range of 10-50 µm but also causes scaffold shrinkage and requires a long solvent sublimation time. In our study, we propose a simple technique to create microporosity in poly(lactic-co-glycolic acid) (PLGA) – Bioglass (BG) composite scaffolds and we investigate the effect of the micropores on the bioactivity of these scaffolds for bone regeneration.

Methods: We prepared BG-PLGA scaffolds either with (BG-mPLGA) or without (BG-PLGA) micropores in the PLGA by solvent casting/particulate leaching, using paraffin microspheres as a porogen and either hexane or citrisolv as leaching agents. We compared the bioactivity of both scaffolds by simulated body fluid (SBF) immersion tests and cell assays to assess the role of PLGA microporosity.

Results: Both BG-mPLGA scaffolds and BG-PLGA scaffolds showed well interconnected spherical pores created by the paraffin microspheres. The main factors determining the polymer microporosity were the solvent used for leaching and the duration of leaching. We found that citrisolv leaching resulted in a microporous PLGA, which was absent when leached with hexane, and a longer leaching time created a higher microporosity. We observed extensive apatite formation in BG-mPLGA after 96 hours in SBF but not on BG-PLGA during the immersion tests.

Conclusion: Our preliminary results show that PLGA microporosity enhances apatite formation on composite BG-mPLGA scaffolds. We are currently doing in vitro tests to study the effect of PLGA microporosity on cell adhesion, proliferation and differentiation on BG-PLGA scaffolds and to understand if the enhanced bioactivity is due to changes in BG reactivity in the BG-mPLGA scaffolds.

## **POSTER ABSTRACTS**

### **Poster #1:**

#### **Title / Titre :**

**REGULATION OF GLIOMA STEM CELL METABOLISM BY ONCOSTATIN M RECEPTOR.**

#### **Authors / Auteurs :**

Matthew Laaper; Jean-Sébastien Joyal; Arezu Jahani-Asl

#### **Abstract / Résumé :**

Glioma stem cells (GSC) possess a unique metabolic phenotype in glioblastoma, relying on oxidative phosphorylation (OXPHOS) as opposed to aerobic glycolysis seen in the bulk of the tumour. The mechanism regulating this metabolic distinction is poorly understood. Here, we report a novel role of oncostatin M receptor (OSMR) in the upregulation of OXPHOS in glioma stem cells. OSMR has recently been shown as a requisite component of the oncogenic EGFRvIII signaling pathway, and RNA seq analysis of OSMR candidate target genes revealed OSMR regulation of key metabolic genes. To determine the effect of OSMR signaling on metabolic phenotype, we performed bioenergetic analysis using a Seahorse XFe96 flux analyser to measure cellular respiration. Interestingly we show that OSMR stimulates OXPHOS in 5 distinct patient-derived GSC lines. OSM ligand treatment results in a prominent increase in maximal respiration (Complex IV activity), while OSMR genetic knockdown displays a global suppression of cellular respiration. Intriguingly, we find the effect of OSMR is maintained irrespective of EGFRvIII mutation status. To determine the mechanism of OSMR regulation, we observed the effect of OSMR signaling on mitochondrial biogenesis. Both OSMR genetic knockdown and CRISPRi-mediated OSMR knockdown are correlated with a reduction in mitochondrial DNA, and a corresponding loss of PGC-1 $\alpha$  expression. Furthermore, the effect of OSMR is consistent in post-mitotic primary neurons, suggesting that OSMR regulation of metabolism is independent of proliferation. Our data suggests that novel therapeutic interventions for inhibition of OSMR signaling in BTSCs can improve outcomes for GB patients via depleting energy supply to tumorigenic stem cells.

**Poster #2:**

**Title / Titre :**

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

**Authors / Auteurs :**

Jamet Solène, Tran Viviane, Côté Jean-François, and Crist Colin.

**Abstract / Résumé:**

During development, trunk and limb skeletal muscle are derived from segmented blocks of somatic mesoderm. As the somite matures, the dorsal dermomyotome compartment of the somite maintains an epithelial structure, and is composed of multipotent progenitors that express Pax3, a transcription factor of the paired homeodomain family. Pax3 plays important roles in both the survival of the multipotent progenitor cells and activation of the myogenic program, but mechanisms guiding Pax3 transcriptional activity within these two contexts remain unclear. Transcription factors commonly interact with cofactors to activate or repress gene expression. We used BioID, an *in situ* proximity biotinylation assay to identify proximity interactors of Pax3 within the context of driving mouse embryonic stem (mES) cells into the myogenic program. Mass spectrometry analysis of proximity dependent biotinylated proteins revealed candidates enriched within functional categories of chromatin binding, transcriptional corepressors, transcription regulatory region binding, signal transduction and mRNA splicing. We further characterize Jmjd1c, a member of the Jumonji domain containing JmjC family, which we show overlaps with Pax3 expression within the multipotent progenitor cells in the dorsal dermomyotome component of E9.5 and E10.5 somites, but not in activated myogenic progenitors expressing Myf5 or MyoD within the emerging myotome. We will use genetic analyses in the developing mouse embryo to further characterize the role of the Pax3-Jmjd1c interaction for activation of gene expression within the myogenic program.

**Poster #3:**

**Title / Titre :**

**THE ORPHAN ADHESION G-PROTEIN COUPLED RECEPTOR GPR116 REGULATES MUSCLE STEM CELL QUIESCENCE AND SELF-RENEWAL.**

**Authors / Auteurs :**

Ryo Fujita<sup>1,2</sup>, Solene Jamet<sup>2</sup>, Arhamatoulaye Maiga<sup>3</sup>, Michel Bouvier<sup>3</sup> Colin Crist<sup>1,2</sup> <sup>1</sup>Department of Human Genetics, McGill University, <sup>2</sup> Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec, H3A 1B1, Canada, <sup>3</sup>Unité de recherche en pharmacologie moléculaire (IRIC), Université de de Montréal.

**Abstract / Résumé :**

Muscle stem cells reside as quiescent cells but activate the myogenic program and the cell cycle in response to injury. These activated myogenic progenitors amplify before differentiating into new myofibers or self-renew to restore the muscle stem cell pool. The muscle stem cell microenvironment is critical for maintaining the muscle stem cell pool, however it is unclear how extrinsic cues from the microenvironment transmit intrinsic signaling in the cytoplasm to maintain muscle stem cell properties. The adhesion G-protein coupled receptor (GPCR) family of GPCRs commonly contain a large adhesion domain in the extracellular region that has been shown to interact with multiple adhesion molecules including integrins, cadherins, and laminins, as well as convert the external stimuli into internal signaling to regulate cellular homeostasis. Amongst the adhesion GPCRS, the orphan receptor GPR116 is highly expressed in quiescent muscle stem cells. Here we demonstrated that the conditional deletion of GPR116 from muscle stem cells leads to a break in quiescent state and gradual loss of a muscle stem cell pool over time. Moreover, GPR116-conditional knockout mice exhibited impaired muscle regeneration ability after repetitive muscle injury accompanied with a decreased number of muscle stem cells undergoing self-renewal. Thus, our results suggest that GPR116 is an integral bridge connecting the extrinsic environment with intrinsic signaling to maintain muscle stem cell quiescence and self-renewal.

**Poster #4:**

**Title / Titre :**

**PANCREATIC  $\beta$ -CELL IMMUNOISOLATION WITH A NOVEL MICROCHANNEL EMULSIFICATION DEVICE**

**Authors / Auteurs :**

Christina Bitar, Karen Markwick, Corinne Hoesli (McGill University)

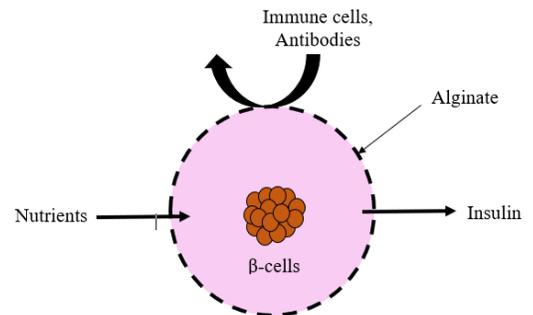
**Abstract / Résumé :**

Background: Islet transplantation has emerged as an attractive long-term treatment option for type 1 diabetic patients involving the implantation of donor pancreatic  $\beta$ -cells. However, this cell therapy requires the administration of lifelong immunosuppressive drugs, which pose a risk of infection, to prevent an immune response against the donor tissue. Alternatively, islet encapsulation with an immunoprotective alginate material (Figure 1) may limit or avoid the need for immune suppression. The objective of this work was to develop and optimize a novel microchannel emulsification (MCE) device that can immobilize  $\beta$ -cells in a semi-permeable alginate matrix.

