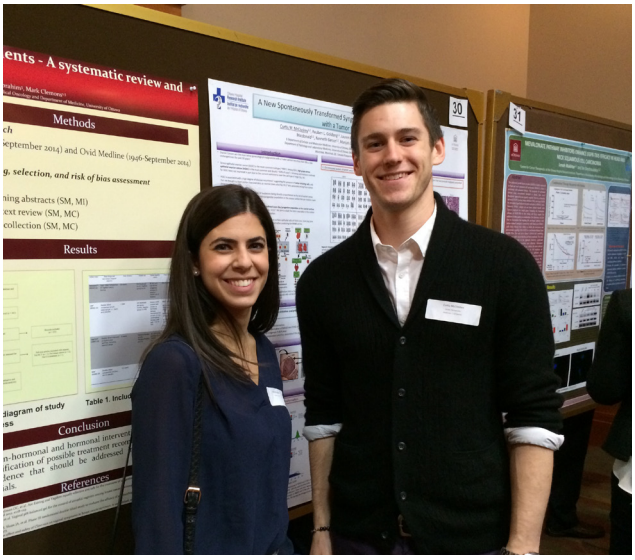
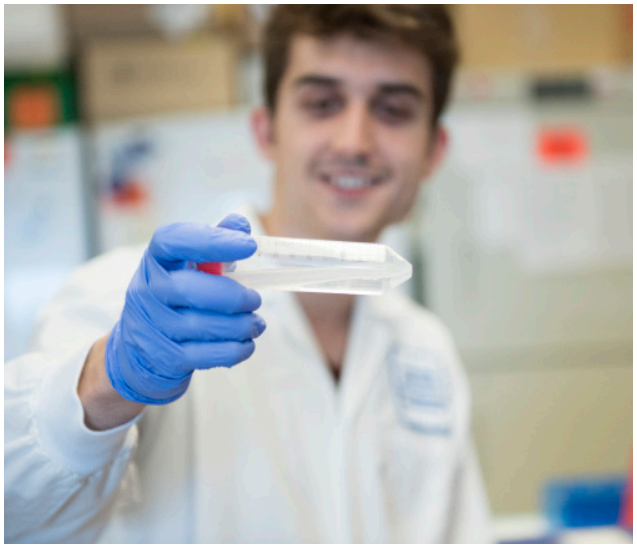
The Ottawa Hospital RESEARCH INSTITUTE | L'Hôpital d'Ottawa INSTITUT DE RECHERCHE

2016 RESEARCH DAY





Program and Abstracts

Thursday, November 10, 2016
7:30 a.m. – 5:00 p.m.

St. Elias Centre
750 Ridgewood Ave.
Ottawa, ON

Research Day is generously supported by:

Research Day Committee

The Ottawa Hospital Research Institute would like to express its appreciation to members of the Research Day Committee for their dedication and hard work in organizing this event, and to the volunteers, whose assistance we could not do without.

Dr. Fraser Scott (Chair)
Dr. Marjorie Brand
Jennifer Ganton
Dr. Luc Sabourin
Dr. Jay Baltz
Dr. Jim Dimitroulakos
Dr. Ian Lorimer
Dr. William Stanford
Amelia Buchanan
Dr. Dean Fergusson
Dr. Tim Ramsay
Dr. Duncan Stewart
Dr. Ketul Chaudhary
Dr. Anouk Fortin
Dr. Catherine Tsilfidis

Volunteers

Greg Canham
Melanie Genereaux
Wayne Lowe

WELCOME TO RESEARCH DAY

Today, we celebrate and showcase the outstanding work of our young researchers. Their insight, passion and commitment to scientific excellence are critical to our success as one of Canada's top research hospitals, ranking 3rd overall in terms of funding from the Canadian Institutes of Health Research.

This day is meant to foster and encourage interaction in a collegial environment for our trainees – to give them experience communicating their ideas clearly and effectively. Even if you are not involved in the judging of posters or oral presentations, I hope you will ask questions of our trainees. As well as providing this learning opportunity, today is a great chance for us all to learn about the exciting research projects taking place across The Ottawa Hospital, in partnership with the University of Ottawa.

Research Day also gives us the opportunity to bring a world-class researcher to Ottawa for the Dr. J. David Grimes Lecture. This year we are truly fortunate that Dr. Carl June of the University of Pennsylvania has agreed to give this lecture, and spend some time ineratcting with our trainees and scientists. Dr. June is world-renowned scientist and a pioneer in the development of CAR-T therapy, which involves genetically engineering a patient's own T cells to attack their cancer. This technology has demonstrated remarkable results, and could become a key pillar of cancer immunotherapy.

On behalf of everyone at the Ottawa Hospital Research Institute, I would like to thank all those involved in making this day happen, from our keynote speaker to our presenters, judges, planning committee and volunteers. I would also like to thank the sponsors for helping to make today's event possible, and I encourage you to visit their tables.

Duncan Stewart, MD, FRCPC

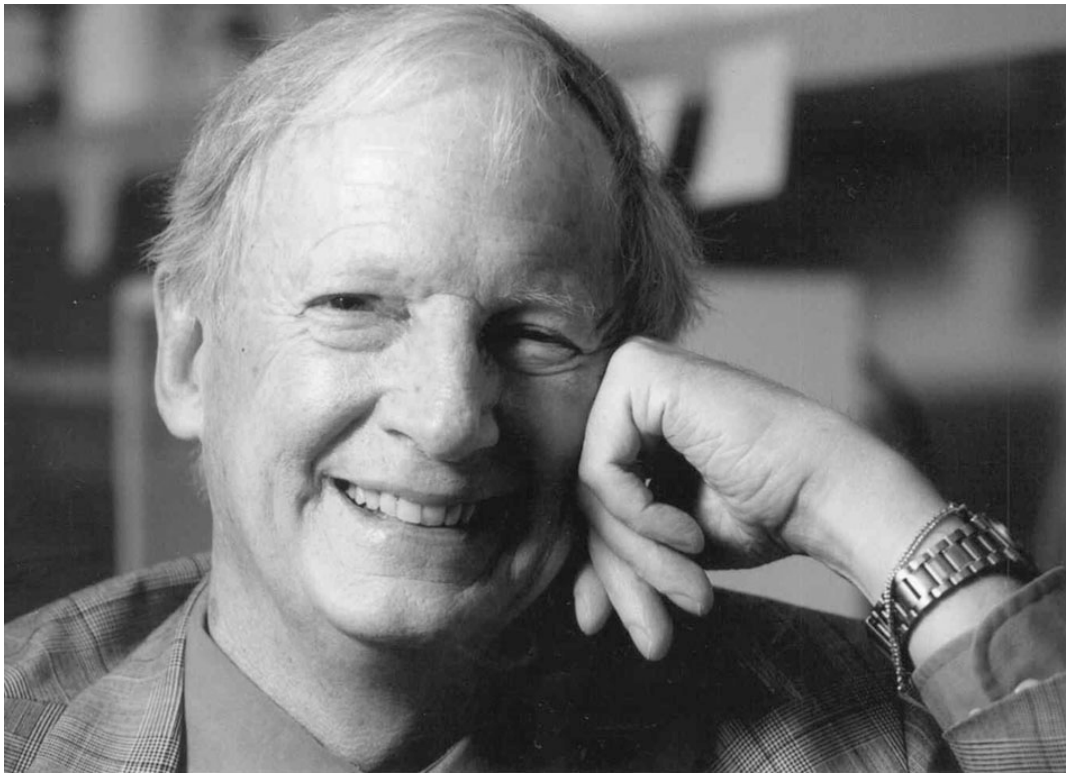
CEO & Scientific Director,
Senior Scientist in the Regenerative Medicine Program,
Ottawa Hospital Research Institute

Executive Vice-President, Research,
The Ottawa Hospital

Evelyne and Rowell Laishley Chair

Professor, Department of Medicine,
Faculty of Medicine, University of Ottawa

DR. J. DAVID GRIMES LECTURE



Dr. J. David Grimes, MD, FRSPC

This annual lecture is named in honour of Dr. J. David Grimes, founder of the Loeb Research Institute, which was the predecessor of the Ottawa Hospital Research Institute at the Civic Campus.

Dr. Grimes served as the Institute's CEO and Scientific Director until he retired in 1997. He recruited and mentored many of Ottawa's leading health researchers. He also practiced neurology for more than 25 years, specializing in Parkinson's disease.

After a long and courageous battle with lung disease, Dr. Grimes passed away on May 9, 2001. A man of great vision and compassion, Dr. Grimes is missed by everyone who knew him. This annual lecture is just one of the ways his memory lives on at the Ottawa Hospital Research Institute. He is also remembered through the Dr. J. David Grimes Research Chair at the University of Ottawa and through the Grimes Career Achievement Award, which is awarded annually at The Ottawa Hospital Gala.

DR. J. DAVID GRIMES LECTURE

"Engineering T cells for cancer therapy"

Dr. Carl June

Carl June is the Richard W. Vague Professor in Immunotherapy in the Department of Pathology and Laboratory Medicine. He is currently Director of the Center for Cellular Immunotherapies at the Perleman School of Medicine, and Director of the Parker Institute for Cancer Immunotherapy at the University of Pennsylvania. He is a graduate of the Naval Academy in Annapolis, and Baylor College of Medicine in Houston, 1979.

He had graduate training in Immunology and malaria with Dr. Paul-Henri Lambert at the World Health Organization, Geneva, Switzerland from 1978-79, and post-doctoral training in transplantation biology with E. Donnell Thomas and John Hansen at the Fred Hutchinson Cancer Research Center in Seattle from 1983 - 1986.

He is board certified in Internal Medicine and Medical Oncology. He maintains a research laboratory that studies various mechanisms of lymphocyte activation that relate to immune tolerance and adoptive immunotherapy for cancer and chronic infection.

In 2011, his research team published findings detailing a new therapy in which patients with refractory and relapsed chronic lymphocytic leukemia were treated with genetically engineered versions of their own T cells. The treatment has also been used with promising results to treat children with refractory acute lymphoblastic leukemia.

He has published more than 350 manuscripts and is the recipient of numerous prizes and honors, including election to the Institute of Medicine in 2012 and the American Academy of Arts and Sciences, the William B Coley award, the Richard V Smalley Memorial Award from the Society for Immunotherapy of Cancer, the AACR-CRI Lloyd J. Old Award in Cancer Immunology, the Paul Ehrlich and Ludwig Darmstaedter Prize, the Novartis Prize in Immunology, the Karl Landsteiner Memorial award, Debrecen Award and lifetime achievement award.

DR. GOODMAN COHEN SUMMER STUDENT AWARDS

Every year, the Ottawa Hospital Research Institute holds the summer student seminar series, which gives students the opportunity to present their research to other students. This year, more than 50 students participated from throughout the Institute, ranging from high-school students to newly graduated Bachelor's students. Awards are given for the best presentations, based on both peer and coordinator evaluations. The students then submit a written paper and the top students are awarded the Dr. Goodman Cohen Summer Student Award. This year they competed in two categories: Senior (returning students) and Junior (new students).

Dr. Jay Baltz, Associate Scientific Director responsible for Trainees, would like to thank Dr. Jane Fenelon and Dr. Lisa Julianfor their excellent job running the summer student program this year.

Winners of the Dr. Goodman Cohen Summer Student Award

Senior Award

Mina Rizk (supervised by David Allan)

"Heterogeneity in studies of mesenchymal stromal cells to treat or prevent GVHD: a scoping review of the evidence"

Junior Award

Joanne Joseph (supervised by Bernard Thébaud)

"Therapeutic potential of endothelial progenitor cells in neonatal pulmonary hypertension"

Dr. Goodman Cohen

The Dr. Goodman Cohen Summer Student Awards are made possible by generous donations made in the memory of Dr. Goodman (Goody) Cohen, one of Ottawa's first and finest cardiologists.

Born in 1922, Dr. Cohen grew up in a tiny rural mining town located between Glace Bay and Sidney in Nova Scotia. The youngest of seven siblings (five boys and two girls), Dr. Cohen was the only one in this family to attend university, starting his post-secondary education at Mount Allison University in Sackville, New Brunswick. He went on to graduate from McGill Medical School in the early 1950s before doing post-graduate work at Harvard and Johns Hopkins universities. He met his future wife, Rita Lambert, a nurse, while training at Massachusetts General Hospital. They settled in Ottawa where they raised three children.

Dr. Cohen, known as Goody, practiced cardiology for almost 35 years, until 1989 when he was diagnosed with cancer. He died in January 1990. Widely known as a kind and caring physician to thousands of patients over the years, he was also highly respected as a clear and forthright professor.

RESEARCH TRAINEE SALARY AWARDS

See more of our trainees' photos on the inside back cover

Atefeh Abedini Najafabadi
Faria Ahmed
Amelia Aitken
Almohanad Alkayyal
Khalid Al-Zahrani
Leonard Angka
Meshach Asare-Werehene
Dr. Vanessa Bacal
Justine Baron
Katherine Baxter
Sean Bennett
Aissa Benyoucef
Anabel Bergeron
Marie-Claude Bourgeois-Daigneault
Caroline Brun
Clair Butler
Chao Chang
Natasha Chang
Ketul Chaudhary
Grace Christou
David Cook
Olivia Cook
Sarah Cummings
Kristin Danko
Marc-Olivier Deguise
Josh Del Papa
Daniel El Kodsi
Peter Feige
Nathan Fergusson
Nicole Forbes
Monica Gad
Karine Gauthier
Bram Gottlieb
Annette Gower
Nathalie Grun
Dhiya Hassan
Mirabelle Ho
Amy Hsu
Hua Huang
Antonella Iaderosa
Mahsa Jessri
Lisa Julian
Janet Jull
Brian Keller
Kaitlin Kharas
Samantha Kornfeld
Ramya Krishnan
Brian Laight
Andrea Lanes
Christopher Lavergne
Hubert Lee
Christine Leung
Yuefeng Li
Marissa Lithopoulos
Chao-Chia Lu
Anisha Lynch-Godrei
Stacey Lynn Fisher
Curtis McCloskey
Brad Mischuk
Sedah Mo
Nikhile Mookerji
Leslie Nash
Fares Ould-Brahim
Adrian Pelin
Benjamin Pryce
Charis Putinski
Daniel Robinson
Elaine Rose
Bratati Saha
Reza Salehi Karashk
Mohammed Selman
Mehdi Shafa
Jordan Sim
Siriwardena, Dylan
Hwan Hee Son
Abera Surendran
Mohamad Taha
Jean-Francois Thibodeau
Matthew Tsang
Faranak Vahid-Ansari
Oliver Varette
Nhung Vuong
Dao Sen Wang
Marie-Ève Wedge

OHRI RESEARCH DAY PROGRAM

7:30 AM **REGISTRATION / POSTER SETUP / CONTINENTAL BREAKFAST**

8:15 AM **OPENING REMARKS** (Duncan Stewart, Bernard Jasmin, Alain Stintzi, Fraser Scott)

8:30 AM **IMMUNE RESPONSE AND MOLECULAR BIOLOGY** (35 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: **Bojan Shutinoski** and **Virgilio Cadete**

- **Marc-Olivier Deguise** (Rashmi Kothary Group) Immune dysregulation may contribute to disease pathogenesis in spinal muscular atrophy mice
- **Hua Huang** (Hsiao-Huei Chen Group) Hepatocytes affect NAFLD development by controlling immune response magnitude
- **Ayden Gouveia** (Jing Wang Group) The aPKC-CBP Pathway Regulates Adult Hippocampal Neurogenesis in an Age-Dependent Manner

9:05 AM **CLINICAL AND PRE-CLINICAL STUDIES** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: **Grace Christou** and **Andrea Lanes**

- **Vignan Yogendrakumar** (Dar Dowlatshahi Group) Location of Intracerebral Hemorrhage Predicts Hematoma Expansion and Clinical Outcome
- **Nhung Vuong** (Barbara Vanderhyden Group) 17ß-Estradiol sensitizes ovarian surface epithelium to transformation by suppressing Dab2 expression
- **Mahsa Jessri** (Douglas Manuel Group) Identification of Dietary Patterns Associated with Chronic Diseases in Canada
- **Olexiy Aseyev** (Susan Dent and Shailendra Verma) Prediction of relapse in patients with locally advanced breast cancer after neoadjuvant treatment

9:55 AM **REFRESHMENT BREAK** (25 minutes)

10:20 AM **STEM CELL BIOLOGY AND THERAPEUTICS** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: **Kaitlin Kharas** and **Yuefeng Li**

- **Natasha Chang** (Michael Rudnicki Group) p38-gamma MAPK is a Critical Regulator of Satellite Stem Cell Self-Renewal
- **Lisa Julian** (William Stanford Group) Patient-derived TSC2-haploinsufficient smooth muscle cells exhibit widespread features of Lymphangioleiomyomatosis disease pathology
- **Mirabelle Ho** (Duncan Stewart Group) Revascularization of Lung Scaffold is Enhanced upon Combined Delivery of Induced Pluripotent Stem Cell Derived Smooth Muscle Cells and Endothelial Cells
- **Joanne Joseph** (Bernard Thébaud Group) ECFCs mediate their therapeutic benefit through the release of exosomes

11:10 AM **POSTER VIEWING / JUDGING OF POSTERS PRESENTED BY PhD, MSc, 4th YEAR HONOURS AND CO-OP STUDENTS** (60 minutes)

12:10 PM **BUFFET LUNCH** (75 minutes)

1:25 PM **TOWARD CANCER IMMUNOTHERAPY** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: **Curtis McCloskey** and **Dominic Roy**

- **Marie-Claude Bourgeois-Daigneault** (John Bell Group) Oncolytic virus-mediated immunotherapy
- **Mohammed Selman** (Jean-Simon Diallo Group) Oncolytic immunotherapy potentiated by phosphatase inhibitor
- **Amelia Aitken** (John Bell Group) An oncolytic virus targeting the RNA interference pathway
- **Almohanad Alkayyal** (Rebecca Auer Group) Eradication of peritoneal carcinomatosis using a Maraba virus infected cell vaccine expressing IL-12 requires recruitment of Natural Killer cells

2:15 PM **POSTER VIEWING / JUDGING OF POSTERS PRESENTED BY POSTDOCTORAL FELLOWS, CLINICAL FELLOWS, RESEARCH ASSOCIATES, RESIDENTS and MEDICAL STUDENTS** (60 minutes)

3:15 PM **REFRESHMENT BREAK** (25 minutes)

3:40 PM **DR. J. DAVID GRIMES LECTURE** (45 minutes plus 20 minutes discussion)
Engineering T cells for cancer therapy
Carl June, Richard W. Vague Professor in Immunotherapy, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania
Moderator: **John Bell**

4:45 PM **POSTER / ORAL PRESENTATION AWARDS AND CLOSING REMARKS**
Moderators: Duncan Stewart, Fraser Scott and Mona Nemer

5:00 PM **RECEPTION AND CASH BAR**

ORAL PRESENTATIONS:

IMMUNE RESPONSE AND MOLECULAR BIOLOGY (8:30 to 9:05)

Moderators: **Bojan Shutinoski** and Virgillio Cadate

1-1

Immune dysregulation may contribute to disease pathogenesis in spinal muscular atrophy mice

Marc-Olivier Deguise[1,2,3], Emily McFall[1,3], Yves De Repentigny[1,3] and Rashmi Kothary[1,2,3,4]

[1.] Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada K1H 8L6

[2.] Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

[3.] Centre for Neuromuscular Disease, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

[4.] Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

Background: Spinal muscular atrophy (SMA) is characterized by selective loss of motor neurons and muscle atrophy. Efforts to date have focused on understanding why motor neurons are preferentially affected in the disease. However, emerging evidence demonstrates intrinsic alterations in numerous other cell types in the context of SMA pathogenesis. Recently, we have noted smaller spleens from two different mouse models of SMA, implying potential immune deficiencies in disease aetiology.