Methods: In the MCE device, a to-be-dispersed cell-containing alginate phase flows through parallel 700 x 200  $\mu$ m microchannels on a hydrophobic polytetrafluoroethylene plate. Alginate droplets float upwards in an acidified continuous organic phase as the alginate exits the microchannels. The decrease in pH causes the gelation of the alginate droplets to form solid microbeads containing immobilized cells. The viability of encapsulated mouse insulinoma 6 (MIN6) cells was measured by Live/Dead staining.

Results: The selection of 3M™ Novec™ 7500 Engineered fluid led to the uniform production of ~1.8 mm alginate microbeads at throughputs reaching 140 mL/h per channel. Preliminary results demonstrated that MIN6 cells survived the encapsulation process at decreased residence times in the continuous phase.

Conclusion: The MCE process is a promising low-cost and high-throughput method not only for  $\beta$ -cell encapsulation, but also for the immunoisolation of a variety of therapeutic cell types.



**Figure 1.** Schematic of  $\beta$ -cell encapsulation in alginate.

**Poster #5:**

**Title / Titre :**

**THE ROLE OF SPINDLE ORIENTATION IN PROSTATE STEM/PROGENITOR CELLS AND DEVELOPMENT**

**Authors / Auteurs :**

Maxwell Shafer, Sophie Viala, Mathieu Tremblay and Maxime Bouchard.

**Abstract / Résumé :**

During prostate development, basal and luminal cell lineages are generated through symmetric and asymmetric divisions of bipotent basal stem cells. However, the extent to which spindle orientation controls the symmetry of divisions and tissue architecture, and the upstream factors regulating this process, are still elusive. Using mouse genetics and microscopy, we show that loss of Gata3 in the developing prostate leads to a mis-localization of PRKCZ, which results 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.

Those observations led us to further investigate the role of spindle orientation in the developing prostate. We use a genetic approach to specifically target mitotic spindle regulators identified in other tissues. Analysis of these mutants leads us to the identification of spindle regulators in the developing prostate stem/progenitor cells. It also suggests that spindle orientation affects tissue architecture. Additional techniques, such as ex-vivo live-imaging of tissue sections, lineage tracing and sphere-forming assays, will be used to explore the role of spindle orientation on cell fate and stem cell potential.

Together, these results advance our understanding of spindle orientation regulation in the stem and progenitor cells of the developing prostate. This project will also improve our comprehension of the connection between oriented cell division and lineage specification, cell fate and tissue architecture.

**Poster #6:**

**Title / Titre :**

**AGE EFFECT ON THE REGENERATIVE CAPACITY OF SKELETAL MUSCLE STEM CELLS**

**Authors / Auteurs :**

Felicia Lazure<sup>1,2</sup>, Graham Lean<sup>1,2</sup>, Colin Crist<sup>1,2</sup>, Krum Asiev<sup>3</sup>, Theodore J. Perkins<sup>4</sup>, and Vahab D. Soleimani<sup>1,2</sup>

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**Abstract/Résumé:**

In skeletal muscle, a rare population of Muscle Stem Cells (MuSCs), also called Satellite Cells, maintains lifelong regenerative capacity of muscle. Upon a signal from their niche, satellite cells can become activated to either maintain the satellite cell pool or differentiate to repair damaged tissue. However, aging has a detrimental effect on satellite cells' regenerative ability. The extent to which the age-related defect in satellite cell function is cell intrinsic or is induced by factors of the aging niche is poorly understood.

Epigenetic mechanisms play a key role in the aging phenotype of adult tissues. In order to determine how the epigenetic landscape is altered during aging, we analyze muscle stem cells within their niche in young and old mice by ChIP-seq for key activating and repressive histone marks. Due to the plasticity of epigenetic marks, we hypothesize that the niche is capable of reprogramming the transcriptome of satellite cells through modification of the epigenome.

To separate niche-mediated from cell-intrinsic age-related modifications, we perform allogeneic muscle stem cell transplantation from old donors to young recipient mice, followed by RNA-seq library preparation of donor cells pre- and post- transplantation using Switching Mechanism at 5' End of RNA Template (SMART) technologies. Importantly, we found that old satellite cells can repopulate the skeletal muscle of young irradiated immunocompromised mice. Donor stem cells also home to the correct sub laminar niche location.

By comparative analysis of gene expression between conditions, we have identified niche-induced changes in the satellite cell transcriptome. Importantly, the integration of epigenetic and transcriptomic data will help identify reversible age-related pathways that can be used as potential epigenetic drug targets in patients with age-related muscle-wasting diseases.

**Poster #7:**

**Title / Titre :**

**HUMAN ANTIGEN R (HUR) MEDIATES CANCER-INDUCED MUSCLE WASTING BY REGULATING PGC1A DEPENDENT MUSCLE FIBER TYPE SPECIFICATION.**

**Authors / Auteurs :**

Anne-Marie Tremblay, Dr Imed Gallouzi

**Abstract / Résumé :**

The Human antigen R (HuR), a well-known master regulator of gene expression post-transcriptionally, is known to promote muscle fiber formation in vitro, but its role in muscle physiology, in vivo, has yet to be explored. Several reports have indicated that the total knockout of HuR in mice is embryonic lethal. Therefore, to investigate the in vivo role of HuR in skeletal muscle formation and physiology, we used the Cre-LoxP system to generate a HuR muscle-specific knockout mice (MyoDCre+;Elav1fl/fl). Here we show that muscle specific HuR knockout (muHuR-KO) mice have higher exercise endurance that is associated with a significant increase in oxygen consumption, carbon dioxide production, and respiratory exchange ratio. Histological analysis demonstrates that the absence of HuR significantly enhances the number of oxidative type I fibers in several skeletal muscles, indicating that under normal conditions, HuR promotes the formation of glycolytic type II fibers. HuR mediates this outcome by acting, unexpectedly, as an mRNA destabilizing factor in skeletal muscle, decreasing the expression of PGC-1 $\alpha$  mRNA, a well-known promoter of type I fiber formation. Furthermore, this oxidative phenotype protects muHuR-KO mice against cancer cachexia induced muscle wasting. Together, our data uncover a novel role for HuR as a key regulator of muscle fiber type specification and provide a proof-of-principle that targeting HuR expression in muscle can be used therapeutically to combat deadly syndromes, such as cachexia-induced muscle wasting.

**Poster #8:**

**Title / Titre :**

**NUCLEAR DEUBIQUITINASE MYSM1 AS A POTENTIAL TARGET FOR C-MYC-DRIVEN HEMATOLOGICAL MALIGNANCIES**

**Authors / Auteurs:**

Yun Hsiao Lin<sup>1,2</sup>, Michael Förster<sup>1,2</sup>, Anastasia Nijnik<sup>1,2</sup>

1. Department of Physiology, McGill University, Montreal, QC, Canada
2. McGill University Centre on Complex Traits, McGill University, Montreal, QC, Canada

**Abstract / Résumé:**

Background: c-MYC, or MYC, is a transcription factor important for regulating genes involved in fundamental cellular processes, such as proliferation and apoptosis, in hematopoietic cells, as well as other cell types. Dysregulation of MYC is common in hematological malignancies, with *Myc* alterations present in ~80% of Burkitt lymphoma cases and ~5-14% of diffuse large B-cell lymphoma cases. As MYC-driven tumours depend on MYC for survival, this transcription factor has been an appealing therapeutic target; however, without a substrate-binding site, MYC proves to be a difficult drug target. Due to the high prevalence of MYC abnormalities in hematological malignancies, it is crucial to explore other strategies to counteract the effects of MYC dysregulation.