Hypothesis and objectives: We hypothesize that morphological defects observed in the lymphoid organs of SMA model mice will lead to immune deficiencies or neuroinflammation and are part of disease burden. To better understand lymphoid organs contribution, we first performed a thorough characterization of gross, histological and molecular changes in the spleens from SMA model mice.

Methods: Lymphoid organs defects were assessed by gross morphology metrics, hematoxylin and eosin staining, immunostaining of common immune cells markers, flow cytometry, and cytokine profiling.

Results: Gross anatomy measurements of the spleens in *Smn*$^{2B/-}$ (P0 to P19) revealed progressively smaller spleens. Hematoxylin and eosin staining showed disrupted white pulp and red pulp organization from P4 to P19 *Smn*$^{2B/-}$ spleens. The abnormal structure observed in the spleen could be the result of depleted B cell and T cell populations in P19 *Smn*$^{2B/-}$ mice. Staining with specific B and T cell markers confirmed that these cells are mislocalized but are still present within the spleen. We next analysed the thymus, where T-cells mature. The *Smn*$^{2B/-}$ thymus appeared relatively spared in gross morphological assessment. However, profound histological abnormalities marked by cortex thinning, increased apoptotic bodies and tangible-body macrophages were present in P19 *Smn*$^{2B/-}$ thymus. Abnormal T-cell maturation could explain both thymic and splenic defects. Flow cytometry analysis of the thymus revealed T-cell misrepresentation in various developmental stages at P19 but not at P4. Lastly, cytokine profiling of the spleen and thymus revealed global misregulation.

Conclusions: Taken together, these results provide evidence that the immune cells are particularly vulnerable to reduced Smn levels. In the scope of this project, further investigation will be required to establish the primary mechanism of lymphoid defects and understand whether they lead to impaired immune functions in SMA patients.

1-2

Hepatocyte affects NAFLD development by controlling immune response magnitude

Hua Huang[1], Xun zhou[1], Aswin Harri[1], Chao Chang[1], Alexandre Stewart[1], Hsiao-Huei Chen[1]
[1]Department of Cellular and Molecular Medicine, University of Ottawa, the Ottawa Hospital Research Institute

The liver is not only the keystone metabolic organ, it is also an immune organ. Due to its unique anatomy, the liver is constantly exposed to microbe-derived ligands and metabolites with inflammatory potential. The dysregulation of hepatic immune and metabolic balance triggers the development of several pathologies including non-alcoholic fatty liver disease (NAFLD). Many studies focus on the activation of hepatic resident immune cell and the consequent metabolic functional changes. How parenchymal hepatocytes, the major cellular component of the liver, react to the metabolic and inflammatory stresses and how this reaction affects the development of NAFLD remains largely unexplored.

Here, we knocked out IRF2BP2, a gene related to both innate immunity and lipid metabolism, in parenchymal hepatocytes to generate liver-specific (LKO) mice. Under inflammatory and metabolic stresses, LKO mice became obese and displayed more severe hepatic steatosis, fibrosis, inflammation, and insulin resistance than littermate controls. That LKO mice display the full spectrum of NAFLD syndromes highlights the importance of the hepatocyte stress response in the development of NAFLD. Microarray studies showed LKO mouse liver expressed less trefoil factor-3 (TFF3), a secreted molecule known to suppress inflammation. We further demonstrated that TFF3 is induced in hepatocytes by inflammatory and metabolic stresses. TFF3 secreted by hepatocytes attenuated an innate immune response of bone marrow-derived macrophages. Over-expressing TFF3 in hepatocytes limited the immune response and increased hepatocyte insulin sensitivity. Our study suggested a loop from hepatocyte to immune cell, and eventually, from immune cell to hepatocyte. To our knowledge, ours is the first study to provide evidence that under the metabolic and inflammatory challenges, parenchymal hepatocytes control the magnitude of the inflammatory response, which in turn, affects hepatocyte metabolic function, and the development of NAFLD.

1-3

The apkc-CBP Pathway Regulates Adult Hippocampal Neurogenesis in an Age-Dependent Manner

Ayden Gouveia,[1,2,10] Karolynn Hsu,[1,10] Yosuke Niibori,[5] Matthew Seegobin,[1] Gonzalo I. Cancino,[5] Ling He,[8] Fredric E. Wondisford,[9] Steffany Bennett,[3,4] Diane Lagace,[2,3] Paul W. Frankland,[5,6,7] and Jing Wang[1,2,3,*]

[1] Regenerative Medicine Program, Ottawa Hospital Research Institute
[2] Department of Cellular and Molecular Medicine
[3] Brain and Mind Research Institute
[4] Department of Biochemistry, Microbiology and Immunology University of Ottawa
[5]Neurosciences and Mental Health, Hospital for Sick Children, Toronto, ON
[6]Department of Psychology
[7]Department of Physiology University of Toronto, Toronto, ON
[8]Division of Metabolism, Department of Pediatrics, Johns Hopkins Medical School, Baltimore, MD
[9]Department of Medicine & Pediatrics, Rutgers-Robert Wood Johnson Medical School, New Brunswick, NJ USA
[10]Co-first author

Background: While epigenetic modifications have emerged as attractive substrates to integrate environmental changes into the determination of cell identity and function, specific signals that directly activate these epigenetic modifications remain unknown.

Objective: Here, we examine the role of atypical protein kinase C (apkc)-mediated Ser436 phosphorylation of CBP, a histone acetyltransferase, in adult hippocampal neurogenesis and memory.

Methods: Using a knockin mouse strain (CBPS436A) in which the apkc-CBP pathway is deficient, we examined the changes in hippocampal neurogenesis between 3 and 6 month old mice by quantifying neuronal differentiation and maturation.

Results: We observe impaired hippocampal neuronal differentiation, maturation, memory and diminished binding of CBP to CREB in 6-month-old CBPS436A mice,but not at 3 months of age. Importantly, elevation of CREB activity rescues these deficits, and CREB activity is reduced whereas apkc. Activity is increased in the murine hippocampus as they age from 3 to 6 months regardless of genotype.

Conclusions: Thus, the apkc-CBP pathway is a homeostatic compensatory mechanism that modulates hippocampal neurogenesis and memory in an age-dependent manner in response to reduced CREB activity.

CLINICAL AND PRE-CLINICAL STUDIES (9:05 to 9:55)

Moderators: **Grace Christou** and **Andrea Lanes**

2-1

Location of Intracerebral Hemorrhage Predicts Hematoma Expansion and Clinical Outcome

Vignan Yogendrakumar MD[1], Andrew M Demchuk MD[2], Richard I Aviv mbchb[3], David Rodriguez-Luna MD[4], Carlos A Molina MD[4], Yolanda Silva Blas MD[5], Imanuel Dzialowski MD[6], Adam Kobayashi MD[7], Jean-Martin Boulanger MD[8], Cheemun Lum MD[9], Gord Gubitz MD[10], Vasantha Padma MD[11], Jayanta Roy MD[12], Carlos S. Kase MD[13], Rohit Bhatia MD[11], Michael D Hill MD[2], and Dar Dowlatshahi MD [1], on behalf of the PREDICT/Sunnybrook ICH CTA study group.

[1]Department of Medicine (Neurology), Ottawa Hospital Research Institute & University of Ottawa, Ottawa, Canada
[2]Calgary Stroke Program, Department of Clinical Neurosciences, Department of Radiology, Hotchkiss Brain Institute, University of Calgary, Calgary, Canada
[3]Division of Neuroradiology and Department of Medical Imaging, Sunnybrook Health Sciences Centre, University of Toronto,
[4]Department of Neurology, Hospital Universitari Vall d'Hebron, Barcelona, Spain
[5]Department of Neurology, Dr Josep Trueta University Hospital, Institut d'Investigació Biomèdica Girona (idibgi) Foundation, Girona, Spain
[6]Department of Neurology, Elblandklinikum Meissen Academic Teaching Hospital of the Technische University, Dresden, Germany
[7]2nd Department of Neurology, Institute of Psychiatry and Neurology, and Department of Experimental and Clinical Pharmacology, Warsaw, Poland [8]Department of Medicine, Charles lemoyne Hospital, University of Sherbrooke, Montreal, Canada [9]Department of Diagnostic Imaging, Neuroradiology Section, University of Ottawa, Ottawa Hospital Research Institute [10]Department of Neurology, Dalhousie University, Halifax, Canada
[11]Department of Neurology, All India Institute of Medical Sciences, New Delhi, India [12]Apollo Gleneagles Hospitals, Kolkata
[13]Department of Neurology, Boston Medical Center, Boston, USA

Background: Intracerebral hemorrhage (ICH) is a devastating form of stroke that is difficult to predict, and treat. Hematoma expansion is common in ICH and significantly worsens mortality and morbidity. With emerging therapies targeting hematoma expansion, there is significant interest in improving patient selection for clinical trials. Hematoma location is a characteristic whose predictive quality for hematoma expansion remains unclear. Our objective was to assess the effect of lobar vs. Non-lobar hemorrhage on hematoma expansion and clinical outcome.

Methods: We analyzed data from the prospective PREDICT study where patients with ICH presenting to hospital under 6 hours of symptom onset received a baseline CT, CTA, 24 hour follow-up CT, and 90-day mrs. ICH location was categorized as lobar vs non-lobar, and primary outcomes were significant hematoma expansion (>6ml) and poor clinical outcome (mrs >3). Multivariable regression was used to adjust for relevant covariates. The primary analysis population was divided by spot sign status and the effect of hemorrhage location was compared to hematoma expansion in exploratory post-hoc analysis.

Results: Among 302 patients meeting the inclusion criteria, lobar hemorrhage was associated with increased hematoma expansion >6ml (p=0.003), poor clinical outcome (p=0.011) and mortality (p=0.017). When adjusted for covariates, lobar hemorrhage independently predicted significant hematoma expansion (aor 2.2 [95% CI: 1.1-4.3], p=0.021) and poor clinical outcome (aor 2.6 [95% CI: 1.2 – 5.6], p=0.019). Post-hoc analysis showed that patients who were spot sign negative had a higher degree of hematoma expansion with baseline lobar hemorrhage (Lobar 26% vs. Deep 11%; p=0.01). No significant associations were observed in spot-positive patients (Lobar 52% vs. Deep 47%; p=0.69).

Conclusion: Hematoma expansion is more likely to occur with lobar ICH and hemorrhage location is associated with poor clinical outcome.

2.2

17ß-Estradiol sensitizes ovarian surface epithelium to transformation by suppressing Dab2 expression.

Nhung H. Vuong[1, 3] and Barbara C. Vanderhyden[1-3]

[1.] Departments of Cellular and Molecular Medicine, University of Ottawa.
[2.] Obstetrics and Gynecology, University of Ottawa.
[3.] Centre for Cancer Therapeutics, Ottawa Hospital Research Institute

Background: Ovarian surface epithelial (OSE) cell proliferation and morphology are tightly regulated by the asymmetrical distribution of polarity proteins that provide positional cues for surface localization and growth inhibition. In a mouse model of ovarian cancer, we have established that prolonged exposure to 17β-estradiol (E2) accelerates tumor onset and increases the incidence of morphologically dysplastic/hyperplastic OSE. We hypothesize that E2 causes OSE hyperplasia by inhibiting a tumor suppressor gene called Disabled-2 (Dab2). Dab2 is critical in mediating the polarized distribution of cell surface proteins and is highly expressed in normal OSE, but is absent in the majority of ovarian carcinomas.

Objective: The goal of this project is to recapitulate the effects of E2 in mouse OSE (mose) with an in vitro model and to elucidate the mechanisms by which E2 sensitizes OSE cells to transformation.

Methods: To reproduce the E2-induced OSE hyperplasia seen in vivo, an in vitro model system was established using primary cultures of mouse OSE (mose). The effects of E2 in this model were assessed in growth curve experiments, by immunofluorescence staining, quantitative RT-PCR, and western blot analyses.

Results: Our results show that E2 can suppress Dab2 via the Esr1 pathway and prolonged E2 exposure causes mose cells to become stratified and hyperplastic; displaying evidence of loss of contact inhibition and polarity. Experiments using conditional Dab2 knockout mose have demonstrated that loss of Dab2 alone is sufficient to increase mose proliferation and cause mose dysplasia. Using primary cultures of human ascites cells, the mechanism of Dab2 suppression by E2 is confirmed to be conserved in humans.
Conclusions: E2 appears to sensitize OSE to tumor initiation by suppressing Dab2, resulting in OSE dysplasia and increased proliferation that may render them more susceptible to transformation

2-3

Identification of dietary patterns associated with obesity in a nationally-representative survey of Canadian adults: application of *a priori*, hybrid and simplified dietary pattern techniques

Mahsa Jessri[1]

[1.] Banting Postdoctoral Fellow, Ottawa Hospital Research Institute; University of Ottawa

Background: Analysing the effects of dietary patterns is an important approach for examining the complex role of nutrition in the etiology of diet-related non-communicable chronic diseases (NCDs).

Objective: The objectives were to characterize the dietary patterns of Canadians using *a priori*, hybrid and simplified dietary pattern techniques, and to compare the associations of these patterns with obesity risk in individuals with and without accompanying chronic diseases (unhealthy/healthy obesity).
Design: Dietary recalls from 11,748 participants (≥18 y) in the cross-sectional nationally-representative Canadian Community Health Survey 2.2 were used. *A priori* dietary pattern was characterized using the previously-validated 2015 Dietary Guidelines for Americans Adherence index (DGAI). Weighted partial least squares (wPLS) (hybrid method) was used to derive an energy-dense (ED), high-fat (HF) and low fiber density (LFD) dietary pattern using 38 food groups. The associations of derived dietary patterns with disease outcomes were then tested using multinomial logistic regression.

Results: An ED,HF,LFD dietary pattern had high positive loadings for fast foods, carbonated drinks, refined grains and negative loadings for whole fruits, and vegetables (≥|0.17|). Food groups with a "high" loading were summed to form a simplified dietary pattern score. Moving from the first (healthiest) to the fourth (least healthy) quartiles of the ED,HF,LFD and the simplified dietary pattern scores was associated with increasingly elevated odds ratios (OR) for unhealthy obesity, with individuals in quartile 4 having an OR of 2.57 (95%CI:1.75,3.76) and 2.73 (1.88,3.98), respectively (p-trend<0.0001). Individuals who adhered the most to the 2015 DGAI recommendations (quartile 4) had 53% lower OR of unhealthy obesity (p-trend<0.0001). The associations of dietary patterns with healthy obesity and being unhealthy non-obese were weaker, albeit significant.

Conclusions: Consuming an ED,HF,LFD dietary pattern and lack of adherence to the recommendations of the 2015 DGAI were associated with significantly higher risk of obesity with and without accompanying chronic diseases.

2-4

Prediction of relapse in patients with locally advanced breast cancer after neoadjuvant treatment

Olexiy Aseyev[1], Lisa Simmonds[1], Maddie Gertler[1], Susan Dent[1] and Shailendra Verma[1]
[1.] The Ottawa Hospital Cancer Center / University of Ottawa

Background. Despite advances in cancer treatment, over 25% of patients (pts) with locally advanced breast cancer (LABC) relapse during first 5 years after treatment.

Objectives. The primary objective was to construct a prediction tool for risk of relapse in patients with LABC after neoadjuvant therapy. Previously published works (Matsuda N. Et al, 2014; Keam B. Et al, 2011; Katz A. Et al. 2008) have also examined this issue.

Material and methods. This was single center, retrospective study of 546 patients with LABC who received neoadjuvant chemotherapy at the Ottawa Hospital Cancer Center between 2005 and 2015. Median follow-up was 49 months. The following data collected: demographics, tumor size, nodal and receptor status, grade, HER-2, stage of disease, cancer treatment and clinical outcomes. Primary endpoints were local and/or distant disease recurrence rate during first 5 years and time to relapse during the first 5 years. A prediction tool was devised based on the Cox regression model.

Results: In 545 patients neoadjuvant chemotherapy was prescribed as follows: FEC-D – 91 (17%), AC-Docetaxel – 330 (60%), other regimens (AC, AC-Paclitaxel, TC, TCH)– 124 (23%). Breast conserving surgery was performed in 67 (12%) pts, mastectomy in 440 (81%) pts. Adjuvant radiotherapy was given in 485 (89%). All patients had trastuzumab – 173 pts (34%) for Her2-positive disease and endocrine therapy (tamoxifen and/or AI) – 356 (44%) pts – for endocrine -sensitive disease. Recurrence rate during first 5 years of follow up was 17.3% (local relapse – 3.2%, distant relapse – 13.2%, local + distant relapse – 0.9%). Over 60 variables were included in primary analysis. Cox regression proportional hazards model analysis resulted in only 5 factors with significant influence on risk of relapse during first 5 years of follow up. Risk factors and their risk prediction value are: 1) residual disease (yes- 4; no-0), (HR = 4.25; p-value=0.000), 2) lymph nodes status (positive-3; negative-0), (HR = 2.27; p-value=0.006), 3) Inflammatory histology (yes-2; no-0), (HR = 1.90; p-value=0.003) 4) estrogen receptors status (positive-2; negative-0), (HR = 2.07; p-value=0.001), 5) Adjuvant radiotherapy

STEM CELL BIOLOGY AND THERAPEUTICS (10:20-11:10) *Moderators:*

Kaitlin Kharas and **Yuefeng Li**

3-1

P38-gamma MAPK is a Critical Regulator of Satellite Stem Cell Self-Renewal

Natasha Chang[1], Fabien Chevalier[1], Melanie Lacaria[1], Michael Rudnicki[1,2]

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Background: During skeletal muscle regeneration, resident muscle stem cells termed satellite cells activate and give rise to myogenic progenitors that repair the damaged tissue. Importantly, a subpopulation of satellite cells undergo self-renewal to ensure maintenance of the satellite cell pool, a process that can occur through either symmetric or asymmetric satellite stem cell divisions. Recent work from our laboratory have highlighted the role of dystrophin in mediating satellite cell polarity during asymmetric satellite stem cell divisions. Dystrophin expression is restricted to the stem cell, though the downstream effectors that regulate self-renewal have not been identified. In contrast, the methyltransferase Carm1 mediates transcriptional activation of the myogenic commitment factor Myf5 in the progenitor cell. How Carm1 activity is regulated in the stem cell to prevent commitment, however, remains unknown.