Our lab and others have characterized MYSM1 as a nuclear protein required for maintaining hematopoietic stem cells (HSCs) and blood cell production in mice and humans. Loss of MYSM1 is associated with dysfunctional HSCs and decreased lymphocyte numbers, both of which are mediated through p53 upregulation. As such, we investigated an indirect approach of targeting MYC in B-cell lymphoma via inhibiting *Mysm1* and activating p53.

Methods and Results: Our data explores the cellular and molecular mechanism of MYSM1 activity in MYC-driven tumours and characterizes the outcome of deleting *Mysm1* on disease-progression in a MYC-driven B cell lymphoma mouse model.

Conclusion and Significance: This work elucidates the mechanism underlying the effects of inhibiting MYSM1 in MYC-driven lymphoma and suggests new strategies for treatment of MYC-driven hematological malignancies.

**Poster #9:**

**Title / Titre :**

**DERIVATION OF FUNCTIONAL AND PHYSIOLOGICALLY RELEVANT IPSC-DERIVED NEURONS**

**Authors / Auteurs :**

Baumann E, Huang J, Ribocco M, Sodja C, Corluca S, Aylsworth A, Tauskela J, Martina M, Stanimirovic D, Bani-Yaghoub M, Jezierski A

Human Health Therapeutics Portfolio, National Research Council of Canada, Ottawa, ON, K1A 0R6, Canada. Mahmud Bani-Yaghoub is deceased. Correspondence and requests for materials should be addressed to A.J. (email: [anna.jezierski@nrc.ca](mailto:anna.jezierski@nrc.ca))

**Abstract / Résumé :**

The functional network of human iPSC-derived neurons (iNs) is a potentially powerful *in vitro* model for evaluating disease mechanisms and drug responses. We have optimized the differentiation and defined the culture conditions and duration required for the full functional maturation of iNs and the establishment of synchronous networks. The mature iNs display characteristic neuronal morphology with defined cell bodies and branching neurite extensions and stain positive for several neuronal markers such as  $\beta$ III-TUBULIN, NCAM, NeuN, VGLUT1, VGLUT2, SYNAPTOTAGMIN and SYNAPTOPHYSIN. We also investigated the development of spontaneous electrophysiological activity demonstrating that the mature iNs are able to generate action potentials that are evoked by depolarizing current pulses. Spontaneous synchronized synaptic activity and pharmacological responses of the iNs were also assessed by multi-electrode arrays (MEAs) over a period of 24 days *in vitro* to evaluate the presence of functional channels/receptors based on the responses to a panel of neuroactive drugs eliciting exogenous and endogenous responses. These results confirm the electrophysiological and pharmacological properties of mature human neurons.

**Poster #10:**

**Title/Titre:**

**MUTATIONS IN HUMAN HISTONE H3 ARE PRE-LEUKEMIC EVENTS THAT PROMOTE HEMATOPOIETIC STEM CELL EXPANSION AND LEUKEMIC AGGRESSIVENESS**

**Authors/Auteurs:**

Meaghan Boileau<sup>1</sup>, Margret Shirinian<sup>2</sup>, Tenzin Gayden<sup>1</sup>, Ashot S. Harutyunyan<sup>1</sup>, Carol C.L. Chen<sup>1</sup>, Leonie G. Mikael<sup>1</sup>, Heather M. Duncan<sup>1</sup>, Andrea L. Neumann<sup>1</sup>, Patricia Arreba-Tutusa<sup>1</sup>, Nicolas De Jay<sup>1</sup>, Michele Zeinieh<sup>1</sup>, Katya Rossokhata<sup>1</sup>, Yelu Zhang<sup>1</sup>, Hamid Nikbakht<sup>1</sup>, Carine Mouawad<sup>2</sup>, Radwan Massoud<sup>2</sup>, Felice Frey<sup>2</sup>, Rihab Nasr<sup>2</sup>, Jean El Cheikh<sup>2</sup>, Marwan El Sabban<sup>2</sup>, Claudia L. Kleinman<sup>1</sup>, Rami Mahfouz<sup>2</sup>, Mark D. Minden<sup>3</sup>, Nada Jabado<sup>1</sup>, Ali Bazarbachi<sup>2</sup>, and Kolja Eppert<sup>1</sup>  
<sup>1</sup>McGill University, Montreal, Quebec, Canada; <sup>2</sup>American University of Beirut, Beirut, Lebanon; <sup>3</sup>Princess Margaret Cancer Centre, University Health Network, University of Toronto, Toronto, Ontario, Canada

**Abstract/Résumé:**

Aberrant epigenetic regulation is often an early step in the development of acute myeloid leukemia (AML). Our ability to prevent or treat AML is limited by our incomplete understanding of this epigenetic disruption, including improper histone methylation. Rare K27 mutations in histone H3 have been identified in T-acute lymphoid leukemia, myelodysplastic syndrome and AML. Here, in a comprehensive analysis of all histone H3 genes, we demonstrate that H3 mutations are early events in AML leukemogenesis.

We sequenced 16 H3 genes in 434 primary AML samples and identified 7 mutations (1.6%) (*HIST1H3F*-K27I, *HIST1H3H*-K27M, *H3F3A*-K27M/A26P, *HIST1H3A*-Q69H, *H3F3C*-R2Q/R8H), with an enrichment in secondary AML (9%). The mutations were present in the founding clones, indicated by high variant allele frequencies. In two cases with corresponding normal samples, we identified Q69H and K27M mutations in pre-leukemic hematopoietic stem cells (HSCs).

Consistent with a role in pre-leukemic HSC clonal expansion, primary and secondary xenotransplantation confirmed that K27M/I mutant histones increased normal human HSC frequency. Furthermore, K27 and Q69 mutations altered myeloid and erythroid differentiation. In established AML, K27M/I mutations increased proliferation and progenitor frequency *in vitro* demonstrated by colony formation unit assay and drastically increased leukemic aggressiveness *in vivo*. We performed RNA-seq and ChIP-seq analyses and observed changes in gene expression, with a global decrease in H3K27 tri-methylation in AML cells with the K27M/I mutations. Furthermore, there was a more drastic loss of H3K27 tri-methylation at promoters of upregulated genes and a concurrent increase in H3K27 acetylation. Gene ontology analysis indicated alterations in regulators of biological processes involved in erythrocyte and myeloid differentiation. These findings place H3 mutations alongside *ASXL1* and *EZH2* mutations as drivers of an altered histone epigenome in pre-leukemia and secondary AML.

**Poster #11:**

**Title / Titre :**

**IDENTIFYING THE ROLE PROTEIN ARGININE METHYL TRANSFERASE 1 IN MUSCLE STEM CELL FUNCTION**

**Authors / Auteurs :**

Claudia Dominici, Stéphane Richard

**Abstract / Résumé :**

Duchenne muscular dystrophy (DMD) is a muscle wasting disease arising from a mutation in the gene encoding dystrophin. Muscle stem cells (MuSCs) have extraordinary regenerative capacity and can differentiate into mature muscle. In patients with DMD, the MuSC pool is eventually exhausted and muscle regeneration is halted. Developing therapies to improve regeneration must involve elucidating which molecular pathways regulate MuSC differentiation and self-renewal. Key components of the molecular machinery in MuSCs are protein arginine methyl transferases (PRMTs). We have shown that PRMT1 effects MuSC fate by regulating the transcriptional activation of MyoD. PRMT1 knockout mouse myoblasts have enhanced proliferation but are unable to differentiate. We therefore hypothesize that transient PRMT1 inhibition will allow for expansion of the MuSC pool, priming the muscle for enhanced regeneration following injury once inhibition is de-repressed. We therefore use MS023, an inhibitor of type I PRMTs. We observed enhanced proliferation of myoblasts in vitro as well as increased MuSC number on cultured muscle fibers. To assess MS023 in vivo, GFP<sup>+</sup> MuSCs will be isolated and treated with MS023, then engrafted into recipient mice that have been injected with cardiotoxin to induce muscle injury 24h prior. Following 3 weeks of regeneration, cross-sections of the injured muscle will be assessed for GFP<sup>+</sup> fibers and MuSC number. We expect that the mice with MS023-treated MuSCs will have an expanded MuSC pool and more GFP<sup>+</sup> fibers. Inhibition of PRMT1 is a promising avenue to explore for MuSC pool expansion and ultimately the treatment of muscle wasting disease.

**Poster #12:**

**Title / Titre :**

**THE ROLE OF PARYLATION IN THE HUR-MEDIATED MODULATION OF MUSCLE FIBER FORMATION.**

**Authors / Auteurs :**

Mubaid S.1,2, Hall D.1,2, Lian X.J. 1,2, Gagné J.P. 3, Carlile G. 1, Di Marco S. 1,2, Poirier G. 3, Thomas D.Y. 1, Gallouzi I.E1,2. Dept. of Biochemistry McGill University<sup>1</sup>, Rosalind & Morris Goodman Cancer Research Centre<sup>2</sup>, Montreal, QC, Canada. Centre de Recherche du CHUQ University of Laval<sup>3</sup>. Quebec, QC, Canada.

**Abstract / Résumé :**

Skeletal muscle is a tissue composed of oriented and dense myofibers. Upon injury, a repair process, known as myogenesis, is initiated to replace damaged fibers. We and others have shown that the RNA binding protein HuR promotes myogenesis by posttranscriptionally regulating mRNAs encoding key players that modulate muscle fiber formation. Importantly, the function of HuR is dependent on post-translational modifications that affect its interaction with mRNAs, as well as other trans-acting factors. Recently HuR was found to be covalently modified by poly(ADP-ribose) ribosylation (PARylation), by an enzyme named Poly(ADP-Ribose) Polymerase 1 (PARP1). However, the impact of this modification on HuR's function in muscle formation is unknown. Here, using in vitro assay, we confirmed that HuR is PARylated by PARP-1, and showed that other PARPs including PARP5a and b are also capable of PARylating HuR. Interestingly, the knockdown of PARP5a but not the others prevented muscle cell differentiation. We observed that PARP5a promotes myogenesis by PARylating HuR, increasing its interaction with mRNAs encoding myogenic regulating factors, resulting in the altered expression of these messages. We also determined that PARylation of HuR modulates its interaction with protein partners such as YB1 and KSRP that are required for the HuR-mediated regulation of target messages as well as HuR promyogenic function. Our work, therefore, reveals a new essential role for PARP5a in muscle cell differentiation and identifies the PARylation of HuR as a key event required for this process.

**Poster #13:**

**Title / Titre :**

**MODELLING OF SETBP1 DISORDERS THROUGH IPSCS AND POTENTIAL TREATMENTS**

**Authors / Auteurs :**

Antonyan, Lilit. Department of Human Genetics, McGill University.  
Douglas Mental Health University Institute.  
Ersnt, Carl. Department of Human Genetics, McGill University.  
Douglas Mental Health University Institute.

**Abstract / Résumé :**

The use of induced pluripotent stem cells (iPSCs) is vital for the study of rare neurological diseases in humans. They allow scientists to use easily obtained cells, such as cells found in the urine or fibroblasts, from any particular patient and covert them to neuronal cell types of interest. In this study, 3 sets of patients and controls with mutations in the *SETBP1* gene were recruited to be studied. *SETBP1* is a gene that encodes for SET binding protein 1. According to the type of mutation found in this gene, different disease phenotypes can arise. Missense mutations in a 4-aminoacid hotspot cause Schinzel-Giedion syndrome (SGS), an autosomal dominant disease characterized by severe mental retardation, facial and renal abnormalities, brain anomalies and neurological degeneration. Conversely, loss-of-function (LOF) mutations cause a disease with a milder phenotype, where children present with mild mental retardation, expressive language impairment, autistic-traits and seizure. Our aim is to use induced pluripotent stem cells (iPSCs) derived from *SETBP1* patients and controls to reprogram them into neuron-like cells to characterize and model these diseases molecularly. The downstream goal is to develop genetic therapeutic tools to treat and correct the pathologic phenotypes according to the disease mechanism.

**Poster #14:**

**Title:**

**ANGIOGENESIS-PRIMED PDMS MICROFLUIDIC DEVICE FOR BRAIN ORGANOID GROWTH AND VASCULARIZATION**

**Authors:**

Alia Alameri, Grant Ongo, David Juncker

**Abstract:**

Three-dimensional hiPSC-derived brain organoids are promising models to investigate the mechanisms underlying neurodegenerative diseases<sup>1</sup>. A major challenge that hinders applications of these organoids is the lack of vasculature, which causes necrosis in organoid cores once they exceed several hundred microns in diameter<sup>2</sup>. Vascularization of spheroids that were 100 and 500- $\mu$ m in diameter has been demonstrated<sup>3, 4</sup>. However, vascularization of organoids on the millimeter scale remains unexplored. To address this, we developed a microfluidic device to promote the in-vitro vascularization of brain organoids. Our device incorporates 3D-printing and optimized microfluidic logic necessary for organoid vascularization. The device consists of a middle channel that contains the organoid, connected to side channels where endothelial cells (ECs) are introduced. The middle channel is filled with a fibrin-based hydrogel to support the proliferation of ECs toward the organoid. Stop valves are incorporated to prevent hydrogel overflow to the side channels and to allow for controlled EC migration. To fabricate the device, positive molds were 3D-printed using a digital-light-processing 3D-printer. Polydimethylsiloxane (PDMS), chosen for its gas permeability, transparency, and biocompatibility, was casted on the molds and polymerized at 60°C overnight<sup>5</sup>. The PDMS device was sealed, and liquids were injected into the middle channel and showed no leakage through the stop valves. Currently, the device is being tested for its biocompatibility for EC cultures, and biochemical factors influencing vascularization. Our device could overcome necrosis allowing organoids to increase in size and complexity, enable longer culture times, and allow perfusion of substances into the organoids mimicking in-vivo brains.

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**Poster #15:**

**Title / Titre :**

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

**Authors / Auteurs :**

Deepak Saini (M.Sc.3) and Yojiro Yamanaka

**Abstract / Résumé :**

Compaction is an essential morphogenetic event for blastocyst formation during preimplantation mouse embryo development. This morphological change is recognized an increase in cell-cell contact area, minimizing the exposed surface area of an embryo. However, the molecular mechanisms driving compaction have not been elucidated.

To identify molecular changes before and after compaction, we investigated the distribution of cortical F-actin, a core regulator of cell shape. We observed a reduction of F-actin at cell-cell contacts after compaction, suggesting the generation two cortical subdomains within a cell. We speculate that the cortical F-actin levels reflect cortical contractility and that the reduction of F-actin at cell-cell contact areas facilitates the flattening of cells.

What regulates this reduction of F-actin at cell-cell contacts? 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 before and after compaction, we observed an increase of  $\alpha$ -catenin at cell-cell contacts during compaction. Since homodimer forms of  $\alpha$ -catenin suppresses actin polymerization by blocking the ARP2/3 activity, we speculate that the increased level of  $\alpha$ -catenin reflects its homodimer conformation to reduce the level of F-actin at cell-cell contacts

Interestingly, E-cadherin null embryos exhibit two phenotypes not related to cell adhesion; (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 contact-free surface to negatively regulate polarization as well as regulating the actocytoskeleton.

**Poster #16:**

**Title / Titre :**

**THE ROLE OF QKI IN MICROGLIA**

**Authors / Auteurs :**

Jeesan Lee, Xiaoru Chen, Stephane Richard

**Abstract / Résumé :**

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) where a person's immune system mistakenly attacks myelin. Research show that the progression of MS is linked to the defects of immune cells in the CNS known as microglia. However, what causes the defects of microglia and how this affects the progression of MS is largely unknown. To understand the role of microglia in de- and re-myelination settings, we focused on the RNA binding protein Quaking (QKI). We successfully generated a mouse model that specifically deletes QKI in microglia. Depletion of QKI resulted in abnormal microglial morphology and alternative splicing patterns in pre-mRNAs. To understand how mice without QKI react to the demyelination, we used a cuprizone diet that causes demyelination and subsequent accumulation of microglia to the demyelinated region. Our results revealed that QKI depleted mice showed an inability of microglia to migrate towards the demyelinated region unlike normal mice where abundant microglia were observed. Based on this cumulative evidence, our results shows that QKI is an important regulator of microglia. Thus these results will deepen the understanding of microglia biology and may provide valuable information towards microglia targeted treatment options in MS.