Objectives: Interestingly, the p38-gamma MAP kinase has been shown to interact with syntrophins, a group of dystrophin-interacting proteins that are part of the dystrophin-associated glycoprotein complex (DGC). We hypothesize that p38-gamma may mediate satellite stem cell functions downstream of the DGC.

Methods: To study the contribution of p38-gamma in satellite cell function we assessed muscle regeneration in satellite cell-specific conditional p38-gamma knock-out mice. Loss of function experiments were performed using sirna to knock-down p38-gamma in satellite cells and assess engraftment capacity and their ability to undergo symmetric or asymmetric divisions. Proximity ligation assays were used to determine protein-protein interactions of p38-gamma with beta1-syntrophin and Carm1. Finally, in vitro kinase assays and mass spectrometry were employed for biochemical analysis of p38-gamma phosphorylation of Carm1.

Results: Genetic deletion of p38-gamma specifically in satellite cells in vivo resulted in impaired muscle regeneration and a significant reduction in satellite cell numbers following muscle injury. As well, satellite cell self-renewal and symmetric stem cell expansion were dependent on p38-gamma Interestingly, we found that p38-gamma negatively regulates Carm1 by direct phosphorylation. Phosphorylation of Carm1 prevents Carm1 nuclear translocation and thereby prevents Carm1-mediated activation of Myf5.

Conclusion: We identify a critical player of satellite stem cell fate, p38-gamma. P38-gamma functions downstream of the DGC to control satellite stem cell self-renewal and negatively regulates Carm1. Ultimately, insight into the molecular pathways that regulate satellite stem cell fates are essential for advancement of therapeutic strategies to treat muscle degeneration.

3-2

Patient-derived TSC2-haploinsufficient smooth muscle cells exhibit widespread features of Lymphangioleiomyomatosis disease pathology

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Background: Lymphangioleiomyomatosis (LAM) is a progressive neoplasm of the lung affecting at least 6 per million women, and is one of the most clinically severe manifestations of the multi-system tumour disorder Tuberous Sclerosis Complex (TSC). LAM is characterized by the growth of abnormal neural crest (NC)-like and smooth muscle cells (smcs) in the lungs, leading to cystic tissue destruction and eventual respiratory failure. LAM and TSC are characterized genetically by inactivating TSC1 or TSC2 mutations. This drives hyper-activation of the mtor signaling pathway, a central regulator of cell growth, proliferation, differentiation, metabolism and survival. Current treatment options for LAM are limited to lung transplant and the mtor inhibitor Rapamycin, which provides some clinical benefit but is not curative.

Objectives and Methods: Our overall objective is to accelerate the development of improved therapeutic options for LAM by generating humanized LAM models, which are currently entirely lacking. Toward this goal, we have generated TSC2+/- induced pluripotent stem cell (ipsc) lines from fibroblasts obtained from patients with TSC and LAM. Subsequently, we undertook an in vivo differentiation approach to allow TSC2+/- ipscs to differentiate into postulated LAM cell lineages in teratoma growth assays. To test the hypothesis that LAM cells exist as smooth muscle-like cells, teratoma explants were cultured under SMC growth conditions, and the resulting cell lines were expanded and characterized for molecular and functional LAM cell phenotypes.

Results and Conclusions: Using this approach, we have successfully established TSC2-deficient SMC lines that can be maintained in culture and, importantly, reflect characteristic LAM phenotypes. These include: expression of NC and SMC-related proteins, mtor dysregulation, increased cell size, expression of LAM biomarkers, and a shift toward glycolytic metabolism. Thus, through our ipsc reprogramming and in vivo differentiation approach, we have established the first humanized cellular disease model for LAM. We show that these cells can be selectively sensitized to death compared to wild-type control SMC lines and a pre-existing TSC2-deficient mammalian cell line in the presence of chemical regulators of glycolysis and autophagy. As LAM cells are thought to be dependent on autophagy and glycolytic metabolism for their survival, these findings demonstrate the high validity and potential of our patient-derived SMC lines to model LAM and aid in the identification of novel therapeutics.

3-3

Revascularization of Lung Scaffold is Enhanced upon Combined Delivery of Induced Pluripotent Stem Cell Derived Smooth Muscle Cells and Endothelial Cells

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Background: Bioartificial lungs represent a novel alternative for organ transplantation; however, inadequate revascularization has limited in vivo function and survival of recellularized lung-scaffolds.

Methods and Results: Smooth muscle cells (smcs), including both ipsc-derived smcs (ismcs) and endogenous pulmonary artery-derived SMC (PASMC) were co-cultured with GFP-huvecs across a range of ratios. Analysis of branching-length and node numbers suggest an optimal SMC:HUVEC ratio of 3:1. Immunoflourescence revealed spontaneous HUVEC-SMC alignment, recapitulating in vivo vessel anatomy, whereby vessel-stabilizing pericytes afford structural support. Correspondingly, flow cytometric data demonstrated >95% expression of pericyte markers CD146 and PDGFR in ismcs but not PASMC (<15%). EC-alone networks collapsed <16Hrs; however, network persistence lasted <36hrs with HUVEC-pasmcs and >72hrs for HUVEC-ismcs. Vessel stabilization is also a function of reciprocal EC-SMC gene-regulation. To study this interaction, HUVEC-SMC co-cultures was performed for 24-72Hrs, prior to $CD144^+$ MACS-based separation. Relative to HUVEC alone, co-cultured huvecs increase expression of EC and angiogenic genes as time progressed. To gain further mechanistic insights underlining network persistence disparity conferred by HUVEC-PASMC and HUVEC-ismc, 72Hr time-point samples were subjected to angiogenesis-focused PCR microarray. A significant increase in pro-angiogenic genes and reduction in angiogenic-inhibitory genes in HUVEC-ismc compared to HUVEC-PASMC co-cultures was noted. Q-PCR and immunostaining also showed marked elevation in extracellular matrix proteins in co-cultured huvecs. Subsequent histological studies of decellularized rat lung scaffold seeded with ismcs and ips-derived endothelial cells (iecs) (3:1 ratio), demonstrated revascularization as rapidly at D3 post-seeding. Ismcs and iecs continue to survive and engraft within the scaffold in the absence of contamination for at least 5 days post-seeding.

Conclusion: Therefore, ismcs improved the stability of vascular network formation in vitro potentially through structural support and the upregulation of endothelial, angiogenic and matrix-related genes.Mechanistic insights underlying this improved revascularization strategy provide viable biotherapeutic targets employable across a range of organ recellularization endeavours.

3-4

Endothelial Progenitor Cell-derived Exosomes for Neonatal Pulmonary Hypertension

Joanne Joseph[1,2] , Arul Vadivel[1], Chanèle Cyr-Depauw[1], Shumei Zhong[1], Dylan Burger[3], Merv Yoder[4], Bernard Thébaud[1,3,5]

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Background: Pulmonary hypertension (PH) is an increasingly recognized complication that doubles the risk of death in severe neonatal lung diseases such as Congenital Diaphragmatic Hernia (CDH) and Bronchopulmonary Dysplasia (BPD). CDH and BPD are characterised by lung vascular hypoplasia. Evidence suggests that angiogenic factors promote alveolar growth and regeneration. Accordingly, we showed that endothelial colony forming cells (ecfcs), a subset of vascular progenitor cells with self-renewal and de novo angiogenic potential, exist in the developing lung, are perturbed in experimental lung injury. Furthermore, exogenous human umbilical cord blood-ecfcs promote lung growth and attenuate PH in nude rats with Monocrotaline (MCT) induced PH through a paracrine effect. Increasing evidence suggest that extracellular vesicles, including exosomes, mediate cellular communication of stem cells.

Hypothesis: Ecfcs mediate their therapeutic benefit through the release of exosomes.

Methods: In the experimental group Sprague-Dawley (SD) rat pups were injected with MCT on postnatal day 6 (P6) to induce PH. The treatment group received 10 million human umbilical cord blood-derived ecfcs/kg or an equivalent dose of exosomes (4.5µg exosome/animal) intravenously on P7. In the control group some animals received similar cell or exosome treatment without the MCT injection. At P21 the animals were sacrificed and the heart and lungs were harvested and processed for various endpoints.

Results: MCT impaired lung growth characterized by fewer and larger alveoli and caused PH characterized by right ventricular hypertrophy (RVH) and pulmonary artery remodelling with increased medial wall thickening (MWT). Exosome treatment significantly decreased alveolar enlargement (as quantified by the mean linear intercept, $P<0.0001$), while ECFC treatment did not show any significant decrease. Exosome treatment significantly decreased MWT ($P<0.001$), while ECFC treatment did not.

Conclusion: MCT injection induced lung hypoplasia and characteristics of PH such as RVH and vascular remodelling, confirming a robust animal model. Exosomes were able to reduce lung hypoplasia and attenuate features of PH. Thus, the previously seen therapeutic potential of ecfcs may be mediated through exosomes as similar therapeutic effects were seen. Exosomes open new allogeneic ECFC-based therapeutic options for PH.

TOWARD CANCER IMMUNOTHERAPY (1:25 – 2:15)

Moderators: **Curtis McCloskey** and **Dominic Roy**

4-1

Oncolytic virus-mediated immunotherapy

Marie-Claude Bourgeois-Daigneault[1,2], Dominic Guy Roy[1,2], Lauren Elizabeth St-Germain[1], Adrian Pelin[1,2], Amelia Sadie Aitken[1,2], Theresa Falls[1], John Cameron Bell[1,2]

Background: The lack of treatment options for patients with chemotherapy-resistant cancers is pushing forward the rapid development of alternative therapies. One such option, especially for disseminated or recurrent disease, is the use of oncolytic viruses, with the first candidate now approved for use in patients. Oncolytic viruses are know to specifically destroy tumors by mediating direct killing and tumor vascular shutdown, but the induction of an efficient and persistent anti-tumor immune response is a facet that is underappreciated.

Objective: To assess the extent of the potential of oncolytic viruses as immunotherapeutic agents and the importance of the virus-induced anti-tumor immunity for treatment efficacy.

Methods: We used the clinical trial candidate rhabdovirus Maraba MG1 in orthotopic murine breast tumor models prior to surgical resection and subsequent rechallenge in immunocompetent and immunocompromised animals. We measured primary tumor growth as well as the natural lung metastasic burden. The anti-tumor immunity was analyzed by ELISPOT, flow cytometry and immunohistochemistry.

Results: Our results show that the administration of MG1 is sufficient to control the growth of subsequent tumors and even cure some animals without the need for further treatment. The virus induces an efficient tumor-specific immune response and recruits immune cells to the tumor. Also, tumor-infiltrating lymphocytes are able to penetrate the tumor more profoundly. Importantly, treatment with MG1 causes the upregulation of PDL1 by tumor cells, and active regulatory T cells were found in greater amounts. We therefore investigated if the combination with immune checkpoint inhibitors could further improve oncolytic virotherapy. Indeed, our data demonstrate the successful combination of MG1 with immune checkpoint inhibitors in tumor models that are usually resistant to the latter.

Conclusions: Here we demonstrate that direct treatment with MG1 protects against subsequent tumor rechallenges. We show that this response is immune mediated and that the combination with immune checkpoint inhibitors further improves efficacy. We believe that our study is the first to reveal the extent of the potential of oncolytic viruses as immunotherapeutic agents.

4-2

Oncolytic immunotherapy potentiated by phosphatase inhibitor

Mohammed Selman[1,2,3], Chris Rousso[1,3], Fanny Tzelepis[1,3], Andrew Chen[1,3], Jean-Simon Diallo[1,2,3]
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Background: Oncolytic viruses (OV) are an emerging class of anticancer bio-therapeutics, based on their selective replication and lysis of tumor cells, in addition to inducing antitumor immunity. However, the efficacy of OV as a single agent is still limited. Numerous studies have shown that viral oncolysis, spread and overall efficacy is improved using pharmacological compounds that manipulate the cellular innate anti-viral immune response. A number of viruses have evolved various strategies to antagonize the antiviral activity. For instance, some viral proteins target phosphatases to evade innate immune recognition.

Objective: We propose a strategy to boost the therapeutic efficacy of OV by combining it with immuno-modulating agents targeting protein phosphatases.

Methods: A phosphatase inhibitor panel was screened for their ability to enhance of OV VSVΔ51 in 786-0 cells, a human renal carcinoma cell line which is highly resistant to infection with VSVΔ51. Promising compounds were tested for cancer specific enhancement of VSVΔ51 in ex vivo in tumours and normal organ samples. The mechanism of action was examined by microarray analysis. In vivo anti-tumor efficacy of the compounds alone and in combination with VSVΔ51 was tested in resistant mouse tumor models was also tested.

Results: We identified PI compounds that could enhance OV infection in vitro and ex vivo, in resistant tumor cell lines. Furthermore, one phosphatase inhibitor tested increased anti-tumor efficacy in combination with OV in several syngenic tumor models, leading to durable responses in models otherwise refractory to OV and drug alone. Microarray analyses suggest this potentiation may occur through enhanced immune-stimulation in addition to improved oncolysis.

Conclusions: We show that PI compounds can maximize anticancer immunity, and the ability to enhance the growth and spread of oncolytic rhabdoviruses, in addition to OV mediated viral lysis in cancer cells leading to increase efficacy of OV treatment in in vivo models. Overall, the present project will lead to a better understanding of important factors of viral infection and development of improved oncolytic therapy strategies.

4-3

An oncolytic virus targeting the RNA interference pathway

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Background: Oncolytic viruses (ovs) are emerging as promising cancer therapeutics due to their ability to selectively target and kill cancer cells and associated vasculature while eliciting an anti-tumor immune response. To be effective therapeutics, ovs must be able to overcome host barriers that hinder establishment of infection within the tumour. In contrast to the protein-mediated antiviral response in mammalian cells, plants and invertebrates use an RNA interference (rnai) mediated pathway. RNA-mediated immunity was thought to be restricted to plants and invertebrates until the recent discovery of a similar mechanism in mammalian embryonic or undifferentiated cells. Given that many properties of cancer cells resemble those of embryonic stem cells, this has led us to hypothesize that cancer cell lines may use rnai as antiviral defense mechanism. More specifically, cancers with interferon pathway defects may rely on this alternate pathway as a primary defense against viral infection.

Objective: We aim to determine whether inhibition of rnai improves OV efficacy in certain tumour types and to characterize the resulting cellular and immune responses as well as mechanism of action of this therapy.

Methods: We have engineered vsvd51 to express an inhibitor of rnai (vsvd51-VSR1) and have screened a panel of human cancer cell lines with this virus to assess efficacy of viral killing relative to control virus. Viral replication in a selection of cell lines was assessed by qpcr of viral genomes and titering. Next Generation Sequencing was used to assess viral genome and microrna processing.

Results: The expression of a rnai inhibitor enhances the efficacy of vsvd51 in approximately 80% of human cancer cell lines tested. Vsvd51-VSR1 shows enhanced replication in sensitive cell lines both in vitro and in vivo. Vsvd51-VSR1 leads to increased activation of the Type I interferon pathway, TNF alpha pathway and expression of a number of cytokines and immune-related genes. Additionally, VSR1 both blocks direct cleavage of viral genomes and alters microrna processing in other cell lines in a cell-line specific manner, as confirmed by Next Generation Sequencing.

Conclusions: We have engineered a novel OV expressing an inhibitor of the rnai pathway and demonstrated its enhanced efficacy in a panel of cancer cell lines. We have begun to unravel the molecular response to this virus and characterize the mechanisms behind its improved efficacy. This work provides insight on the biology of viral defense mechanisms as well as contributes to our knowledge on the rational design of OV therapies.

4-4

Eradication of peritoneal carcinomatosis using a Maraba virus infected cell vaccine expressing IL-12 requires recruitment of Natural Killer cells

Almohanad Alkayyal [1,3,7], Lee-Hwa Tai[1], Michael A. Kennedy[1], Christiano T de Souza[1], Jiqing Zhang[1,2], Charles Lefebvre[8], Shalini Sahi[1], Anu Abhirami Ananth[1,3], Ahmad B. Mahmoud[3,9], Andrew P. Makrigiannis[3], Greg O. Cron[5,6], Blair Macdonald[5], David F. Stojdl[3,8], John C. Bell[1,3] and Rebecca C. Auer[1,4].
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Introduction: Peritoneal carcinomatosis (PC) is the most common cause of death for abdominal cancers. Immunotherapies have demonstrated efficacy in selected solid malignancies but their potential in PC has only been explored to a limited extent. Here we report the utility of an intraperitoneal (IP) injection of the infected cell vaccine (ICV), consisting of autologous tumor cells infected *ex vivo* with an oncolytic Maraba MG1 (MG1) virus engineered to express Interleukin-12 (IL-12) in a murine model of peritoneal carcinomatosis.

Method: Interleukin-12 (IL-12) was cloned into The MG1 virus. The infected cell vaccine (ICV) was created by infecting autologous tumour cells *ex-vivo* with MG1 or MG1-IL12 following irradiation. The measured immune response to ICV on NK cells included migration (transwell assay), activation (flow cytometry) and cytotoxicity (chromium release assay) *in-vivo* and *in-vitro*. PC was generated by inoculating mice IP with CT26 or B16F10 cells. The treatment was administered IP on day 3-17 following tumor seeding or when a measurable tumor was confirmed by MRI imaging.

Results: MG1-IL12-ICV promoted a robust migration and activation of NK cells in the presence of Dendritic cells and was dependent on their secretion of the chemokine IP10 and this was not seen with MG1-ICV vaccine. In a murine model of PC, IP treatment with MG1-IL12-ICV stimulated recruitment of IFN-γ secreting NK cells to the peritoneal cavity associated with a dramatic reduction in tumor burden and durable cures in >90% compared to 0% survival in mice treated with MG1-ICV. Even in mice with bulky PC (tumours >8 mm), a complete radiological response was demonstrated in a 100% of mice within 14 weeks. The effect was dependent on intact NK cell function as NK cell depletion abrogated any survival advantage. The effect of MG1-IL12-ICV on NK cell migration, activation were recapitulated in-vitro using human lymphocytes.