**Poster #17:**

**Title / Titre :**

**THE ROLE OF OXYGEN AND GLUCOSE ON THE SURVIVABILITY OF MOUSE BONE MARROW MESENCHYMAL STEM CELLS**

**Authors / Auteurs :**

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1.  
2.

**Abstract / Résumé :**

A key limitation for bulk tissue engineered scaffold development is the inability to deliver oxygen to cells found within scaffolds – which can result in hypoxia and tissue necrosis. The objective of this study was to determine the necessary requirements for anoxic MSC survival up to 13 days.

Initially, unmodified and Erythropoietin (Epo)-gene modified mouse bone marrow MSCs were cultured for 7 days in normoxic (21%) and anoxic (<1%) oxygen conditions. In the second set of experiments, unmodified MSCs were cultured in anoxia for up to 13 days in high (4.5g/L) and low (1g/L) glucose media. Cell viabilities were determined for up to 13 days.

We observed from our oxygen experiments, that while both groups of MSCs showed similar viabilities (78-88%) when compared to normoxic controls (85-91%), Epo-MSCs showed a significantly higher cell count ( $10.1 \times 10^4 \pm 4.5$ ) compared to unmodified MSCs ( $7 \times 10^4 \pm 3.8$ ) when cultured in anoxia. In anoxia, addition of high glucose media to unmodified MSCs led to a significant increase in cell viability percentage when compared to low glucose media at 13 days.

Our results indicate that both groups of MSCs can survive for up to 13 days in the absence of oxygen, with higher cells counts seen for Epo-MSCs. Protein production is unaffected by the absence of oxygen compared to normoxic controls. Additional glucose allowed for a 37% increase in MSC survival in anoxia. These results may lead to future research in scaffold designs that do not require vasculature to maintain cell survival for extended periods of time.

**Poster #18:**

**Title / Titre:**

**A NOVEL HUMAN INDUCED PLURIPOTENT STEM CELL BLOOD-BRAIN BARRIER MODEL: APPLICABILITY TO STUDY ANTIBODY TRIGGERED RECEPTOR-MEDIATED TRANSCYTOSIS**

**Authors / Auteurs:**

Jez Huang, Maria Ribecco-Lutkiewicz, Caroline Sodja, Julie Haukenfrers, Arsalan S. Haqqani, Dao Ly, Peter Zachar, Ewa Baumann, Marguerite Ball, Marina Rukhlova, Marzia Martina, Qing Liu, Danica Stanimirovic, Anna Jezierski & Mahmud Bani-Yaghoub Human Health Therapeutics Portfolio, National Research Council of Canada, Ottawa, ON, K1A 0R6, Canada. Mahmud Bani-Yaghoub is deceased. Correspondence and requests for materials should be addressed to A.J. (email: anna.jezierski@nrc.ca)

**Abstract / Résumé:**

We have developed a renewable, scalable and transgene free human blood-brain barrier model, composed of brain endothelial cells (BECs), generated from human amniotic fluid derived induced pluripotent stem cells (AF-iPSC), which can also give rise to syngeneic neural cells of the neurovascular unit. These AF-iPSC-derived BECs (i-BEC) exhibited high transendothelial electrical resistance (up to  $1500\Omega\text{ cm}^2$ ) inducible by astrocyte-derived molecular cues and retinoic acid treatment, polarized expression of functional efflux transporters and receptor mediated transcytosis triggered by antibodies against specific receptors. In vitro human BBB models enable pre-clinical screening of central nervous system (CNS)-targeting drugs and are of particular importance for assessing species-specific/selective transport mechanisms. This i-BEC human BBB model discriminates species-selective antibody-mediated transcytosis mechanisms, is predictive of in vivo CNS exposure of rodent cross-reactive antibodies and can be implemented into pre-clinical CNS drug discovery and development processes.

**Poster #19:**

**Title / Titre :**

**CHARACTERIZATION OF THE MYSM1 REGULATED TRANSCRIPTION NETWORK IN HEMATOPOIETIC STEM AND PROGENITOR CELLS**

**Authors / Auteurs :**

HanChen Wang<sup>1,2,3</sup>, Jad I Belle<sup>1,2</sup>, Jessica C Petrov<sup>1,2</sup>, #David Langlais<sup>2,3,4</sup>, #Anastasia Nijnik<sup>1,2</sup>

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**Abstract / Résumé :**

MYSM1 is a histone H2A deubiquitinase and a regulator of gene expression. In both humans and mice, MYSM1 is essential for the function of hematopoietic stem cell (HSC), the maintenance of hematopoiesis, and the differentiation of blood cells. We have previously shown that MYSM1 represses the activity of the tumor suppressor and transcription factor p53 in hematopoietic stem and progenitor cells (HSPC). The loss of MYSM1 leads to p53 activation, p53-dependent HSPC depletion, and bone marrow failure. The deletion of p53 in MYSM1-deficient mice reverts the phenotype to normal, indicating an antagonistic role of MYSM1 to p53. Currently, the mechanism of how MYSM1-deficiency activates the p53 pathway remains unknown.

In recent unpublished work, we acquired the transcriptional signatures of MYSM1-deficient and control mouse HSCs with RNA-Seq and mapped the genome-wide MYSM1 DNA-binding sites in hematopoietic progenitor cell-lines with ChIP-Seq. I analyzed these datasets and identified an important group of genes regulated by MYSM1. This group of genes is essential to HSC biology and hematopoiesis, and its dysregulation has been described to activate the p53 pathway in many studies.

This project provides insights into the transcriptional changes leading to bone marrow failure in MYSM1-deficiency and facilitates future studies on the molecular mechanisms of MYSM1 in the regulation of transcription. In long-term, this knowledge will contribute to the better understanding and management of the human MYSM1-deficiency and possibly other disorders of hematopoiesis.

**Poster #20:**

**Title / Titre :**

**COMPARISON OF STEM CELL THERAPEUTIC POTENCIES TO HUMAN INTERVERTEBRAL DISC DEGENERATION THROUGH THE SUPPRESSION OF TOLL-LIKE RECEPTORS**

**Authors / Auteurs :**

Li Li, Derek Rosenzweig, Lisbet Haglund

**Abstract / Résumé :**

Background: Low back pain (LBP) is often related to intervertebral disc (IVD) degeneration and linked to a loss of integrity and failure of the central portion. Cell-based therapies such as stem cell implantation have recently been proposed as minimally invasive approaches to reduce LBP and repair degenerate discs. Previous studies indicated that toll-like receptors activation can induce degeneration in human discs, as they regulate cytokine, neurotrophin, and protease expression and control key factors in disc degeneration. Therefore, blocking these receptors may counteract the inflammatory environment and potentially provide new therapeutic options for early degeneration prior to or concurrent with cell supplementation strategies. Both human bone marrow-derived mesenchymal stem cells and adipose stem cells are reported as suitable candidates for cell therapy. Here, we will compare and determine the superior cell type and evaluate them in combination with drugs blocking inflammation regulated by toll-like receptors.

Hypotheses: 1) Toll-like receptor suppression will improve differentiation of the stem cells to IVD-like cells. 2) Human adipose stem cells will have a stronger capacity than bone marrow derived stem cells to decelerate IVD degeneration.

Methods: Therapeutic indicators of degeneration will be evaluated. Cell matrix formation will be verified by IHC and DMMB assays. Bioactive, inflammatory, and degenerative factors will be detected via protein arrays. Cell markers that indicate cell differentiation conditions will be detected through FACS.

Prospective results: Human adipose stem cells will have the strongest capacity to differentiate to IVD cells and subsequently have the best potential to prevent or reduce IVD degeneration.

**Poster #21:**

**Title / Titre :**

**CAN SURFACE CHEMISTRY POLARIZE MACROPHAGES TOWARDS WOUND-HEALING PHENOTYPES?**

**Authors / Auteurs :**

Emily Buck, Seunghwan Lee, Laura Stone, and Marta Cerruti

**Abstract / Résumé :**

Background: Macrophages help direct the process of tissue repair with their ability to polarize, or change their phenotype, throughout the wound healing process<sup>1</sup>. During the early stages, macrophages take on a pro-inflammatory state and promote angiogenesis while at later stages, they shift towards an anti-inflammatory state and initiate wound healing. Since implanting a biomaterial creates a wound, macrophages are recruited to the implant site to direct the repair process where they interact with the implant's surface and either promote normal repair or foreign body reactions (FBR)<sup>2</sup>. Many implants with hydrophobic surfaces elicit FBR, and surface modifications can help reduce the FBR<sup>3-4</sup>. However, there is little understanding of how implant surface properties impact macrophage-mediated FBR. Some work has demonstrated that altering wettability can affect macrophage polarization<sup>3-4</sup>, but there are inconsistent results because wettability depends on both surface chemistry and topography. To better understand the effect of surface properties on macrophage polarization, surfaces with well-characterized properties are required.

Methods: Polystyrene substrates functionalized with primary amine (NH<sub>2</sub>), carboxylic acid (COOH) or phosphonic acid (PO<sub>3</sub>H<sub>2</sub>) groups were prepared, and their surface properties, including surface composition, wettability and topography, were characterized. THP-1 human macrophage-like cells were then cultured on the surfaces, and their gene expression, cytokine production and adhesion were measured after 4, 24, and 48h.

Results: All surfaces promoted similar macrophage adhesion and spreading. However, the COOH surfaces led to significant increases in the anti-inflammatory cytokine, IL-10, after 48h.

Conclusions: COOH groups could be applied to the surface of implants to promote a wound-healing phenotype in macrophages and improve implant integration.

References: 1) *Semin. Immunol.* (2008) 20:86-100; 2) *Mater. Today* (2016) 19:304-321; 3) *Biomaterials* (2009) 30:5497-5504; 4) *J. Biomed. Mat. Res. A* (2014) 102A:60-67

**Poster #22:**

**Title / Titre:**

**REGULATION OF GLIOMA STEM CELL SELF-RENEWAL AND TUMORIGENESIS BY CARBOHYDRATE BINDING PROTEIN**

**Authors / Auteurs:**

Ahmad Sharaneek<sup>1</sup>, Idris Fatakdawala<sup>1</sup>, Arezu Jahani-Asl<sup>1</sup>

**Abstract / Résumé:**

Glioma Stem Cells (GSC) are a population of malignant self-renewing stem cells in glioblastoma (GBM) tumors, the most common and aggressive primary brain tumor in the adult brain. GSC promote tumor growth and recurrence and acquire resistance to therapy. Strategies to target GSC in the tumor bulk are urgently needed in order to develop more effective therapeutic strategies for GBM. EGFRvIII/STAT3 signaling is a major oncogenic pathway in GBM. Here, we report our discovery that Galectin1, a family member of carbohydrate-binding proteins with affinity for b-galactosides, promotes glioma stem cell self-renewal and tumor growth in EGFRvIII-expressing subset of tumors. Analysis of RNA-Seq data shows that LGALS1, the gene encoding Galectin1, is highly expressed in human EGFRvIII-expressing GSC and its expression correlates with the expression of transcription factor STAT3. Importantly, STAT3 directly binds the promoter of LGALS1 to upregulate its expression and knockdown of STAT3 significantly attenuates LGALS1 mRNA levels. Genetic knockdown of LGALS1 impairs the ability of GSC to form spheres, as assayed in limiting dilution assay, suggesting that LGALS1-STAT3 signaling regulates glioma stem cell fate in EGFRvIII tumor subset. Strikingly, we employed genetic and pharmacological approaches in patient derived xenografts and found that LGALS1/Galectin1 impairs gliomas stem cell growth and tumorigenesis. Treatment of GSC with OTX008, a specific inhibitor of galectin-1, significantly attenuates the self-renewal ability of these cells and impairs tumorigenesis. Together, our findings suggest that targeting LGALS1/Galectin1 in combination with inhibitors of EGFRvIII/STAT3 pathway may provide an effective strategy for the future treatment of these deadly brain tumors

**Poster #23:**

**Title / Titre :**

**RSPO1 ENHANCES INTEGRATION OF NEPHRON PROGENITOR CELLS INTO GLYCEROL-DAMAGED RENAL TUBULES**

**Authors / Auteurs :**

Dickinson, K (McGill). Hammond, L (McGill). Carroll, T (UT Southwestern). Goodyer, P (McGill).

**Abstract / Résumé :**

Background: Primed WT1(+); CD24(+) cells from embryonic mouse kidneys are able to integrate into damaged adult kidneys after glycerol-induced acute kidney injury (AKI). Pre-treating the cells with an inhibitor of the Wnt/ $\beta$ -catenin signalling pathway significantly diminished tubular integration, highlighting the importance the Wnt/ $\beta$ -catenin pathway in this process. We hypothesize that embryonic nephron progenitor cells (NPCs) have been primed to respond to WNTs, and these cells are competent to integrate into an injured adult kidney. In this study, we used M15 cells, derived from early embryonic mouse kidney to identify critical molecules in the Wnt/ $\beta$ -catenin signalling pathway that define the primed NPC.

Methods: To measure activation of the canonical WNT-pathway, we transfected M15 cells with 8X TOPFlash. AKI was induced by intramuscular injection of glycerol. M15 cells were administered to the mouse by tail vein injection.

Results: We treated M15 cells with recombinant WNT9b, resulting in minimal luciferase activity suggesting a signalling component is missing. We analyzed M15 cells for components of the WNT-pathway and detected mRNA expression of Frizzled1-6, Lrp6 but not R-spondin1 (Rspo1). To ascertain whether absence of RSPO1, a WNT agonist, accounts for the lack of responsiveness, we transfected M15 cells with Wnt9b in the presence of recombinant RSPO1 and observed a 4.77-fold increase in luciferase activity. Given the rescue of WNT-responsiveness upon RSPO1 addition, to test whether RSPO1 treated M15 cells integrate better than M15 cells alone in an AKI model, we injected red-labelled cells into glycerol-injured mice. Mice that received M15+RSPO1 displayed a marked increase in cells integrated into the damaged kidney compared to the M15 alone condition.

Conclusion: These data suggest RSPO1 is a critical component to render NPCs responsive to a WNT9b signal and also enhances cell integration in an AKI model. These data have implications in future cell-based therapies to treat human AKI.

**Poster #24:**

**Title / Titre :**

**BIOPRINTING ALGINATE-GELATIN SOFT BIOMATERIAL BIOINK FOR THREE-DIMENSIONAL CANCER CELL CULTURE.**

**Authors / Auteurs:**

Salvador Flores Torres<sup>1</sup>, José Gil Munguia López<sup>1</sup>, Tao Jiang<sup>1</sup>, Jacqueline Kort Mascort<sup>1</sup>, and Joseph M. Kinsella<sup>1</sup> .

**Abstract / Résumé :**

Malignant neoplasms or cancer occurs when cells acquire genetic instability causing uncontrolled cell growth with the potential of invading healthy tissues around the body. Interrogating cancer biology has been an exhausting task for many scientists. Cancer research and drug discovery depend upon robust and clinically relevant models. Most of our experiments are based on traditional, two-dimensional (2D) cell models in which cells are grown on flat, non-representative environments. Although 2D cell-based experiments had been the pillar of many ground-breaking discoveries, cells naturally grow in a volumetric space to form soft tissues and organs structures along with a complex extracellular matrix (ECM) arrangement. Fundamental understanding of cancer cannot be fully concluded from 2D studies due to the lack of physiologically-relevant conditions such as cell-cell and cell-ECM interactions. In this work, we evaluate the use of a naturally-derived biomaterial as an ECM analog to perform 3D cancer cell culture for long periods of time (>80 days). We rely on extrusion bioprinting techniques to produce cancer cell-laden scaffolds with high-reproducibility and control over the initial conditions. This project aims to integrate both engineering approaches and biological principles to present a novel technique to apply biomaterials in cancer research.

**Poster #25:**

**Title / Titre :**

**G PROTEIN-COUPLED RECEPTOR 56 (GPR56/ADGRG1) AS A POTENTIAL FUNCTIONAL REGULATOR OF LEUKEMIC AND HEMATOPOIETIC HUMAN STEM CELLS**

**Authors / Auteurs :**

Heather Duncan, MSc<sup>1\*</sup>, Karin G. Hermans, PhD<sup>2</sup>, Sara Chisling, BSc<sup>3</sup>, Mark D. Minden, MD, PhD<sup>4</sup>, John E. Dick, PhD<sup>4</sup>, Kolja Eppert, PhD<sup>5</sup>

<sup>1</sup>Division of Experimental Medicine, McGill University, Montreal, QC, Canada; <sup>2</sup>Program of Developmental and Stem Cell Biology, Peter Gilgan Centre for Research and Learning, The Hospital for Sick Children, Toronto, ON, Canada; <sup>3</sup>Faculty of Medicine, University of Montreal, Montreal, QC, Canada; <sup>4</sup>Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada; <sup>5</sup>Department of Pediatrics, McGill University, Montreal, QC, Canada.