Conclusions: MG1-IL12 ICV treatment IP recruits activated NK cells to the peritoneal cavity resulting in a dramatic reduction in tumor burden and improved survival. Our promising results in preclinical models of PC provide a proof of principle that an MG1-IL12-ICV platform has the potential to provide a personalized immunotherapy patients who are diagnosed terminal PC each year

Role of WNT signaling pathway in ovulatory wound repair

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Background: The ovarian surface epithelium (OSE) is a monolayer of cells surrounding the ovary and is ruptured during ovulation. After ovulation, the ovulatory wound is repaired, but this process and the consequences of improper healing are poorly understood. Stem cells are the source of proliferating cells during wound healing in many epithelial tissues and several OSE characteristics suggest the presence of a stem population. Microarray analysis on OSE cells isolated two days post-ovulation vs. From normally cycling mice determined a notable increase in WNT pathway activation in post-ovulation OSE which was suggested to occur through the down-regulation of several genes encoding suppressors of WNT signaling (Frzb, Sfrp1 and Sfrp2) and increased Wnt4 and Wnt5a as well as the WNT pathway activator Prl2c2 (proliferin-1).

Objective: In light of these findings, our aim is to characterize OSE stem/progenitor cells and the signaling mechanisms involved in ovulatory wound repair.

Methods: To study the effect of wnts on OSE stem cell characteristics, we treated mouse OSE (mose) with WNT5a and WNT4 recombinant proteins (selected because they were found to be increased in post-ovulatory OSE by microarray) for different lengths of time. In addition, biological assays, including morphology assessment, proliferation, and viability, were evaluated.

Results: Candidate stem/progenitor cell markers shown to be expressed in human tissues include CD44, CD133, and NANOG as well as ALDH2 and LGR5. Challenging OSE cells with WNT4 in vitro increased Cd44 mrna expression, while WNT5a dramatically decreased Cd44 expression. Using conditional gene targeting, we found that OSE-specific inactivation of Wnt5a (not Wnt4) results in higher CD44 expression. Interestingly, Cd44 is modestly increased 1.2-fold after ovulation as well, supporting the investigation of this protein as a potential stem/progenitor cell marker in the OSE. In addition, WNT4 increased Nanog expression and WNT5a significantly increased Sca1 and Aldh2 mrna levels. WNT5a appears to signal by upregulating the WNT/Ca(2+) pathway and inhibiting the canonical pathway, β-catenin, whose expression was increased after ovulation. Biological assessment of cells did not show any significant changes in the presence or absence of WNT4-5A, except for cell proliferation which was significantly decreased by WNT5a.

Conclusions: In summary, these data suggest that the WNT family is able to regulate the expression of stem cell biomarkers in OSE cells in vitro and in vivo. Further investigation will increase our understanding of their role in ovulatory wound repair and could clarify the consequences of disruption of this process on fertility.

Heterologous prime-boost vaccination approach using Measles Virus as an oncolytic prime vector for the Maraba boost vaccine

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Background: Originally used as tumor lytic agents, oncolytic viruses (ovs) are now considered as potent stimulators of anti-tumor immunity. Indeed, most ovs are able to induce immunogenic death of tumor cells characterized by the release of tumor antigens in an immunostimulatory environment that triggers the activation of anti-tumor specific T cell responses. However, a part of the immune response will be directed towards viral antigens, also produced in the tumor environment during the infection. In case of multiple administration of the same OV for patient treatment, the anti-viral immune response could dominate the anti-tumor immune response.

Objective: To avoid this and to generate a large population of memory CD8 T cells against the tumor, a heterologous oncolytic prime-boost strategy has been developed. In this approach, two different ovs expressing the same tumor-associated antigen (TAA) are used: one to prime a specific TAA immune response and the other to boost it. Treating patients with these two ovs should efficiently destroy the tumor and elicit a strong immune response against the OV-encoded tumor antigen. This strategy is already under clinical investigation based on the use of a non-replicating adenovirus as a priming vector followed by the oncolytic Maraba virus to boost the TAA-specific immune response (NCT02285816). We aim at strengthening this strategy by using a replicating oncolytic measles virus (mev) as a priming vector rather than a non-replicating and non-oncolytic adenovirus.

Methods: We will generate an oncolytic mev encoding a fusion protein containing epitopes of E6 and E7 proteins of both serotypes 16 and 18 of the Human Papillomavirus (HPV), which is the causative agent for different cancers. E6/E7 proteins are foreign viral antigens, therefore naturally immunogenic unlike most autologous taas. Furthermore, these oncoproteins interfere with the type I interferon responsiveness, making HPV^+ tumors highly susceptible targets for ovs. After in vitro characterization of mev-E6/E7 we will assess its therapeutic efficacy in an immunocompetent mouse model bearing HPV-driven TC-1 tumors.

Conclusion: The main focus of this project is to evaluate the therapeutic potential of mev as a priming vector used in combination with the Maraba virus in the heterologous oncolytic prime-boost strategy. Due to its replication in tumor cells and its capacity to generate an inflammatory environment, we hypothesize that the T cell priming with mev is more efficient than with the adenovirus.

The role of the Ste20-like kinase, SLK in erbb2-mediated breast cancer

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Background: The Ste20-like kinase, SLK, has previously been implicated in regulating cell motility, focal adhesion dynamics and is activated downstream of HER2/erbb2/Neu. HER2 is overexpressed in approximately 30% of human breast cancers, the majority of which are highly metastatic. Recently, SLK deletion has been shown to be embryonic lethal and has been shown to be activated downstream of HER2. These data suggest that SLK may play a potential role in HER2-driven breast cancer progression.

Objective: As SLK has been shown to signal downstream of HER2, we aimed to assess whether SLK deletion had any impact in tumor progression and overall survival in the context of HER2-induced breast cancer, as well as whether SLK deletion had a major impact on the physiology and/or molecular signaling within these tumors.

Method: As SLK is activated downstream of HER2 we aimed to conditionally knockout SLK in the mammary epithelium of a murine model of HER2-induced tumorigenesis (NIC). Tumor bearing mice were palpapated weekly in order to track tumor growth. Once mice had reached humane endpoint (tumor volume > 1.7 cm^3), mice were sacrificed and tumors were harvested for protein, RNA, FACS and formalin-fixed paraffin sections.

Results: $slk^{fl/fl}$ NIC mice show significantly reduced time to tumor onset and overall survival as compared to NIC controls. SLK deletion also results in a significant reduction in apoptosis within the tumor epithelium, with no differences observed in proliferative index. Consistant with these observation no differences in proliferation or migration are observed in vitro. In order to further explore this phenotype, we assessed the number of tumor infiltrating leukocytes by both IHC and FACS analysis. The total percentage of Iba1+ infiltrating macrophages is significantly reduced in $slk^{fl/fl}$ NIC tumors suggesting that epithelial specific SLK-deletion may regulate immune cell recruitment, engagement and/or proliferation.

Conclusions: SLK plays an important role in regulating macrophage infiltration in HER2-induced mammary tumors, as well as being required for efficient apoptosis. The combination of these two phenotypes results in an accelerated tumor onset and worsened survival in HER2-positive tumors following SLK deletion.

Perioperative arginine supplementation prevents natural killer cell dysfunction ex vivo and reduces surgery-induced metastases in a murine tumour model.

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Background: Cancer patients with large, bulky tumours must often undergo invasive surgical procedures. Unfortunately, there are prometastatic consequences associated with the physiological stress of surgery. Surgery causes a dramatic suppression in natural killer (NK) cell cytotoxicity resulting in an increased number of metastases in mouse tumour models. This is due, in part, to surgery-induced myeloid derived suppressor cells (mdscs). Mdscs metabolize arginine via arginase which is upregulated in some disease states and can lead to a meaningful drop in plasma arginine levels. The importance of arginine for proper T-cell function and proliferation has been studied extensively in literature, however, substantially less is known about arginine and NK cell mediated cytotoxicity.

Objective: To determine the importance of arginine for NK cell function following surgical stress.

Methods: The following experiments used C57BL6 mice in our validated model of surgical stress (abdominal laparotomy followed by nephrectomy). MDSC populations and arginase expression were assessed by flow cytometry. Arginase activity was assessed via a colourimetric assay which measures urea production. NK cell cytotoxicity was assessed with the ^{51}Cr-release assay. Mice were given supplemental arginine (arginine-enhanced diet) and serum arginine levels were determined in collaboration with Newborn Screening Ontario. Lastly, b16lacz cells were injected I.V. and lung metastases were quantified on post-operation day 3 (POD3).

Results: We observed a significant increase in granulocytic mdscs (gmdscs; CD11b^{+}, Ly6G^{+}, Ly6C^{dim}) with increased arginase-1 expression and activity following surgery. Since gmdscs have elevated levels of arginase-1, we hypothesized that gmdscs were depleting their microenvironment of arginine. The addition of exogenous arginine (2mm) to our ex vivo NK-MDSC overnight co-culture assay abrogated the suppressive effects of mdscs. To assess the in vivo efficacy of a perioperative arginine therapy, mice were fed either a control diet or a diet enhanced in arginine. Surgery was performed on a cohort of mice from each group and b16lacz cells were inoculated I.V. for 3 days in all mice. Lung metastases were significantly reduced on POD3 in arginine-fed vs control diet mice (41 vs 83 lung metastases). Furthermore, all mice suffered a similar drop in plasma arginine post-operation, but those on the arginine-enhanced diet had higher arginine levels pre-operation.

Conclusion: The immunosuppressed state following surgery is a critical period for minimal residual disease to grow in the absence of anti-tumour immunity. Arginine represents a potential perioperative intervention that can improve NK cell function and prevent increases in metastases.

THE INTERACTION OF GLIOBLASTOMA WITH MACROPHAGES

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Background: Glioblastoma is the most common and aggressive type of adult brain tumour with a need for new treatments. CD47 has been shown to be overexpressed on some human cancers but its role in glioblastoma has not yet been fully explored. CD47 is a ligand for sirpα, a protein expressed on macrophages.

Objective: The objective of the study is to look at the interaction of glioblastoma with macrophages and examine the role of CD47.

Methods: In order to understand the interactions between macrophages and glioblastoma cells, co-culture conditions with a dual labelling system were established. Primary glioblastoma cell lines were obtained from patients undergoing surgery and macrophages were obtained by differentiating monocytes isolated from human blood.

Results: Co-culture of macrophages and glioblastoma cells resulted in increased glioblastoma cell growth compared to control. Addition of anti-CD47 to co-cultures of primary glioblastoma cells blocked macrophage stimulated growth of glioblastoma cells. Furthermore, addition of anti-sirpα also blocked macrophage stimulated glioblastoma growth. Addition of a SHP-1/2 inhibitor to co-cultures, thus blocking downstream signalling of sirpα resulted in no change in macrophage stimulated glioblastoma growth suggesting the observed increase in growth is through CD47. To explore downstream signalling pathways of CD47, selective inhibitors of the PI3Kα and PI3Kβ were added to co-cultures. Addition of the PI3Kα inhibitor resulted in no change in glioblastoma growth while addition of the PI3Kβ inhibitor resulted in a partial blockage of macrophage stimulated cell growth.

Conclusion: This indicates that upon interaction with sirpα, CD47 may signal through PI3Kβ to increase cell growth however, further characterization is still needed. The implication of this research is the possibility to develop new therapies targeted at CD47 to decrease glioblastoma cell growth.

ATF3 as a key regulator of cisplatin cytotoxicity in non-small-cell lung cancer

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Lung cancer is responsible for the majority of cancer-related morbidities and mortalities, with non-small-cell lung carcinomas (NSCLC) accounting for 85% of all lung cancers. Platins, such as cisplatin and carboplatin, are commonly used as anti-cancer agents against NSCLC, with platin combination chemotherapy displaying enhanced efficacy. However, resistance has kept the overall survival rates modest, therefore, there is an urgent need for alternative and more effective therapeutic regimens against NSCLC.

Our laboratory has identified Activating Transcription Factor 3 (ATF3), a stress-inducible gene, as a key regulator of the cytotoxic and apoptotic effects of cisplatin. The overall aim of this project is to further evaluate ATF3 as a key regulator of cisplatin response. This will be evaluated by determining the effect of overexpression and knockout of ATF3 in NSCLC cell lines.

Furthermore, the combination of platins with novel inducers of ATF3, which were identified following screening of a library of 1200 FDA approved compounds, will be evaluated as a therapeutic strategy. One of the compounds identified was vorinostat, an HDAC inhibitor, which was determined to induce ATF3 and increase the levels of cytotoxicity in combination with cisplatin in parental and resistant NSCLC cell lines. Lastly, the role of ATF3 as a potential treatment-induced biomarker will be evaluated in NSCLC patients that will be undergoing platin treatment.

Investigation of a new anti-cancer vaccine strategy for pancreatic cancer

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Identification and Evaluation of Novel Sarcoma Antigens for Use with Oncolytic Vaccines

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Background: Cancer immunotherapies focused on tumor-specific T cell response are promising therapeutic alternatives for cancer because in addition to eliminating cancer cells, they can also establish an active and long-term surveillance against relapsing tumours. The efficacy of such therapies depends primarily on the immunogenicity of the tumours. However, the lack of targetable and known specific antigens for osteosarcoma poses as a limitation for the development of active immunotherapies against this cancer. Thus, identification of immunogenic antigens is a critical step. Current methodologies for antigen discovery are time consuming involving peptide-MHC separation, generation of T cell clones, etc. Furthermore, most of these methods are ineffective in identifying a breadth of truly immunogenic antigens and provide limited opportunities to identify antigen/T-cell receptor pairs. Therefore, the development of a new or improved strategy would be a tremendous benefit for the design of immunotherapies against osteosarcoma.

Objective: The goal of this project is the development of a methodology for identification of immunogenic osteosarcoma antigens that efficiently isolates a breadth of truly immunogenic tumour specific antigens.

Methods: This methodology will consist of 3 steps: 1) generation of target cells through transduction with cdna library developed with RNA extracted from the tumor; 2) expansion of tumor-specific T cells (without the necessity of generating T cell clones); and 3) isolation of target cells recognized by tumor-specific T cells through development of a novel approach involving the utilization of CD107a as a marker for analysis by flow cytometry.

Results: Initial optimization was done using the B16 melanoma model expressing known antigen SIINFEKL from the chicken ovalbumin (OVA) protein and GFP serving as our target cells and co-cultured with transgenic OVA-specific CD8+ T cells (called OT-1) serving as our effector cells. A significantly higher percentage of GFP+ doublets expressing CD107a and CD8 were observed in co-culture with B16-OVA-GFP compared to B16-GFP when using effector cells previously cultured in IL-7+IL-15 for 7 days. We also observed a higher percentage of CD107a+CD8+ doublets when a greater number of effector cells are used.

Conclusion: CD107a can be used as an effective method for identifying antigen mediated cytotoxic synapses consisting of effector cells specific for antigens presented by target cells.
There is an improved capacity of memory-like effector cells to recognize antigen specific target cells co-cultured at higher effector: target ratios. Following further optimization, antigens discovered for the osteosarcoma tumour model K7M2 will be incorporated into an oncolytic vaccine therapy.

Regulation of Ovarian Surface Epithelium Stem Cells by Transforming Growth Factor Beta 1 is Mediated by Ptgs2 activation and Brca1 Repression

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Background: Ovulation is the primary non-hereditary risk factor for ovarian cancer. During ovulation, the ovarian surface epithelium (OSE) is ruptured and subsequently repaired, however these repair processes are poorly understood. We have previously shown the mouse OSE (mose) contains a Stem Cell Antigen 1-expressing population of cells that exhibit increased stem cell characteristics. Further characterization of these cells has identified CD44 as an additional stem cell marker. Treating OSE cells with Transforming Growth Factor Beta 1 (TGFB1), a factor found in the follicular fluid, enhances their stem cell phenotype. These data suggest that components in the follicular fluid can increase OSE stem cell characteristics, perhaps to aid in ovulatory wound repair.

Objective: To elucidate the mechanism for TGFB1-induced stemness in the OSE.

Methods and Results: TGFB1 induced an epithelial-to-mesenchymal transition (EMT), with a 3.5-fold increase in SNAI1, 80% decrease in CDH1 and increased cell migration. Overexpression of SNAI1 in mose cells also decreased CDH1 and increased sphere forming capacity. A TGFB signaling targets PCR array identified an 8-fold increase in Ptgs2 in mose cells treated with TGFB1, which is also induced in mose cells overexpressing SNAI1. Treating mose cells with Prostaglandin E2, the downstream product of PTGS2, or activating its receptor Ptger4, increased CD44 expression. These data suggest that the increase in stemness by TGFB1 is through an EMT mediated by Snai1, increasing Ptgs2 expression and PGE2 secretion, which increase stemness characteristics through the Ptger4 receptor. Since BRCA1 regulates stemness in mammary epithelium, we explored the possible role of BRCA1 in modulating OSE stem cells. TGFB1 treatment of mose cells decreased Brca1 expression by 50%. Deletion of Brca1 also increased CD44 expression 3-fold, suggesting that the increase in stemness induced by TGFB1 is mediated by repression of Brca1 expression. TGFB1 treatment of human OSE cells also showed an increase in SNAI1, PTGS2 and CD44 expression as well as enhanced sphere forming efficiency and reduced BRCA1 expression, suggesting the results in mose cells can be translated to human OSE cells.

Conclusions: This data suggests that the increase in stemness by TGFB1 is through an EMT mediated by Snai1, repressing Brca1 and increasing Ptgs2 expression, which increases the stem cell phenotype through the Ptger4 receptor. Rapid proliferation of OSE cells during ovulatory wound repair, accompanied by increased stem cell characteristics and repressed Brca1 expression could make cells more susceptible to transformation.

FGL2 is involved in the regulation of inflammatory response and leukocyte activation within the preeclamptic placenta

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Background: Impaired placental development early in pregnancy is at the root of placental dysfunctions such as preeclampsia, that cause symptoms at late stages of pregnancy. Crucial to early placentation is the establishment, at the maternal-fetal interface, of a balance between pro- and anti-inflammatory cytokines secreted by various subsets of leukocytes. Fibrinogen-like protein 2 (FGL2), expressed by the trophoblast and by some leukocytes subsets, has known roles in the regulation of this immune balance and in the formation of fibrin clots. We hypothesize that FGL2 mediates leukocyte recruitment and cytokine secretion at the maternal-fetal interface, and could act as a biomarker of an impairment in early placental development. We have previously determined that cases of preeclampsia can be further classified into distinct clusters according to placental gene expression patterns, thus identifying molecular subtypes of preeclampsia.

Objectives: This study aims to determine mechanisms by which FGL2 regulates the immune environment during early pregnancy and placentation. It also aims to determine if FGL2 could be used as a reliable biomarker in early pregnancy, predicting the appearance of preeclampsia symptoms at later stages.

Methods: Using novel in vitro transgenesis techniques, the role of FGL2 in trophoblast function will be defined. In vivo placenta-specific overexpression will be used to determine how FGL2 modulates leukocyte populations at the maternal-fetal interface and how these changes affect early development. Bioinformatics analysis of FGL2 expression in human placenta samples will be used to determine its potential as a biomarker.

Results and conclusion: Microarray analysis revealed differential expression of FGL2 between molecular subtypes of preeclampsia. Compared to healthy controls, FGL2 expression is significantly lower in samples exhibiting classical markers of preeclampsia and significantly higher in samples showing upregulated inflammation-related genes. Gene Ontology (GO) term analysis revealed that genes whose expression best correlates with that of FGL2 are predominantly involved in immune response (215 genes). Differential correlation analysis identified gene expression correlations that are stronger in a diseased state than in a healthy state. GO term analysis determined those genes to be mostly involved in leukocyte activation pathways (1005 genes). FGL2 overexpression in invasive trophoblasts HTR-8/svneo was found to have no effect on proliferation, migration or invasion. Preliminary data suggests FGL2 overexpression in bewo trophoblasts hinders their ability to syncytialize. These data suggest an important role for FGL2 in the regulation of immune pathways and of the inflammatory state within the placenta.

Searching for Synergy: Focal Adhesion Kinase Inhibition in Metastatic Breast Cancer Treatment

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Background: Breast cancer is the most common cancer among Canadian women and 14-20% of these patients will develop lethal metastases within 5 years. Therefore, there is an urgent need for new therapies for metastatic breast cancer patients. A potential novel therapeutic target is Focal Adhesion Kinase (FAK). FAK is a cytoplasmic tyrosine kinase known to regulate cell migration, proliferation and invasion. Critically, is overexpressed in metastatic breast tumours and its expression is inversely correlated with survival. However, as with many tyrosine kinase inhibitors, it is likely to be more effective when used in combination with other drugs. As such, it would be useful to identify additional drugs with which FAK inhibitors may synergize to more effectively manage breast cancer cells.

Objective: We hypothesize that using FAK inhibitors in combination with other pharmacological agents commonly used to treat metastatic breast cancer patients will result in enhanced anti-tumor activity.

Methods: The ability of FAK inhibition to enhance the anti-cancer effects of a panel of commonly used metastatic breast cancer chemotherapies was assessed. Three breast cancer cell lines were used to evaluate effects of treatment with a FAK inhibitor alone, a potential drug candidate alone, as well as in combination. Viability was measured using MTT assay, and western analysis was used to confirm FAK inhibition in response to the chemical inhibitor.

Results: While most chemotherapeutics examined have shown no increased cytotoxicity when paired with the FAK inhibitor, there has been some success with the DNA damaging subclass of chemotherapeutics. Thus far, potentially enhanced cytotoxicity in combination was observed with cisplatin, and significantly enhanced combination effect was observed with doxorubicin. However, when higher doses of FAK inhibitors were used the enhanced combination effect was eliminated.

Conclusions: High doses of FAK inhibitor are highly effective at decreasing breast cancer cell viability. However, at lower doses of FAK inhibitor, which are more achievable in patients, a similar decrease in cancer cell viability can be observed when combined with certain commonly used chemotherapies, like doxorubicin. The observed enhanced anti-tumor activity of DNA damaging agents, and the lack of enhanced activity when combined with other classes of chemotherapeutics, suggests possible synergy of FAK inhibition with this drug class.

SNF2H-mediated chromatin remodelling and its regulation of the pluripotent state

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Background: The pluripotent state is maintained by a variety of regulatory factors that coordinate the expression of pluripotency-associated gene expression, while repressing the expression of lineage-associated factors. ATP-dependent chromatin remodellers chromatin regulators responsible for mobilizing nucleosomes, and are broadly implicated in transcriptional regulation, DNA repair and replication, and maintaining higher-order chromatin structure. The SWI/SNF, CHD, and Ino80 subfamilies of remodellers have been shown to be critical to the pluripotent state, but the ISWI subfamily has yet to be studied, despite the ISWI homolog SNF2H being essential for the development of early embryos and in other biological contexts.

Objective: The objective of this study is to perform loss-of-function experiments and high-throughput genomics to explore SNF2H-mediated chromatin remodelling and its impact on the pluripotent state in mouse embryonic stem cells (mescs).

Methods: SNF2H loss-of-function experiments were performed using a mesc line derived from $Snf2h^{fl/fl}$ mice, allowing for conditional deletion following the transfection of Cre recombinase. Apoptosis and cell death were measured by flow cytometry with Annexin V staining. SNF2H localization across the genome was mapped using chip-Seq, and various public data sets were used to determine enrichment of SNF2H at regulatory regions and transcription factor binding sites.

Results: Deletion of Snf2h in mescs results in aberrant changes to colony morphology, reduced alkaline phosphatase activity, and coincides with the repression of Oct4, Sox2, and Nanog expression—changes typically indicative of the loss of pluripotency. The emergence of this phenotype is not associated with increased apoptosis or cell death. Additionally, Snf2h expression is seemingly critical for the reprogramming of somatic cells to induced pluripotent stem cells. Chip-Seq analysis demonstrated that SNF2H is broadly distributed across the genome, but is preferentially enriched at active regulatory regions and at the binding sites of key pluripotency transcription factors.

Conclusion: These data suggest that SNF2H is a critical regulator of pluripotency and may facilitate gene expression through maintaining accessible chromatin for transcription factors to bind, and in its absence, the pluripotency gene expression program can no longer be maintained. It has now been demonstrated that all families of chromatin remodellers are critical to pluripotency, and this work provides a foundation for exploring how diverse remodellers work together to coordinate chromatin accessibility across a variety of biological contexts.

Immune Profiling the Infected Cell Vaccine for the Treatment of Acute Leukemia

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Background: Acute lymphoblastic leukemia (ALL) is a very difficult blood cancer to treat using current treatment methods. The development of a novel treatment by infecting ALL cells with an oncolytic virus, followed by gamma-irradiation creates an immunotherapeutic infected cell vaccine (ICV). Administration of the ICV to murine recipients provides anti-tumour immunity and promotes survival when these recipients are further challenged with ALL.

Objective: Elucidate the molecular mechanisms that are created and required by the ICV to provide anti-tumour immunity. Immune monitor the effects of the ICV to determine how it creates a specific and long term T-cell response. Further, analyze each subset of T-cells to determine what subset is required for the ICV to create anti-tumour immunity.

Methods: Murine ALL cells infected with a rhabdovirus, followed by gamma-irradiation will be used to create the ICV. Intravenous injections of the ICV will be administrated to mice weekly for 3 weeks followed by ALL challenge. The first experiment will be a survival analysis after ALL challenge in the following cohorts: a) CD4 depleted ICV immunized mice, b) CD8 depleted ICV immunized mice, c) CD4/CD8 depleted ICV immunized mice, d) unimmunized mice as controls. Spleen collection and saphenous bleeding will be done on these cohorts to perform flow cytometry and ELIPSPOTS to monitor the effects of the ICV on the host's immune system. Differences in cytokine production and surface proteins will also be determined.

Results: It has been shown that the administration of the ICV following ALL challenge provides long-term anti-tumour immunity. However, this model does not provide anti-tumour immunity in athymic mice signifying that T-cells play an important role. Administration of different doses of CD4 and CD8 antibody has been done in mice to determine the optimal antibody dose needed to deplete each T-cell subset. Flow cytometry analysis has shown that the optimal antibody dose to deplete each T-cell subset is 100ug. This optimal dose, 100ug, will be used in downstream experiments. Based on the CD4/CD8 depletion experiment, further experiments will be done to characterize the immune system.

Conclusions: Rhabdovirus-infected ALL cells can be used to create a vaccine that produces a long-term anti-tumour immune response in murine recipients. The ICV requires the presence of T-cells to protect against leukemic challenge. Profiling the host's immune system to the ICV will provide insight to create a pre-clinical package to translate this immunotherapeutic vaccine to future human clinical trials.

The Forgotten Women's Cancer; a molecular profile of Vulvar Squamous Cell Carcinoma (VSCC) in HPV positive and HPV negative cancers

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Background: Vulvar squamous cell carcinoma (VSCC) is a neglected woman's cancer that suffers from high rates of surgical morbidity and has few treatment options in the advanced/recurrent setting. VSCC is characterized by two distinct etiologies, HPV-associated and HPV-independent disease. While personalized therapies have improved outcomes in other cancer types, the genomic landscape of VSCC remains poorly defined and is hampered by the inconsistent reporting of HPV status and the limited application of NGS technologies.

Methods: This was a retrospective chart review and molecular analysis of patients diagnosed with VSCC between 2000-2016 at The Ottawa Hospital Cancer Centre (Ottawa, Canada). Patients were included in the study if they had adequate tumor for molecular analysis from a pretreatment biopsy or tumor resection. Clinicopathological data and surgical and treatment details were collected from the medical charts. DNA was isolated from the tumor blocks to test for the presence of high-risk HPV by nested PCR and were amplified and sequenced using the Ion ampliseq Cancer Hotspot v2 Panel of 50 genes of oncogenic significance. Clinical and molecular data was analysed separately by HPV status. Progression-free and overall survival was estimated using the Kaplan-Meier approach and compared between groups using the log-rank test and Cox proportional hazards.

Results: The overall prevalence of one or more oncogenic mutations in the full study cohort was X%, with a high rate of mutations noted in both HPV-associated (%) and HVP-independent disease (%). The molecular profiles were found to differ by HPV status, with HPV-associated cancers characterized by oncogenic mutations in PIK3CA X/Y (Z%), KIT: X/Y(Z%), KDR (VEGR-2) X/Y (Z%), FGFR3 X/Y (Z%), PTEN X/Y (Z%), CTNNB1 X/Y(Z%), TP53 X/Y (Z%), while HPV-independent cancers were found to have mutations in TP53 X/Y (Z%), HRAS X/Y (Z%), CDKN2A X/Y (Z%) and PI3KCA X/Y (Z%). The novel FGFR3 mutations (all S249C) were established as somatic by mutational analysis of adjacent normal tissue and were validated by Sanger sequencing.

Conclusions: VSCC is characterized by a high mutation burden. HPV-associated and HPV-independent disease display different molecular profiles. FGFR3 maybe be a novel therapeutic target in the future management of HPV-associated VSCC. Given its molecular diversity, biomarker identification and screening in VSCC will be critical in guiding the future management of this forgotten woman's cancer.

Small Molecule Viral Sensitizers Enhance Oncolytiv Virus Therapy by Suppressing the Innate Antiviral Response

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Background: Oncolytic Viruses (ovs) are often attenuated to increase their safety profile, however this can lead to reduced efficacy in heterogeneous malignancies and result in resistance to OV therapy. Many strategies have been developed as a means to increase the efficacy of OV therapy while maintaining selectivity. One such strategy utilizes small molecule enhancers of OV therapy termed viral sensitizers. These small molecules have been shown to enhance the replication and spread of oncolytic rhabdovirus VSVΔ51 in vitro and prolong survival in tumour-bearing mice.

Objective: In this study we evaluate the effect of these viral sensitizers on the innate antiviral response in order to identify the mechanism of action responsible for their viral-sensitizing properties.

Methods: In order to evaluate the effect of our viral sensitizers on the antiviral response, cells were pre-treated with Viral Sensitizers for 2-4 hours. The cells were then infected with VSV?51 or treated with IFNβ in order to induce a robust antiviral response. Components of the antiviral response are analyzed using several assays such as immunoblots, ELISA, real-time qpcr and competition assays with IFNβ.

Results: Our data suggests that our viral sensitizers dampen the transcriptional expression of IFN-β and interferon-stimulated genes (isgs), a subset of antiviral genes expressed in response to Type-I ifns. Sandwich elisas were used to verify that the viral sensitizers also impaired the secretion of IFN-β. Western blots suggest that the compounds may interfere with the activation of key antiviral transcription factors, including STAT1 and IRF3, which are responsible for the up-regulation of isgs and IFN-β respectively. Finally, we found that these viral sensitizers were capable of enhancing VSV?51 infection even in cells pre-treated with IFNβ, indicating that the compounds inhibit both the induction of and response to Type I ifns. Other components involved in the regulation of Type I ifns have yet to be investigated, namely NFκB and AP1.

Conclusions: Identifying the mechanism of action is crucial for the development of a new generation of viral sensitizers with enhanced efficacy and retained safety. We have demonstrated that our current viral sensitizers significantly dampen the Type I IFN response, and that this effect may be linked to the viral sensitizing properties of these compounds. These viral sensitizers have the potential to be used in combination with ovs and other cancer gene therapy approaches to induce a more robust anti-tumour effect in patients.

Impact of myeloma induction therapy on hematopoietic stem-cell collection outcomes, the Ottawa Hospital experience.

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Introduction: Multiple myeloma (MM) induction treatment has evolved over the last 15 years with the introduction of new agents, but patients continue to receive consolidation with autologous hematopoietic stem cell transplant (ahsct).

Objectives: We examined the impact of changes in induction therapy on stem cell (SC) mobilization and collection outcomes among transplant-eligible MM patients.

Methods: Retrospective cohort study using data from MM Clinical Management Tool (MMCMT) consisting of 482 consecutive MM patients who underwent successful SC collection for first ahsct from 1st-January-1994 to 30th-September-2015 at The Ottawa Hospital. Patients that received syngeneic or allogeneic SC transplants before ahsct were excluded.

Results: The most common induction regimens were VAD (n=233), Velcade with or without steroids (n=88) and CYBORD (n=78). Fewer patients experienced admissions because of toxicity after mobilization chemotherapy if they had received Velcade (12.5%) compared to VAD (27.5%) or CYBORD (19.2%) (p=0.014). The most common toxicity was febrile neutropenia followed by bone pain. SC collection successfully reached a target of >5x10^6 $CD34^+$cells/kg in 93% of Velcade treated patients, 81% of CYBORD treated patients and 78% of VAD treated patients (p=0.0057). While 72% of Velcade and 68% of VAD treated patients needed one apheresis, 56% of CYBORD treated patients had successful single collections (p=0.04). More patients achieved a very good partial response or better after cyclophosphamide mobilization if they received CYBORD (58.3%) versus Velcade (29.5%) or VAD (11.5%).

Conclusion: Myeloma induction regimens influence both the toxicity and success of SC mobilization. Velcade induction resulted in the least toxicity and the optimal SC collection after cyclophosphamide mobilization at the cost of lower response rates when compared to the current preferred regimen, CYBORD.

Investigation of GREB1 as a potential therapeutic target for ovarian cancer

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Background: Estrogenic hormone replacement therapy is a known risk factor for ovarian cancer, but the mechanisms underlying this are unknown. Estrogen (E2) accelerates tumour initiation and progression in mouse models. We have shown that Growth Regulation by Estrogen in Breast Cancer 1 (Greb1) is highly E2-upregulated in these tumours. GREB1 is required for E2-driven proliferation of several hormone-responsive cancer cell lines, and is a transcriptional cofactor with ESR1 but may have additional functions. We propose that GREB1 is an estrogen receptor alpha (ESR1)-upregulated protein which mediates some of the estrogenic effects in hormone-sensitive ovarian cancers, and it may be a good biomarker of hormone-sensitive cancers.

Objective: The role of GREB1 in ovarian cancer is not well defined, but our preliminary studies suggest a pro-tumourigenic role. We therefore will determine if GREB1 expression correlates with ESR1 expression in human tissue microarrays (tmas) and in primary cultures from patient ascites, then compare GREB1 expression to patient outcomes to determine the clinical relevance of GREB1.

Methods: To examine GREB1 and ESR1 expression in human ovarian cancers, we will assess protein levels by immunohistochemistry on a TMA containing 200 high-grade serous ovarian carcinomas (Canadian Ovarian Experimental Unified Resource). We will compare expression of GREB1 to ESR1 and levels of GREB1 to patient outcome. This data will be compared to preliminary data acquired from a TMA with 4 ovarian cancer subtypes with 20 cases each (Cooperative Human Tissue Network) and to expression levels in primary cultures from human ascites.

Results: The preliminary analysis showed that GREB1 expression correlated with ESR1 at the mrna level (qpcr), but not at the protein level (TMA). It also showed that 81% of ESR1- and 78% of ESR1+ tumours were GREB1+ by IHC, with no major differences across subtypes. This suggests estrogen-associated GREB1 expression in some cases, but provides the first evidence of estrogen-independent GREB1 in others. Samples from human ascites reflect this trend.

Conclusions: GREB1 is frequently expressed in epithelial ovarian cancers of all subtypes. It is normally expressed mainly in the reproductive tract, suggesting that it may be a useful diagnostic biomarker. Furthermore, inhibiting GREB1 activity may prevent tumour-promoting estrogen signalling pathways downstream of ESR1. Therefore, targeting GREB1 may prove more effective than the current anti-estrogen therapies.

Does Ectopic Expression of Germ Cell Proteins Contribute to Genomic Instability in Cutaneous T-Cell Lymphoma?

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Purpose: Cutaneous T-cell lymphoma (CTCL) is a heterogeneous group of non-Hodgkin lymphomas with primary localization to the skin originating from CD4+ T cells. Patient-derived CTCL cells exhibit extensive and inconsistent chromosomal abnormalities, suggestive of a high level of genomic instability which is thought to be a driving force behind the progression of cancers. The ectopic expression of proteins normally expressed by germ cells, aka Cancer/Testis antigens, have been detected in various cancers including CTCL. It has been hypothesized that aberrant activation of meiosis pathways could result in genomic instability in CTCL.

Experimental Design: In the present work, we use immunofluorescence to detect the expression of DNA double strand breaks (dsbs) in various patient-derived CTCL cell lines compared to healthy donor T-cells. To elucidate potential mechanisms that contribute to genomic instability, we test the expression and cellular localization of various germ cell proteins.

Results: Extensive foci of DNA dsbs were detected in five CTCL cell lines representative of Mycosis Fungoides and Sezary Syndrome, while none were detected in the CD4+ T Cells from a healthy donor. An assortment of proteins associated with meiosis initiation (SPO11, STRA8), homologous recombination (RAD51, DMC1, HOP2, MND1), synaptonemal complex formation (SYCP1, SYCP3, HORMAD1), and meiotic cohesins (REC8, SGO2, STAG3) were found to be ectopically expressed in CTCL cells with unique staining patterns. The retrotransposon LINE1, as well as its associated protein L1TD1, and transposon repressors (GTSF1, PIWIL2) were also detected.