**Abstract / Résumé :**

Leukemic stem cells (LSCs) sustain acute myeloid leukemia (AML). Improved understanding of LSCs is required to improve therapy. We identified G protein-coupled receptor 56 (GPR56/ADGRG1) among a gene expression signature common to LSCs and normal hematopoietic stem cells (HSCs). GPR56 is a novel marker of high LSC frequency in AML patients, was shown to accelerate leukemogenesis in mice, and may be involved in murine HSC development and regulation. This study aims to establish the functional role of GPR56 in human LSCs and HSCs.

GPR56 expression was associated with worse survival across three microarray AML cohorts ( $p < 0.01$ ). GPR56 expression was higher in samples with worse prognosis ( $p < 0.0001$ ) predicted by cytogenetics. We confirmed higher expression of GPR56 in human LSC and HSC fractions by qRT-PCR. Colony forming cell assays were conducted to determine effects on progenitor proliferation and differentiation. Overexpression of GPR56 increased colony formation in AML cell line MOLM-13 ( $p < 0.05$ ) and cultured AML sample 8227 (GPR56dN,  $p < 0.0001$ ), but did not significantly alter normal human progenitor cell activity. Long-term xenograft assays were performed via intrafemoral injection in immunodeficient mice to determine effects on stem cell function. GPR56 overexpression conferred a significant engraftment advantage ( $p < 0.0001$ ) maintained in secondary 12-week transplants ( $p < 0.05$ ) with sustained hierarchical organization indicating self-renewal of stem cells.

These data suggest that GPR56 enhances HSC function *in vivo*, and may regulate human leukemic progenitors, but not hematopoietic progenitors *in vitro*. Further functional studies will be performed to determine the role GPR56 in LSC function *in vivo* and chemotherapy resistance.

**Poster #26:**

**Title / Titre :**

**IDENTIFYING MALE GERMLINE STEM CELLS: TOWARDS FUNCTIONAL MAPPING OF THE STEM CELL FATE**

**Authors / Auteurs :**

Joëlle Desmarais<sup>1</sup>, Xiangfan Zhang<sup>1</sup>, Makoto Nagano<sup>1</sup> 1. Research Institute of the McGill University Health Center and McGill University, Department of Obstetrics and Gynecology, 1001 Decarie Blvd., Montreal, QC, H4A 3J1.

**Abstract / Résumé :**

Spermatogonial stem cells (SSCs) are the foundation of high-throughput, life-long production of sperm. Clinically, SSCs are expected to become an important resource to restore male fertility, particularly for childhood cancer patients whose fertility is at risk. Yet, fundamental characteristics of SSCs remain elusive, making mechanistic studies difficult to control SSC fate for preserving stem cells or encouraging sperm production. Our ultimate goal is to determine a fate map of SSCs and to visualize them. Using a functional transplantation assay and multi-parameter flow cytometry, we found that loss of THY1 or ITGA6 indicates the exit from the SSC state, while the expression of widely-used SSC markers (GFRA1 and CDH1), as well as KIT, denotes the transition to commitment. To enrich the SSC population from “adult intact” mouse testes, which has been hard to achieve, we fractionated primitive germ cells using five surface antigens simultaneously (THY1, CDH1, GFRA1, KIT, and ITGA6). SSC transplantations showed that two cell fractions, the THY1+ / ITGA6<sup>Hi</sup> / GFRA1- / CDH1- / KIT- and the THY1+ / ITGA6<sup>Hi</sup>, exhibit the degrees of SSC enrichment that exceed any of those reported in the past two decades. Further characterization of these cell fractions is underway (e.g., cell cycle, protein/mRNA expression). We are also in preparation for single-cell RNA sequencing to understand the SSC fate commitment process at the transcriptome level. Our data should establish a powerful platform to dissect SSC fate decision mechanisms and to identify SSC niches, leading to clinical SSC applications for male fertility preservation and restoration. (Supported by CIHR and FRSQ)

**Poster #27:**

**Title / Titre :**

**REGULATION OF NORMAL AND CANCEROUS STEMNESS BY A GATA3/BMP AXIS IN THE PROSTATE**

**Authors / Auteurs :**

Mathieu Tremblay, Maxwell Shafer, Sophie Viala, Alana H.T. Nguyen, Adda Lee-Graham, Maxime Bouchard

**Abstract / Résumé :**

Adult stem cells are found in prostate tissue and can act as the cells-of-origin for prostate cancer. Loss of the tumor suppressor PTEN is a common occurrence in prostate cancer. We showed 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 primary prostate stem cells enhanced their long-term self-renewal capacities both *in vitro* (serial sphere formation assay) and stem cell frequency *in vivo* (limiting dilution transplantation assay). Using RNAseq analysis, we found that BMP signaling is important for stem cell maintenance and loss of GATA3 is associated with an autocrine up-regulation of Bmp5. In addition, BMP5 treatment increased normal stem cell potential and numbers *in vitro* and *in vivo* whereas its inhibition (Noggin) corrects GATA3-deficient sphere forming potential to normal levels as well as the aberrant Pten-deficient cancerous sphere forming potential. Finally, loss of Bmp5 in the mouse affect prostate tissue homeostasis which is associated with a reduction in stem cell potential. Together, these data establish GATA3 as an important regulator of normal and cancerous prostate stem cells homeostasis through a Bmp5 dependent mechanism.

**Poster #28:**

**Title / Titre :**

**CELLULAR SENESENCE COMPROMISES THE RESPONSE TO OXIDATIVE STRESS**

**Authors / Auteurs :**

Ossama Moujaber, Dana Abou Samhadaneh, Ines Colmegna and Ursula Stochaj

**Abstract / Résumé :**

The proper response to stress is crucial to survive physiological and environmental insults. Cellular senescence promotes aging and impairs stress responses. 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. 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.

The formation of cytoplasmic stress granules (SGs) is a conserved response to various forms of stress. I have used senescent cultured kidney cells (LLC-PK1) to analyze SG formation in a quantitative fashion. Based on my results with LLC-PK1 cells, I have extended these studies to MSCs.

LLC-PK1 cells undergoing senescence in vitro display specific hallmarks of aging. This is exemplified by the irreversible loss of cell proliferation, increased cell size and nuclear dysmorphism. We identified two mechanisms that contribute to these senescence-associated changes: depletion of factors required for SG biogenesis, and altered cell signaling.

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. I expect that my results will provide measurable parameters that evaluate MSC quality for therapeutic intervention.

**Poster #29:**

**Title / Titre :**

**DEVELOPING CELL MODELS TO TEST THE ROLES AND MECHANISMS OF BAP1 ACTIVITY IN B CELL DEVELOPMENT AND IMMUNITY**

**Authors / Auteurs :**

Yue Liang<sup>1,2</sup>, Yun Hsiao Lin<sup>1,2</sup>, Mansen Yu<sup>2,3</sup>, Anastasia Nijnik<sup>1,2</sup> Affiliation: 1. Department of Physiology, 2. McGill University Research Centre on Complex Traits, and 3. Department of Microbiology and Immunology, McGill University, Montreal, Quebec

**Abstract / Résumé :**

BAP1 is a deubiquitinating enzyme (DUB) of the ubiquitin C-terminal hydrolase family with specificity for the histone mark H2AK119ub. It is involved in the regulation of gene expression, DNA repair, and cell cycle progression. Since these processes are important in blood and immune cell differentiation, we hypothesize that BAP1 may play a key role in hematopoiesis and immunity. Previous studies showed that BAP1 is essential for the myeloid lineage differentiation, however its activity in the lymphoid lineage is poorly understood. Our lab focussed on the potential involvement of BAP1 in B cell physiology. With mouse models, we demonstrated that upon BAP1-deletion, B cell development is impaired at the pro-B to pre-B cell stage. Moreover, the production of antigen-specific antibodies and the formation of memory B cells are also impaired in the BAP1-knockout mice. The aim of the current project is to establish BAP1-deficient B cell lines using CRISPR/Cas9 approach, and to use them to analyze the roles and mechanisms of BAP1 activity. CRISPR gRNAs targeting BAP1 exons 4 and 5 were designed (<http://crispr.mit.edu>) and cloned into the pSpCas9(BB)-2A-GFP vector. The constructs will be transfected into pre-B cell line Ba/F3 and mature B cell line CH12F3, to generate BAP1-deficient lines. Following validation, the lines will be analyzed for defects in survival, proliferation, activation, and antibody class switching. Changes in gene expression, stability of BAP1-binding proteins, and their recruitment to DNA will also be analyzed. The novel BAP1-deficient cell models generated using CRISPR technology will help to further investigate the mechanisms of BAP1 activity in B cell differentiation and function.