Conclusions: Patient-derived CTCL cells exhibit endogenous DNA dsbs consistent with the notion of enhanced genomic instability in this cancer. The ectopic expression of proteins normally restricted to germ cells, such as meiosis regulators and the LINE-1 retrotransposon, may contribute to genomic instability and malignant progression in CTCL. Due to their specificity to cancer, germ cell proteins are likely to be useful as diagnostic/prognostic markers and represent potential therapeutic targets in CTCL.

Activating transcription factor 3 as a novel regulator of chemotherapy response in breast cancer

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Background: Doxorubicin is a commonly used chemotherapy for the treatment of breast cancer. Understanding the mechanisms regulating doxorubicin cytotoxicity will help improve its effectiveness. Activating transcription factor 3 (ATF3) has recently been identified by our laboratory as a mediator of cisplatin cytotoxicity.

Hypothesis: The goal of this study is to determine the role of ATF3 in mediating doxorubicin cytotoxicity in breast cancer, as well as to identify novel ATF3 inducing agents for combination therapies.
Results: ATF3 expression was upregulated in both a time and concentration dependent manner by doxorubicin treatment in the human breast cancer cell lines tested. Doxorubicin induced ATF3 expression occurred through the activation of both JNK and ATM signaling pathways. In ATF3-/- mefs there was a significant reduction in sensitivity to doxorubicin treatment. Through a 1200 FDA approved compound library screen, several drugs were identified that could enhance doxorubicin cytotoxicity, including the HDAC inhibitor Vorinostat and 6-mercaptopurine. Additionally, we have demonstrated that doxorubicin can induce ATF3 expression in ex-vivo human breast and ovarian tumor samples which represents a potential novel screening strategy for identifying responsive tumors.

Conclusion: ATF3 is a novel mediator of doxorubicin cytotoxicity and represents a new therapeutic target for the treatment of breast cancer. The use of other ATF3 inducing compounds, such as HDAC inhibitors, can enhance doxorubicin cytotoxicity.

Random mutagenesis of Vaccinia virus uncovers novel clinical candidate oncolytic backbones with unique phenotypes.

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Background: Oncolytic viruses (ovs) are an emerging class of multi-mechanistic biological cancer therapeutics designed to: (1) directly lyse cancer cells, (2) destroy tumour vasculature, and (3) induce anti-tumour immunity. This latter effect has been demonstrated to have therapeutic efficacy on existing sites of disease, prevent further metastases, and provide immune-mediated surveillance.

Objectives: Poxviruses are being tested clinically in a variety of settings, however a systematic analysis of the types of virus gene mutations/deletions that favour both oncolytic activity and immune stimulation remains to be completed. We aim to define important Vaccinia virus (vacv) genes for their role in modulating characteristics that may be important in next-generation clinical candidate ovs.

Methods: We have identified Copenhagen as the most rapidly replicating clinical candidate strain of vacv in several patient-derived models of cancer, and utilized a transposable element system to randomly mutagenize the Copenhagen genome. With next-generation sequencing, we confirmed that we have generated a library of 89 unique vacv clones and inserted into 52 unique genes. We have generated clonal stocks of each of these clones and made progress in phenotypically characterizing them.

Results: We have demonstrated transposable element-induced loss-of-function of vacv genes and have begun characterizing our library of novel clones. We have identified dozens of clones with differential growth properties compared to wild-type Copenhagen as well as the current clinical candidate vacv product. We have demonstrated unique ability of these clones to induce cytotoxicity and cell-to-cell spread. We are now gaining an understanding of the immunogenic potential of the viruses in our library. We have identified 3 lead candidate viruses, which are being more completely characterized and whose gene functions are being more completely understood.

Conclusions: We have generated a unique library of viral clones that have allowed us to probe the vacv genome more comprehensively than previously possible. Using this tool, we have identified novel functions of several vacv genes and gained fundamental insights into the eradication and prevention of human cancers. We have identified several candidate clones that demonstrate more desirable oncolytic characteristics than the current clinical standard. This tool has the potential to impact the fields of virology and vaccine development by providing novel fundamental insights into vacv biology.

First-in-class small molecule potentiators of cancer virotherapy

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Background: Pre-clinical and clinical studies have shown that oncolytic virus (OV) therapy is safe, well tolerated and effective in a broad range of cancers. Still, resistance in a subset of tumors highlights areas for improvement in oncolytic virus based therapeutics. Combining OV therapy and drug therapy is a promising strategy to selectively enhance OV-mediated tumour cell death. To this end, we have previously identified the synthetic compound Viral Sensitizer 1 (vse1) that enhanced the spread of oncolytic vesicular stomatitis virus (VSVΔ51) in resistant cancer cell lines up to a 1000-fold, resulting in synergistic cell killing and improved efficacy in vitro and in vivo. While we know vse1 suppresses the ability of cancer cells to defend against viral infection, its mechanism of action and biological targets are unknown.

Objectives:
1. Characterize the structure-activity-relationship of vse1
2. Identify vse1 analogues with improved pharmacological and pharmacokinetic properties.

Methods: To study the structure-activity-relationship (SAR) of vse1, enhancement of VSVΔ51 by vse1 analogs was assessed by a novel high-throughput titration assay. Plasma stability and electrophilicity of analogs were assessed by mass spectrometry and reactivity with glutathione, respectively. Selectivity of analogs for cancerous tissue was assessed by ex vivo treatment of murine tissues. In vivo tolerability of vse1 analogs was assessed in a dose escalation study, and in vivo efficacy was assessed in murine and human xenograft tumour models.

Results: In vitro assays and a rational design approach allowed us to identify modifiable functional groups on vse1. Lead compounds increased VSVΔ51 growth up to 2000-fold in vitro and demonstrated remarkable selectivity for tumors over normal tissue. Some analogs possess improved potency and stability with reduced electrophilicity. Analogs were better tolerated than vse1 in vivo and also enhanced survival and in vivo VSVΔ51 replication.

Conclusions: We have developed a novel class of small molecules for enhancing VSVΔ51 replication in tumors. SAR studies led to the identification of compounds with favourable pharmacological properties that significantly enhance VSVΔ51 propagation selectively in resistant cancers in vitro, ex vivo, and in vivo. Ultimately, this study demonstrates the feasibility of using these first-in-class small molecules with favourable pharmacological properties to enhance OV efficacy in resistant cancers.

Towards Novel Effective Combination Therapy for KRAS Mutant Non-small Cell Lung Cancer

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Background: Non-small-cell lung cancer (NSCLC) accounts for 80–85% of all lung cancers and is associated with significant mortality. As epidermal-growth-factor receptor (EGFR) is over-expressed in 80- 90% of NSCLC and is associated with poor outcomes and reduced survival, its inhibition via EGFR-Tyrosine Kinase inhibitors (EGFR-tkis) is a main therapeutic strategy for these patients. However, it has been shown that patients with mutations in KRAS are resistant to EGFR-tkis. A recent study in mutant KRAS-driven lung cancer in transgenic mice showed that tumor growth was dependent on the activity of focal adhesion kinase (FAK). Therefore, we hypothesized that KRAS mutant NSCLC will be sensitive to FAK tkis, and given known cross-talk of FAK with the EGFR signaling pathway, FAK inhibition will sensitize KRAS mutant NSCLC cells to EGFR-tkis.

Methods: We thus performed cell viability assays of mutant H460, A549, and A427 compared to wild type H1299, H1563, H1975 KRAS NSCLC cell lines following treatment with FAK TKI alone or in combination with a clinically relevant EGFR-TKI.

Results: As predicted we found KRAS mutant cells were more sensitive to FAK inhibitors than KRAS wild-type NSCLC. We additionally found that the use of FAK and EGFR tkis in combination resulted in reduced tumor cell viability as compared to treatment with either drug alone. Previous results led us to speculate that this enhanced anti-tumor response could be a result of the ability of FAK inhibition to block the FAK- EGFR cross talk and prevent activation of downstream signaling thereby affecting cell survival, or potentially via altering endosome recycling, thereby affecting levels of surface or internalized EGFR and thus its signaling. Our preliminary data suggests that in KRAS mutant cells, the combination of FAK and EGFR TKI drugs appears to more effectively inhibit AKT activity, as evidenced by significantly reduced levels of phospho-AKT, than does treatment with either drug alone, suggesting enhanced ability to impair cell survival following treatment with the drug combination. These proposed mechanisms are currently being further tested by assessing activity of other important cell viability and proliferation pathways, and by measuring the EGFR surface expression pre/post FAK treatment using FACS analysis.

Conclusion: Our results suggest that FAK and EGFR tkis used in combination are an effective therapeutic combination for KRAS mutant NSCLC, and elucidation of the mechanism of enhanced activity has uncovered unique and previously unrecognized roles of FAK modulation of EGFR activity.

Regulation of breast cancer subtypes by Periostin and Prolactin-Induced Protein expression in an erbb2-positive animal model.

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Background: erbb2 is an oncogene overexpressed in about 30% of all breast cancer tumors. About 50% of these tumors acquire epithelial Periostin (Postn) expression. This has been correlated with a more aggressive phenotype.

Objective: Therefore, we assessed the role of Postn in erbb2+ tumors using a global knockout model. Loss of Postn results in a shift from a luminal to a molecular apocrine tumor subtype.

Results: Consistent with this, we observed an upregulation of Prolactin-Induced Protein (PIP) in Postn-null tumors. We hypothesize that Postn is driving erbb2-mediated tumor growth through a feed forward activation loop while PIP is driving the molecular apocrine subtype.

Conclusion: We believe that the expression of these two proteins is mutually exclusive and important for the resulting subtype, as we have observed consistent reciprocal expression of these two proteins in vivo and in vitro. A double knockout tumor model will dissect their role in subtype determination and tumorigenesis.

Ovulation-associated fibrosis promotes a pro-tumor initiation niche in the ovary.

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Background: Ovarian cancer is a high fatality cancer with a mortality rate >45%, partly due to a lack of early detection methods and frequent disease recurrence within 2 years of diagnosis. Regardless of the cell-of-origin, ovarian cancer takes root within the ovary and up to 30% of cancers found in the ovary are metastases from other primary sites, highlighting the ovary as a pro-tumor niche. Although the early events in ovarian cancer are unknown, we know the number of lifetime ovulations is the primary non-hereditary risk factor. Ovulation results in cycles of extensive tissue remodeling and excess connective tissue deposition over time, also known as age-associated ovarian fibrosis.

Objective: We hypothesized that regulatory T cells (Tregs) would be recruited to quench autoimmunity that could result from chronic inflammation in response to the fibrosis, but also create an immune privileged pro-tumor niche in the aged ovary.

Results: We have identified a novel tissue-resident FOXP3+ Treg population in the murine aged ovary, which is not present in the ovaries of young mice. Using the 4-vinylcyclohexene diepoxide model of ovarian aging, no Tregs were present showing that ovulation is required for Treg accumulation. Fibrinogen-like protein 2 (FGL2), a potent inducer of Treg proliferation is upregulated in ovarian stromal cells during ovulation, a potential recruitment mechanism we will be investigating with FGL2 knockout and overexpression mice. Using correlation analysis of The Cancer Genome Atlas and human tmas, we found FGL2 expression correlates with Tregs (FOXP3, CD39, PRDM1, NRP1, CTLA4) immune checkpoint targets and pro-inflammatory cell exhaustion (PD1, PDL1, PDL2, TIM3, LAG3) in human ovarian cancer, suggesting FGL2-mediated Treg regulation is also present in tumors. The syngeneic murine STOSE model of ovarian cancer expresses both FGL2 and FOXP3, as shown by IHC. We have therefore begun testing anti-FGL2 immunotherapies with the goal of perturbing Treg homeostasis to promote a more robust anti-tumor response. Preliminary results using MG1 oncolytic virus prolonged STOSE tumor survival by 25% which we predict will be improved by anti-FGL2 therapy.

Conclusion: This study has identified a novel Treg population that may facilitate age-associated immune privilege in the ovary to create a pro-tumor niche, and FGL2 as a new immunotherapeutic target in ovarian cancer.

Inhibition of ggtase1 Enhances Tarceva Efficacy in Head and Neck Squamous Cell Carcinoma Cells: Potential Novel Combination Therapeutic Approach

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Targeting the epidermal growth factor receptor (EGFR), with inhibitors such as tarceva, represents a promising therapeutic option in advanced squamous cell carcinomas (SCC). However, they lack significant efficacy as single agents. Recently, we identified the ability of statins, which target mevalonate synthesis, to induce synergistic cytotoxicity with tarceva in SCC cells through activation of cellular stress pathway regulated by the activating transcription factor 3 (ATF3).

A resulting Phase I trial of rosuvastatin and tarceva, while showing clinical activity, statin induced muscle pathology limited this approach, likely the result of diminished ubiquinone levels. Downstream mevalonate pathway enzymes may represent novel therapeutic targets in SCC cells. Geranylgeranyl pyrophosphate (GGPP), a common protein lipid membrane anchor that is downstream of ubiquinone synthesis, was a key regulator of lovastatin-induced synergy in combination with tarceva. Employing an inhibitor of the ggtase I enzyme, GGTI-298 treatments induced ATF3 expression in SCC9 and SCC25 cells and in a cohort of ex-vivo tumour tissues.

Furthermore, GGTI-298 and tarceva induced synergistic cytotoxicity in SCC cells that was dependant on ATF3 expression as ATF3 deficient murine embryonic fibroblasts displayed attenuated cytotoxicity in response to GGTI-298 alone and in combination with tarceva. Similarly, SCC9 sub-lines that were selected as resistant to GGTI-298 through prolonged exposure to this agent, also failed to demonstrate synergy with GGTI-298 in combination with tarceva. These results suggest the potential clinical utility of combining GGTI inhibitors with tarceva in SCC patients as a novel and more refined combination therapeutic approach.

Envelope exchange in oncolytic measles virus for neutralization escape

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Background: The use of oncolytic viruses (OV) to specifically target and eliminate tumors is a quickly growing approach in tackling cancer. A major obstacle in oncolytic viral therapy is the host immune response when systemic therapy is administered. In the case of measles virus (mev), for which most individuals have been immunized against, the patient's pre-existing neutralizing antibodies will impair delivery of the OV before it can attack the tumor. The neutralization response towards mev targets predominantly the fusion (F) and hemagglutinin (H) envelope glycoproteins displayed at the particle surface.

Objective: We plan to circumvent the immune response to mev by replacing the two envelope proteins with the glycoprotein G of the distantly related Vesicular Stomatitis Virus (VSV) against which no neutralizing antibodies in most individuals exist due to a low incidence of contact.

Methods: The F and H proteins of mev have been substituted for different variants of the VSV-G protein. Three variations of chimeric VSV-G glycoproteins were generated through PCR overlap and fusion techniques: 1) unmodified VSV-G, 2) VSV-G ecto- and transmembrane domains fused to the cytoplasmic tail of mev-F, and 3) VSV-G ectodomain fused to mev-F transmembrane and cytoplasmic domains. Furthermore, both the F and H open reading frames (ORF) of mev were deleted from the viral backbone and each chimera was subsequently inserted in the available F gene slot while EGFP was inserted into the H position of the mev backbone.

Results: Recombinant infectious particles are now ready to be produced in Vero cells. We will introduce these modified viruses in various cancer cell lines and analyze the viral production, virus stability, replication kinetics and cytotoxicity in vitro, in order to identify the best candidate for improved and effective oncolytic therapy. For in vivo studies, we will use an immunocompetent murine model consisting of immunization and viral challenge assays to assess host neutralization effects.

Conclusion: A neutralization evasion strategy may improve the delivery process of the virus to the tumor location in patients immunized for measles. Therefore, by effectively shielding an attenuated oncolytic measles virus against the host's immune response, we aim to take full advantage of its effectiveness as an anti-tumor agent.

Bioengineering an optimal oncolytic Vaccinia virus variant for cancer therapy

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Background: We have shown previously that Pexa-Vec (formerly JX-594), a Vaccinia based oncolytic virus encoding the immune-stimulating transgene GM-CSF, can be safely administered to cancer patients by either direct tumour injection or intravenous infusion. In a small randomized trial, Pexa-Vec was shown to provide a survival benefit to patients suffering from hepatocellular carcinoma in the front line setting. Pexa-Vec is now being tested in this same patient population in a pivotal Phase III study called PHOCUS.

Objectives: These clinical results have encouraged us to begin the development of a second generation Vaccinia virus based oncolytics with improved therapeutic properties.

Methods: To this end, we compared five common vaccine strains of Vaccinia virus in co-infection experiments both in vitro and in vivo and analyzed the output by next generation sequencing. We are broadening this analysis to determine the growth properties of the virus collection on a spectrum of malignant cell types and patient explants. This approach will identify an optimal “virus engine” to form the basis for a new oncolytic platform. Aside from tumour cell growth and killing, a second critical therapeutic attribute of an ideal oncolytic is the ability to stimulate anti-tumour immunity.

Results: We have identified virus variants with large deletions in the Vaccinia virus genome encoding genes with immunosuppressive activity. In in vitro assays, the deletion mutants have enhanced tumour killing activity and retain their ability to replicate at the same rate as parental viruses. Unlike Pexa-Vec, our large deletion mutants do not suppress activation of the immune system and thus the combination of enhanced cancer cell killing, improved immune stimulation and ability to rapidly replicate and spread in tumours suggests that our new variants will be superior oncolytic viruses. Ongoing studies in a variety of in vitro and in vivo assays will be presented illustrating the strategy we have used to create an optimal oncolytic virus platform.

Conclusions: We expect these viruses to surpass the current clinical candidates for cancer OV-immunotherapy in terms of both safety and efficacy.

Deletion of the Ste20-Like Kinase in Dystrophic Muscle Reduces Fibrosis and Increases Myoblast Differentiation

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Background: Muscular Dystrophies are often caused by the loss of cytoskeletal proteins that are critical for myofiber integrity. Deficits in these proteins results in progressive muscular damage and accumulating fibrosis. Recent evidence suggests that the release of tgfb from infiltrating cells further decreases muscle repair due to inhibition of the myogenic program. Additionally, tgfb targets, such as fibronectin and periostin, increase fibrosis and decrease muscle regeneration. Recently, we have disocvered that deletion of SLK can partially resuce the inhibition of myogenesis downstream of tgfb. Therefore, inhibition of SLK may be a means to inhibit the tgfb response and rescue differentiation in dystrophic skeletal muscle.

Objectives/Methods: In order to test the efficacy of SLK deletion on restoring the myogenic program we will treat C2C12 and primary myoblasts with tgfb following SLK deletion. We will then test the capacity for these cells to differentiate and initiate the expression of fibrotic genes. Furthermore, we will assess known substrates of SLK to provide insight into the mechanism of action SLK deletion has on myogenesis. Additionally, we will cross our skeletal muscle SLK null animal model with the mdx model and assess various features of muscular dystrophy. We predict an increase in fiber diameter, and a decrease in collagen deposition, suggesting an increase in muscle regeneration and decrease in the fibrotic response.

Results: To date, we have assessed the response of SLK deletion on myogenic differentiation in C2C12 and primary myoblasts. Although levels of differentiation markers, such as myog and myhc, were lower than controls, there was still a significant upregulation in SLK null myoblasts treated with tgfb. Interestingly, activation of Smad2/3, as well as the expression of tgfb target genes, was unchanged in SLK deleted cells, suggesting that SLK has an effect independent of the tgfb signalling cascade. Our preliminary results from the mdx/SLK null animal model shows a decrease in collagen deposition, decreased circulating creatine kinase levels, and increase myofiber diameter. Collectively these results suggest that deletion or inhibition of SLK infers a protective effect in a dystrophic environment,

Conclusions: Our results demonstrate that SLK may be viable therapeutic target for muscular dystrophy. Designing inhibitors may be a means to treat late stages of muscular dystrophy and improve muscle function.

Ontario Tumour Bank Initiative at The Ottawa Hospital

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The Ontario Tumour Bank (OTB) is a province-wide biorepository and data bank focused on the collection of tumour-related human biospecimens. Operating at four academic teaching hospitals across Ontario (Kingston General Hospital, London Health Sciences Centre, St. Joseph's Healthcare Hamilton, and The Ottawa Hospital (TOH)), the OTB is a program of the Ontario Institute for Cancer Research (OICR) which is funded by the Government of Ontario. The OICR is a not-for-profit corporation that supports research on the prevention, early detection, diagnosis, treatment and control of cancer. Collaboration with the OTB provides academic and industry cancer researchers with a diverse selection of high quality tumour-related specimens and data obtained directly by dedicated tumour bank staff, who follow a stringent set of procedures and ethical guidelines. The biospecimens and clinical data are an important resource for scientists engaged in translational research who are developing better diagnostic tools and new drug therapies. Researchers depend on the OTB to provide research biospecimens of high quality, diversity, and integrity.

The OTB coordinates the collection, storage, analysis, annotation, and distribution of tumours and peripheral blood samples. Working alongside local pathologists, medical oncologists, surgeons and other hospital personnel, specially trained OTB staff obtain patient consent, collect tissue and assemble comprehensive clinical information for each donor and their corresponding samples.

TOH-OTB site has been acknowledged in many scientific journals over the past years. The most recent publication was in the January 2016 issue of The New England Journal of Medicine for the contribution to the TCGA (The Cancer Genome Atlas) study. The researchers involved in this study reported molecular characterizations for papillary renal-cell carcinoma.

TOH-Ontario Tumour Bank team, Dr. Harman Sekhon (PI); Dr. Angel Arnaout and Dr. Sebastien Gilbert (Co-PI's); Marc Venturi, OHRI Project Analyst; TOH-OTB staff, Nikita Rayne and Marta Sienkiewicz.

Optimization of infected cell vaccines in the treatment of acute leukemia

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Background: Acute leukemia (AL) is a malignancy characterized by the uncontrolled proliferation of immature cells, leading to immune dysregulation among other effects. While many patients can reach complete remission with chemotherapy, about half of adults will relapse. Leukemic patients would benefit from less cytotoxic therapies personalized to the patient's cancer that stimulate a robust anti-tumour immune response. An oncolytic virus-infected leukemia cell vaccine (ILCV), combining oncolytic virotherapy with anti-tumour immune activation from inactivated leukemia cells, could be a less toxic treatment modality.

Objective: Previous research found that ILCV is a potentially effective treatment for AL. My objective is to improve the efficacy of this ILCV so it can be applied to preventing disease progression and relapse in AL patients. To do so, I require a detailed understanding of how the immune system responds to ILCV treatment leading to a long-term response. I hypothesize that a combined anti-ILCV response through immune cell expansion and peptide expression leads to long-term, anti-tumour immunity.

Method: Elucidating the immune response to a stimulus involves characterizing changes in immune cell populations as well as knowing whether or not these cells are specifically against the desired target(s). Therefore, I aim to run various panels of surface markers through flow cytometry using peripheral blood and splenocytes from immunized vs. Unimmunized mice to quantify immune cell populations. In addition, intracellular cytokine staining will assess the specificity of individual immune cells following target re-stimulation.

Results: It has been shown that athymic mice lack protection from L1210 leukemic challenge in this ILCV model. Furthermore, mice of a bone marrow transplant had significantly lower numbers of CD8+ T cells, which correlated with lower survival following L1210 challenge. From the studies proposed, I can further reveal the potential importance of all immune cell subsets and their contribution to conferring anti-tumour immunity when exposed to ILCV. Similarly, I can infer their roles and major pathways involved by considering expressed peptides, like cytokines, chemokines and cell surface markers.

Conclusion: It is clear that the adaptive immune response is not only important, but also necessary for the development of ILCV-mediated anti-tumour immunity. Future work will continue to expose more specific functions within the many likely involved subsets of immune cells, leading to the treatment's optimization.

Identification of artificial microrna that increase oncolytic virus using a novel rnai screening approach

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Background: Oncolytic viruses (ovs) infect and kill tumor cells while leaving normal cells unharmed. Specificity towards cancer cells can be a natural feature of the virus, or it can be selected for or engineered into the virus. Ovs are often genetically attenuated by reducing their ability to antagonize antiviral defenses, therefore increasing tumor specificity. This strategy leads to enhanced replication in tumor cells, which often possess defects in antiviral pathways, while sparing normal cells. However, not all tumors have defects in their antiviral defenses and thus OV replication in these tumors is rather limited. Identifying and modulating host factors that regulate OV replication in OV-resistant cancer cells, but not normal cells, will lead to increased OV replication in these tumors and potentially improved therapeutic outcomes.

Objectives: To identify artificial microrna (amirnas) that increase OV replication in cancer cells using an rnai screening approach.

Methods:
1. A Sindbis virus-based amirna library screen was conducted in various cancer cells. Cell selected virus populations were analyzed by deep sequencing and bioinformatics analysis was used to identify amirnas that were enriched.
2. Selected amirna sequences were tested for enhancement of OV growth in cell culture as well as in vivo.
3. The cellular mrna targets of the enriched amirnas will be identified.

Results: Serial passage of the Sindbis virus amirna library in various cancer cell lines followed by deep sequencing of the selected virus populations led to the identification of several amirna sequences that were enriched. Follow-up experiments demonstrated that the identified amirnas increase replication of Sindbis virus, as well as other ovs, including VSVΔ51, Maraba MG1, and Vaccinia virus. Amirna sequences identified in the in vitro screen also enhance virus replication in vivo, thus leading to improved tumor control in a murine tumor model.

Conclusions: The Sindbis virus rnai screening approach is a useful tool to identify amirnas, and potentially their corresponding cellular mrna targets, which modulate OV replication in OV-resistant cancer cells. Viruses expressing the identified amirnas demonstrate an enhancement in replication both in vitro and in vivo. Future studies will be aimed at identifying the cellular mrna targets of the identified amirnas.

Molecular and histopathological determinants of successful oncolytic virotherapy

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Background: Pre-clinical and clinical data indicate that oncolytic viruses(OV) such as the attenuated Maraba-rhadovirus MG-1 can lead to durable anti-cancer responses with minimal adverse effects; however, heterogeneous therapeutic response is evident. Therefore, it would be advantageous to gain insight regarding the factors important for successful OV therapy, such as to eventually use this information to select patients most likely to benefit from these promising therapies.

Objective:To screen a panel of mouse tumor cell lines(n=10) to (1) characteerize OV infection ex vivo and in vivo and (2) assess how hisological and molecular features and anti-tumor efficacy of MG-1 correlate with in vivo infectivity of tumors.

Methods: Mouse tumor cells were implanted in mice, and MG-1 was injected intratumorally or intravenously. At 24 hours post infection(hpi), tumors were excised and cored. For ex vivo infection, tumors were cored before the administration of MG-1. 24 hpi (ex vivo) or following tumor tissue processing (in vivo), virus was quantified. Also, the cores were stained with various markers. In vivo anti-tumor efficacy was assessed by measuring tumor size and survival following MG-1 administration.

Results: Ex vivo infectivity of tumors did not correlate with in vivo infection irrespectively of the route of virus delivery. However, the number of blood vessels positively correlated indicating MG-1 was delivered more efficiently when tumors were better vascularized. In vivo efficacy data revealed little correlation with tumor infection and a potential role of innate immune response upon OV injection for tumor control in some models.

Conclusions: The histological assessment of tumor microenvironment identified high tumor vascularization as a potential biomarker for infection. However, the lack of correlation between tumor infection and therapeutic efficacy study highlights the complexity of therapeutic response to OV therapy.

Understanding the role of adipose tissue and fat cells in the tumour microenvironment and its impact on virus-based therapy responses.

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Background : The tumour microenvironment is an important determinant of successful drug intervention. Increasing global incidence of obesity has highlighted the role of fat cells in cancer progression and aggressiveness. In breast cancer, fat cells are abundant and active in the tumour microenvironment and ovarian tumours have demonstrated preferential metastasis to the omentum, a site of abdominal fat storage. While adipocytes are known for their rich source of energy and endocrine function, there is growing evidence for a role in treatment resistance.

Objective: Here, we aim to characterize the phenotype of cancer cells that have been primed by cancer associated adipocytes (CAA) and provide a mechanistic understanding of how CAA-cancer cell crosstalk influences virus-based therapy responses.

Methods: An in vitro model of high-grade ovarian serous adenocarcinoma (OVCAR8) was cultured with growth media or breast adipocyte conditioned media (BACM) to mimic a fatty tumour microenvironment. These cultures were then infected with a fluorescently-tagged oncolytic virus (OV), vesicular stomatitis virus (VSVΔ51-GFP). Propensity for viral infection was determined by immunofluorescence and cellular responses in the cancer cell, at the RNA level, were determined by microarray analysis. In vivo studies of tumour OV infectivity in an obesity model were conducted in high-fat diet fed mice that were inoculated with tumours in a fat-pad or subcutaneously and subsequently administered OV therapy intratumourally.

Results: Immunofluorescent microscopy reveals markedly lowered OV infection of in vitro BACM-cultured cancer cells in comparison to its growth media cultured counterpart. Microarray data suggests priming of uninfected, BACM-cultured cells with a non-permissive and anti-viral phenotype. These cells also do not exhibit the strong interferon response elicited by cancer cells cultured in growth media. In vivo studies show that tumours localized in the fat pad have lower OV infectivity in comparison to subcutaneously seeded tumours.

Conclusions: In vivo results recapitulate in vitro results that suggest that adipocyte secreted factors confer virotherapy resistance. Future work will aim to determine if this is caused by neutralization of the virus by a biomolecule secreted by the adipocytes or alternatively, adipocyte-secreted factors are conferring structural changes in the cancer cell that prevent the attachment, entry or replication of ovs or both. To elaborate on preliminary in vivo results, immunohistochemistry of immune cell infiltration immediately surrounding tumours inoculated in the fat pad or subcutaneously can shed light on how metabolic diseases such as obesity can shape the tumor microenvironment and impact virotherapy responses.

Identifying Chemical Enhancers of Adenoviral Transgene Expression for Gene Therapy Applications

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Background: Gene therapy is a therapeutic approach that aims to treat genetic illnesses at the source, by replacing or correcting deleterious genes that are causal for disease. Adenoviruses are a desirable vector for gene therapy due to their ability to transduce both replication-competent and –deficient cells, and the fact that the viral genetic material does not integrate into the host genome. However, these therapeutics can be rendered ineffective by inadequate infection of target cells and restricted transgene expression.

Objective: To identify adeno-sensitizing drug compounds that improve target cell infection and or the efficiency of adenovirus transgene expression.

Methods: A high-throughput screening assay was optimized to screen 1280 FDA approved compounds in a human A549 lung carcinoma cell line. Candidate compounds were identified by measuring the expression of a luciferase transgene from a non-replicating adenoviral vector. Drugs that dramatically enhanced the observed luminescent signal were selected to proceed to the validation stage.

Results: Several of the identified drugs were validated in multiple in vitro cell lines, as well as in ex vivo murine tissue samples. The drugs appeared to significantly increase the level of adenovirus transgene expression, in addition to maintaining relatively low levels of cytotoxicity at their active doses.

Conclusion: The compounds identified in this screening process demonstrate the possibility of combining small molecule treatment to potentiate the efficacy of adenoviral gene therapy. Ideally, these approaches will contribute to more potent treatment options for several human diseases, including cancer. Future aims involve transitioning these pharmacoviral systems to a relevant in vivo model, as well as exploring different transgenes.

Tailoring oncolytic viruses for the treatment of pancreatic cancer

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Macrophage subset phenotype is altered in chronic hcv infection & may contribute to generalized cd8+t-cell dysfunction

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Background and Objectives: Our lab and others have previously shown that chronic HCV infection causes generalized $CD8^+$T-cell impairment, not limited to HCV-specific $CD8^+$T-cell populations. In such an inflammatory hepatic disease, infiltrating monocyte-derived macrophages (MDM) contribute to a micro-environment that could influence cells trafficking through the liver, including $CD8^+$ T-cells. These MDM can differentiate into M1 (classically-activated) and m2a, m2b, M2c (alternatively-activated) with pro- and anti-inflammatory functions, respectively. Whether MDM subset generation in chronic HCV infection is altered in the liver is unknown. Furthermore, how these subsets influence $CD8^+$T-cell function needs investigation. We hypothesize that MDM subset phenotypes are altered in chronic HCV infection, thereby contributing to $CD8^+$ T-cell dysfunction.

Methods: MDM subsets were generated from blood collected from healthy controls and HCV-infected individuals. Phenotypes were confirmed using surface receptors (CD163, CD206 and CD86) and quantification of secreted cytokines (IL-6, IL-10, IL-12, IFN-y and TNF-α). Autologous co-culture of MDM subsets and isolated $CD8^+$T-cells in health enabled the assessment of $CD8^+$T-cell functions.

Results: MDM subset phenotyping in chronic HCV infection suggests M2a cells have a higher percentage of $CD206^+$ than M0 subset whereas in health, they showed no significant difference. The percentage of $CD86^+$ cells showed no significant difference between subsets in infected individuals, while in health, M1 and M2a were significantly higher than M0. In HCV-infection, the concentration of IL-6 in M2a subset supernatants was significantly higher than healthy controls. In infection, TNF-α release by any MDM subset was undetectable, whereas in health, M1 cells produced significantly higher amounts of TNF-α compared to M0 and m2a. No differences were observed in the concentration of IL-10, IL-12p70 and IFN-y between the subject groups. In uninfected controls, co-culturing $CD8^+$ T-cells with M1 macrophages significantly increased the percentage of $perforin^+$, $CD107a^+$ and IFN-y^+ $CD8^+$ T-cells, compared to $CD8^+$T-cells alone. Co-culturing with M1 significantly increased the percentage of $perforin^+$ cells and $CD107a^+$ cells compared to m2a. Co-culturing with M2c cells significantly increased the percentage of $CD107a^+$ cells compared to $CD8^+$ T-cells alone.

Discussion: Phenotypic alterations in health and chronic HCV infection are evident both in terms of surface receptors and secreted cytokines suggesting impairment of MDM subsets. The importance of an M1 phenotype, in being able to prime $CD8^+$T-cells and induce perforin and CD107a is evident. How the altered phenotype of MDM subsets in chronic HCV infection will influence the $CD8^+$T-cell function, needs to be further investigated.

The role of pgsn in ovarian cancer cell survival and CD8+ T-cell inactivation

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Background: Ovarian cancer (OVCA) is the most lethal gynaecological cancer, due predominantly to late diagnosis, recurrence and chemoresistance. The standard first line treatment for OVCA is the combination of surgical debulking and chemotherapy with cisplatin (CDDP) and taxane derivatives. Although these treatments are successful initially, chemoresistance is a major hurdle for long term therapeutic success. The responsiveness of cancer cells to chemotherapy is dependent on its microenvironment encompassing a plethora of interactive cells (e.g. CD8+ T-cells) and their secretory products. We have demonstrated together with our collaborators that elevated circulatory levels of plasma gelsolin (pgsn) in head-and-neck and OSCC patients are significantly associated with chemoresistance and tumour recurrence respectively; raising the possibility that pgsn may play a key role in OVCA chemoresistance. These findings led us to hypothesize that pgsn regulates OVCA responsiveness to CDDP and increased pgsn secretion from OVCA attenuates the cytotoxic effects of CD8+ T-cells resulting in OVCA chemoresistance.

Objective: To investigate how pgsn promotes cancer cell survival through an autocrine manner and paracrine inactivation of the cytotoxic functions of CD8+ T-cells.

Methods: Lysates and spent media of chemosensitive (a2780s) and chemo-resistant (a2780cp) paired OVCA cells were assayed for pgsn content using western blot and ELISA. Using loss- and gain-of-function approaches, the role of pgsn in the regulation of CDDP-induced apoptosis was examined. Standard molecular and immunological techniques will be used to investigate pgsn-induced OVCA cells' survival and anti-tumour functions of ovarian tumour-derived CD8+ T-cells, respectively. Xenograft studies coupled with human studies will be used to validate the in vitro studies. We will also extend this study to include other chemosensitive (PA-1) and chemoresistant cells (Hey, skov-3) in key experiments for validation.

Results: Pgsn content was higher in both lysates and spent media of chemoresistant (a2780cp) compared to its chemosensitive counterpart (a2780s); CDDP decreased the levels of pgsn content in A2780s but not a2780cp cells in a concentration-dependent manner. A similar phenomenon was observed in other chemosensitive and chemoresistant OVCA cells. Pgsn content was significantly higher in the spent media compared to lysates in both sensitive and resistant OVCA cells. In addition, CDDP induced concentration-dependent apoptosis in sensitive cells but not the resistant cells.

Conclusion and Future direction: The preliminary data suggest that pgsn might play a key role in OVCA chemoresistance and in addition to promoting cancer cell survival in an autocrine manner, might also inactivate CD8+ T-cells functions in a paracrine manner (Supported by CIHR).