**Poster #30:**

**Title / Titre :**

**MODELING NEUROLOGICAL DISORDERS WITH hiPSCs DERIVED MINIBRAINS**

**Authors / Auteurs :**

MOHAMED Nguyen-Vi\*, HAN Chanshuai\*, MATHUR Meghna, CHEN Carol X.-Q. , FON Edward, DURCAN Thomas

Montreal Neurological Institute, iPSC platform, McGill University, Montreal, Québec, Canada \*These authors equally contributed to the poster

**Abstract / Résumé :**

Often promising, therapies developed with mouse models fail due to differences between mice and humans, which leads us to focus on developing more relevant human disease models. Advances of stem cell technology have made 3D human models feasible, with induced pluripotent stem cells (iPSCs) that can generate a 3D human neuronal network (minibrains) with similar architecture and composition to the brain. In our group, we attempt to use minibrains to model neurodevelopmental disorders and neurodegenerative disorders such as Parkinson's disease.

Loss-of-function mutation in a gene called DENND5A leads to a neurodevelopmental disorder named epileptic encephalopathy. Patients with DENND5A mutation have global developmental delay, epilepsy and microcephaly. To model this disorder, we use CRISPR/Cas9 technology to create DENND5A knockout in iPSCs. We observe morphologic differences in the DENND5A derived cerebral organoids compared to the isogenic controls. This project will provide a potential model to study the pathological mechanisms involved in epileptic microencephaly.

Parkinson's disease (PD) is characterized by the presence of  $\alpha$ -synuclein ( $\alpha$ -syn) aggregates, which have been demonstrated to spread throughout the brain. The regions affected are interconnected implying that  $\alpha$ -syn is being propagated. Developing therapies to impede this process represents a therapeutic avenue. The global aim is to build upon existing 2D cell cultures and mouse models by investigating the process of  $\alpha$ -syn propagation in a 3D human model. To achieve this goal, we investigate the propagation of  $\alpha$ -syn in human midbrain organoids (hMOs) derived from iPSCs lines from a PD patient carrying SNCA triplication and its isogenic control, produced by CRISPR/Cas9 technology. This work will provide new insights on the propagation of  $\alpha$ -syn in a 3D human model.

**Poster #31:**

**Title / Titre :**

**GOLD NANOURCHINS IMPAIR CANCER CELL HOMEOSTASIS**

**Authors / Auteurs:**

Dana Abou Samhadaneh<sup>1</sup>, Adam Smart<sup>1</sup>, April Kuang<sup>1</sup>, Ossama Moujaber<sup>1</sup>, Dusica Maysinger<sup>2</sup> & Ursula Stochaj<sup>1\*</sup>

<sup>1</sup>Department of Physiology, McGill University, Montreal, Canada

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**Abstract / Résumé:**

BACKGROUND: Due to their unique physical properties and chemical non-toxicity, gold nanoparticles are particularly promising tools for cancer diagnosis and therapy. These applications are limited by the current lack of knowledge as it pertains to the effects of gold nanoparticles on cellular homeostasis.

Gold nanoparticles often concentrate in endosomes/lysosomes, organelles that are in close contact with the endoplasmic reticulum (ER). To date, little is known about the impact of gold nanoparticles on the ER and the unfolded protein response (UPR). This information is important, because the UPR can promote cell survival. On the other hand, a severe and persistent UPR may trigger cell death. Gold nanoparticles have also been shown to induce oxidative stress in cancer cells. However, the extent of oxidative damage to RNA, specifically, is unknown. Here, we investigated the impact of gold nanoparticles on the UPR, RNA oxidation and overall cellular proteostasis.

METHODS: HeLa cells were treated with different concentrations of gold nanourchins and UPR inhibitors for defined periods of time. Cell viability, apoptosis, protein aggregation, UPR activation and RNA oxidation were assessed.

RESULTS: We show that gold nanourchins reduced HeLa cell viability in a dose-dependent manner. Our results indicate that gold nanourchins promoted severe protein aggregation, suggesting the impairment of proteostasis. Furthermore, gold nanourchins induced ER stress and caused a branch-specific activation of the UPR. Finally, oxidative stress induced by gold nanourchins promoted RNA oxidation and granule formation in the cytoplasm.

CONCLUSIONS: Our results suggest that non-functionalized gold nanoparticles induce several stress responses that affect the ER and other subcellular compartments. With persistent cellular stress, gold nanourchins are likely to promote cancer cell death. This knowledge is crucial for gold nanoparticle-related therapeutic applications and a promising new strategy to eliminate tumor cells.

**Poster #32:**

**Title / Titre :**

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

**Authors / Auteurs :**

**Amanda Fiore**<sup>1,3</sup>, HanChen Wang<sup>1,3</sup>, Jad I. Belle<sup>1,3</sup>, Philippe Gros<sup>2,3</sup>, David Langlais<sup>4,5</sup>, Anastasia Nijnik<sup>1,3</sup>

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5 McGill University and Génome Québec Innovation Centre, McGill University

**Abstract / Résumé :**

Myb-like SWIRM and MPN Domains 1 (MYSM1) is a chromatin-binding histone H2A lysine 119 deubiquitinase that is essential for normal hematopoiesis in both mice and humans. Mym1- 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 Mym1-deficient hematopoietic stem cells. Additionally, our ChIP-seq analyses performed in the Ba/F3 hematopoietic progenitor cell line revealed that a significant portion of MYSM1-binding sites across the genome are associated with these genes. My current aims are to validate and establish the role of MYSM1 in the regulation of the expression of these genes, understand whether this regulation operates through MYSM1's catalytic domain, and investigate the crosstalk between MYSM1 and other transcription factors in their regulation. This work will expand our 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 #33:**

**Title / Titre :**

**CD271 A MORE PRECISE MARKER FOR CANCER STEM CELL IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS THAN CD44**

**Authors / Auteurs :**

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**Abstract / Résumé :**

Head and neck squamous cell carcinoma (HNSCC) is the seventh most common cancer with over 500,000 new cases diagnosed yearly, and 4.6% of cancer cases. Despite the improvements in treatment modalities, the five-year survival rate for SCCHN has remained unchanged at ~50% over the past 30 years. One reason for HNSCC treatment failure is related to a subpopulation of cells in the tumor called cancer stem cells (CSCs) which are suggested to have tumor initiating potential, combined with the self-renewal ability and multilineage differentiation. According to many studies, CD44 surface marker can be used to identify CSCs. The purified CD44+ cells from the primary tumors can give rise to tumors faster and by injecting less cell number in xenograft model compared to CD44- cells, and these xenograft tumors subsequently reproduce the original tumor heterogeneity observed in the primary tumor. Recently, CD271 was identified as a marker of CSCs in many tumors, such as human melanoma and hypopharyngeal carcinoma. In our study, we investigated if CD271 is a more precise marker for CSCs than the widely used CD44. Our results revealed that CD271+ cells are a subpopulation from the CD44+ cells. The CD44+/CD271+ cells have a faster dividing rate, higher proliferation rate, higher self-renewal ability and chemo/radio-resistance when compared to CD44+/CD271- and the total population. CD44+/CD271+ cells showed higher expression of stemness genes such as BMI1, SOX2, OCT4 and GLI1 when compared to CD44+/CD271-. Our results suggest that CD271+ is a more accurate marker to purify the CSCs from HNSCC.