Generalized cd8+ t cell dysfunction in chronic viral hepatitis in relation to liver damage

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Background: Chronic hepatitis C virus (HCV) infection affects ~170 million people worldwide and can result in fibrosis, cirrhosis, hepatocellular carcinoma (HCC), and end stage liver disease (ESLD). Current HCV antiviral treatment rates remain too low to eliminate HCV transmission and/or ESLD and HCC, in part due to high drug costs and restrictive drug reimbursement policies. Thus, there is a need for identification of currently unknown host-specific predictors of negative liver outcomes and post-HCV antiviral treatment liver fibrosis regression. Critical antiviral immune responses by HCV-specific $CD8^+$ T cells exhibit impaired cytotoxic potential, survival, and proliferation during chronic infection. Our lab has expanded on this knowledge by showing that bulk $CD8^+$ T cells from HCV-infected individuals have decreased cytokine signalling, regardless of their specificity. In addition, bulk $CD8^+$ T cell survival was inversely correlated with liver fibrosis. The immunological consequences of bulk $CD8^+$ T cell impairment in HCV infection remains an important area of study and may yield insights into the predictors of liver disease outcomes.

Objective: The objective of this study is to assess the effector functions of circulating bulk $CD8^+$ T cells in HCV-infected individuals with either minimal or advanced fibrosis.

Methods: Circulating $CD8^+$ T cells from untreated, HCV-infected individuals with high or low liver fibrosis scores are being assessed for subset distribution, cytokine expression, and cytolytic activity. Subsets of $CD8^+$ T cells will be distinguished based on CD45RA, CCR7 and CD27 expression by flow cytometry. The concentration of cytokines released into culture supernatants will be quantified by a multiplexing immunobead assay. The effector functions of $CD8^+$ T-cells, such as effector molecule expression and target lysis, will be evaluated by flow cytometry using direct labelling and mixed lymphocyte reaction assays, respectively.

Results: Thus far, results indicate that $CD8^+$ T cells from HCV-infected patients express lower levels of cytotoxic molecules (CD107a and perforin) and less IFN-γ compared to healthy controls. We expect to identify a further relationship between this functional impairment of bulk $CD8^+$ T cells in HCV infection and the extent of liver fibrosis, supporting the notion that chronic HCV infection is a potential model of liver damage associated immune dysfunction with potential relevance to other liver diseases (e.g. Non-alcoholic fatty liver disease).

Conclusion: Identification of the relationships between HCV infection, liver fibrosis and generalized immune function could identify indicators of immune impairment as well as predictors of fibrosis progression and post treatment fibrosis regression.

Regulation of cell volume signaling pathways in the preimplantation embryo

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Background: At fertilization, the embryo must switch to regulation of its own physiology and during this process, if the embryo is stressed development is blocked at the two cell stage. The exact pathway by which this arrest occurs is currently unknown. One component the embryo must activate is regulation of its cell volume. Regulation of cell volume is essential for multiple cellular processes, including cell cycle progression.

Objective: The Na+/H+ exchange (NHE1) transporter can mediate recovery from cell volume decrease via inorganic ions transport. However, the exact mechanisms by which the embryo senses changes its cell volume and translates this into activation of NHE1 is not understood. JAK2 signaling can activate NHE1 and preliminary results indicate that FAK signaling could also activate NHE1. We aim to confirm the role of FAK in activation of NHE1 and to determine if FAK and JAK2 work in conjunction to activate NHE1.

Methods: The expression of pFAK(Y861) and pFAK(Y397) in one and two cell embryos at normal osmolarity (250 mOsM) and at an increased osmolarity (550 mOsM) with or without the presence of 10 uM inhibitor (PF562271) was examined by western blot. A dose response for the inhibitor concentration was also performed and the results examined by western blot. Two concentrations of inhibitor were then used to assay NHE1 activation using intracellular pH measurements.

Results: The expression of pFAK(Y861) was specifically expressed at the 2 cell stage at 550 mOsM and this was prevented by the presence of 10uM inhibitor. However, since the western blot band size of pFAK(Y861) was higher than the band for total FAK, and very similar to the size of pJAK2, the expression of another phosphorylation site pFAK(Y397) was also examined. Although pFAK(Y397) had the same size band as total FAK and the expression of pFAK(Y397) was completely removed by the presence of 10uM inhibitor, pFAK(Y397) was expressed at both one and two cell stages at both osmolarities. A dose response for the inhibitor was performed and two concentrations chosen to re-examine the activation of NHE1 by FAK.

Conclusion: It is difficult to confirm whether the pFAK(Y861) activation is specific for FAK or is instead detecting JAK2. pFAK(Y397) whilst expressed at all stages, is an autophosphorylation site and may be constitutively active. By assaying for NHE1 at lower concentrations of FAK inhibitor we hope to determine the specific role of FAK in cell volume regulation in the early embryo.

Impaired regulation of Hexokinase II (HKII) by deficient p53 suppresses cisplatin sensitivity via modulation of aerobic glycolysis in ovarian carcinoma cells

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Background: Ovarian cancer (OVCA) is the 5th leading cause of cancer death in women, mainly due to late diagnosis and chemoresistance. Cisplatin (CDDP) resistance is a major hurdle to successful therapy. Cancer cells preferably obtain energy via aerobic glycolysis (Warburg effect). The key glycolytic enzyme, Hexokinase II (HKII) converts glucose to glucose-6-phosphate and is highly associated with tumorigenesis. We have previously shown that p53 is required for overcoming of chemoresistance in OVCA and is a critical determinant for induction of apoptotic response to CDDP. However, it remains unknown if HKII plays an etiologic role in chemoresistance, and whether and how HKII-mediated aerobic glycolysis may be involved.

Objective & Hypothesis: The overall objective is to investigate the role of HKII in dysregulated cellular metabolism and CDDP sensitivity in OVCA. Our hypothesis is that HKII plays an important role in cell survival of chemoresistant OVCA.

Methods: Using paired CDDP sensitive (a2780s) and counterpart resistant (a2780cp) ovarian cancer cell line, protein content and mrna level of HKII in response to CDDP were examined using qpcr and western blot. With confocal microscopy, cellular localization of HKII was examined. For cellular metabolism, glucose consumption and HKII enzymatic activity were measured. Transcriptional regulation of HKII by p53 was examined using chip assay. With gain- and loss-of-function approaches, the regulatory role of p53 upon HKII and effect of HKII depletion upon apoptosis and cell viability were examined.
Results: Despite no significant differences in basal HKII protein levels of between the chemosensitive and chemoresistant cells, CDDP down-regulated HKII mrna abundance and protein content in chemosensitive cells, but not in chemoresistant cells, suggesting that CDDP-mediated HKII responsiveness is the determinant factor for chemosensitivity. P53 is a transcriptional regulator of HKII expression and its promoter binding activity is compromised in chemoresistant OVCA cells. While silencing p53 markedly enhanced HKII mrna and protein levels, HKII down-regulation (sirna or 2-DG) sensitized p53-wt (but not p53-deficient) chemoresistant cells to CDDP-induced apoptosis. CDDP treatment resulted in the P-p53-mediated translocation of HKII from the mitochondria to the nucleus and decreased HKII enzymatic activity in chemosensitive but not in chemoresistant cells.

Conclusion: Taken together, these findings demonstrate the novel regulatory mechanism of HKII in OVCA cells and suggest that HKII-mediated aerobic glycolysis can be a therapeutic target in treatment of chemoresistant OVCA (supported by a grant from the Canadian Institutes of Health Research).

Peri-Ovulatory L-Ornithine Supplementation to Reduce Aneuploidy Rates and Increase Reproductive Success

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Background: It is understood that, as maternal age increases, conception becomes difficult and comes with increased risk of birth defects and spontaneous abortion. Oocyte aneuploidy is a common cause of spontaneous abortion and birth defects. Due to the nature of oogenesis, women have a set number of oocytes at birth and quality decreases with age. A reduction of ovarian ornithine decarboxylase (ODC) and its product putrescine has been observed in older mice. Supplementation with putrescine has been observed to decrease the aneuploidy rate in older mice, identifying putrescine as a possible treatment for aneuploidy in older women. However, putrescine supplementation has never been used in humans and, therefore, no safety data are available. Additionally, putrescine is a biogenic amine of multiple functions. Systemic increase of putrescine may cause unintended consequences. L-ornithine has been used extensively and safely in humans, mainly as health supplements, although the exact health benefit of L-ornithine supplementation is not well established. In addition, L-ornithine supplementation likely specifically increases putrescine levels in tissues/organs where ODC is active (e.g. Ovaries) whereas putrescine supplementation is expected to increase putrescine systemically.

Objective: The objective of this study was to determine if L-ornithine supplementation is capable of increasing peri-ovulatory putrescine resulting in similar reproductive benefits to putrescine supplementation.

Methods: Old C57BL/6 mice (approximately 9 months old) were given L-ornithine via oral gavage, sub-cutaneous injection (500mg/kg), or in water (4% w/v) to determine the impact of supplementation on peri-ovulatory putrescine concentrations in the ovaries. Mice were sacrificed at specific times to determine ovarian putrescine concentrations. Additionally to determine the effect on reproduction mice were mated with young BDF1 male mice to determine the number of resorptions at 9.5 days post-coitum (DPC) and the effect on litter size.

Results: When L-ornithine is delivered via oral gavage it is capable of increasing putrescine levels in the ovaries, and it only does so during ovulation when ODC is active. Mating experiments are ongoing in which old mice are supplemented with L-ornithine in drinking water during ovulation. Preliminary data indicate that at 9.5 DPC, the ratio of normal vs. Resorbed fetuses is greater in mice treated with L-ornithine. However, the number of total implants per pregnancy appears to be slightly smaller in the L-ornithine group.

Conclusions: Peri-ovulatory L-ornithine supplementation can increase ovarian putrescine levels. Continuing work will determine if L-ornithine supplementation has any reproductive benefit.

Hyperadrogenemia in PCOS: triggering the polarization of ovarian M1 macrophages

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Background: Polycystic Ovarian Syndrome (PCOS) is a reproductive condition characterized by hyperandrogenism, antral follicle growth arrest and chronic inflammation. The role of immune system in PCOS is unclear. Macrophages play key role in inflammation and the balance between M1 (inflammatory) and M2 (anti-inflammatory) macrophages determines the physiological/pathological outcome. As in human PCOS, female rats chronically treated with 5α-dihydrotestosterone (DHT) exhibit elevated serum chemerin, an adipokine that function as chemoattractant to macrophages expressing its receptor, CMKLR1. Hypothesis: We hypothesize that hyperandrogenism alters chemerin expression and macrophage polarization in ovaries, leading to ovarian follicle dysfunction.

Methods: Female rats were treated with DHT (83ug/day during 3, 7, 15 and 28 days). The incidence of M1 (CD163-CD68+) and M2 (CD163+CD68+) macrophages expressing CMKLR1 (immunofluorescence and flow cytometry) and ovarian follicle apoptosis (TUNEL) were quantified. Ovarian and serum chemerin were analyzed (western blotting).

Results: DHT significantly decreased ovarian but not body weight until 28 days. 15 days of DHT altered the ovary dynamics: increased early antral follicles ($p< 0.005$), absence of pre-ovulatory follicles and up-regulated ovarian chemerin ($p< 0.0001$). Unhealthy antral follicles were observed in DHT-treated but not in control ovaries. Ovarian M1 and M2 macrophage polarization was follicular stage-specific and influenced by DHT. DHT significantly increased M1 macrophages abundance in antral [7 days ($p<0.05$) and 15 days ($p< 0.001$)] and pre-ovulatory follicles [7 days ($p<0.001$)] but reduced M2 ($p< 0.05$) and increased apoptosis in pre-ovulatory follicles ($p<0.05$). DHT (15 days) increased the frequency of total CMKLR1+ M1 macrophages ($p= 0.030$). Human ovarian stromal macrophages (M1: MHCII+CD86+; M2: CD163+CD206+) and follicular fluid and serum chemerin were also assessed (immunofluorescence and western blotting, respectively) in PCOS (n=16 and n=16, respectively) and non-PCOS patients (n=11 and n=20, respectively). Lower abundance of M2 macrophages was found in PCOS ovaries ($p=0.003$). While M1 macrophages abundance did not differ, a higher M1/M2 ratio was evident in PCOS ($p= 0.005$). There was no difference in M1 macrophages expressing CMKLR1. Follicular fluid chemerin, but not serum was significantly higher in PCOS compared to non-PCOS patients ($p= 0.0043$).

Conclusions: Our results indicate that hyperandrogenemia affects the immunological niche of ovaries and may be important in the pathophysiology of PCOS. Increased expression of chemerin in PCOS ovaries may regulate macrophages polarization and function, and follicle cell fate. Studies are ongoing to investigate how granulosa cells and macrophages interact under androgenic influence, which may contributes to a better understanding of PCOS etiology and development of new therapeutic strategies.

Nanodomain calcium signaling in Xenopus meiotic spindle

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Previous works demonstrate global calcium level or homeostasis is related to spindle fitness and first polar body formation. The underlying mechanism, however, is largely unknown and to our knowledge specific functional calcium transient has never been detected during oocyte maturation. Our recent experiments showed that a fast calcium chelator BAPTA, but not EGTA(slow), could induce immediate depolymerization of spindle microtubule during oocyte maturation, which strongly suggests a highly localized, or nanodomain calcium signaling may be involved in formation and maintenance of meiotic spindle in Xenopus.
To investigate the possible calcium in the spindle, we used a BAPTA-based calcium indicator combined with live cell confocal imaging and successfully detected a specific signal in the spindle region, which was then shown to be insensitive to EGTA but disappear after BAPTA injection. With in situ IP3R immunofluorescent staining and caged-IP3 calcium release experiments focused on the spindle, we demonstrate that ER-IP3R could be a possible calcium source. We further propose a calmodulin- and microtubule- binding protein, Aurora A, may be responsible for spindle calcium signaling effector.

Relationship between Body Mass Index (BMI) and Lipophilic Persistent Organic Pollutants (L-POPs) Levels in Women of the Maternal-Infant Research on Environmental Chemicals (MIREC) Study

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Background: Mechanisms through which obesity may cause congenital anomalies remain unclear. Evidence suggests that lipophilic persistent organic pollutants (L-POPs) are able to reach the fetus across the placenta. Even though, data concerning the effects of these toxins in neonates is still scarce, they are known to have detrimental effects in adults. Knowledge concerning the relationship between body mass index (BMI) variations during pregnancy and L-POPs serum levels is limited.

Objective: This study aimed to determine whether first trimester maternal BMI and L-POPs serum levels were associated.

Methods: A cross-sectional study was performed using data from the Maternal-Infant Research on Environmental Chemicals (MIREC) Study, a prospective cohort that enrolled pregnant women from 10 different sites across Canada between 2008 and 2011. We used first trimester maternal BMI and serum levels of 41 L-POPs to create unadjusted and age-adjusted linear regression models. Pairwise comparisons of L-POPs levels between BMI categories were conducted when linear regression tests were determined significant.

Results: The most abundant L-POPs were found to be PCB (0.494 µg/kg lipid) and DDE (0.589 µg/kg lipid), whereas the lowest PARLAR26 (0.005 µg/kg lipid) and PARLAR50 (0.005 µg/kg lipid). 19 L-POPs (PBDE100, 153, 47, and 99, PCB138, 146, 153, 156, 163, 167, 170, 180, 183, 187, 194, 201, and 203, PCB, and PARLAR26) had concentration levels that significantly varied across BMI classes, and were used for pairwise comparisons between BMI classes.

Conclusion: Our findings show that several L-POPs concentrations in first trimester maternal serum are associated with first trimester maternal BMI. Most associations seem to be negative. Subsequent research is needed to evaluate L-POPs levels variations during pregnancy given gestational weight changes and potential teratogenic effects.

TRPC5 is required for NADPH oxidase 5 activity in podocytes

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Background: The transient receptor potential cation channels (trpcs) are non-selective cationic channels that modulate intracellular calcium. TRPC5 and TRPC6 predominate in podocytes and are associated with glomerular injury. NADPH oxidase 5 (NOX5) is a unique NOX family member as its 4 EF-hand calcium-binding domains prime its ability to produce superoxide (ROS).

Objective: To determine whether TRPC5/6-associated calcium signaling regulates NOX5-derived ROS production in podocytes.

Methods: Glomerular lysates were isolated from nontg and mice with podocyte-specific NOX5 expression ($NOX5^{pod+}$ mice). Human podocytes were transduced with NOX5 containing adenovirus (adnox5) for 48 hours, incubated with the TRPC5 inhibitor, ML204 (30μM) or TRPC6 inhibitor, La^{3+} (50μM), for 1 hour and suspended in a HEPES-tyrode solution to maintain intact cells. A lucigenin assay was employed in the presence of ATP (200μm) to determine ROS. For angiotensin II (angii) experiments, podocytes were incubated with angii (500nm) for 24 hours. Mrna levels were determined by qpcr.

Results: TRPC5 (but not TRPC6) mrna levels were significantly elevated in glomerular lysates from $NOX5^{pod+}$ mice. To determine if TRPC5 or TRPC6 contributes to NOX5 activity in vitro, a pharmacological inhibition strategy was employed. TRPC5 inhibition with ML204 in adnox5-infected podocytes resulted in a significant decrease in ROS. TRPC6 inhibition with La^{3+} did not alter ROS. Next, we tested whether TRPC5 or TRPC6 inhibition attenuates NOX-derived ROS production in the presence of an upstream agonist of trpcs and NOX5. Incubation of uninfected or adnox5-infected podocytes with angii did not result in increased ROS, despite NOX5, TRPC5 and TRPC6 mrna induction. As such ATP was used as an agonist to evoke ROS (through P2Y receptor/diacylglycerol/TRPC signaling). Stimulation with ATP yielded increased ROS in adnox5-infected podocytes. La^{3+} did not reduce ATP-evoked ROS production in adnox5-infected podocytes. Conversely, ML204 attenuated ATP-induced ROS production in adnox5-infected podocytes, suggesting that TRPC5 signaling may be required for NOX5 activation in podocytes.

Conclusions: These data are the first to link TRPC5-derived calcium signaling as a potent upstream regulator of NOX5 activity in podocytes.

A primary culture of Sertoli cells from adult mice: unique differences in their properties as compared with Sertoli cells from 20-day old mice

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Background: Sertoli cells are reproductive cells that pivotally provide spatial and nutritional support to developing male germ cells as well as degrade remnants of residual bodies and apoptotic germ cells that they endocytose/phagocytose. A primary culture system of Sertoli cells is therefore desirable for studies of their biochemical/physiological properties. However, in adult rats/mice (>10 weeks old), Sertoli cells constitute only 5% of total cells in the seminiferous tubules (sfts), thus challenging their pure population preparation following SFT enzymatic digestions. Hence, in ex vivo studies, Sertoli cells are usually isolated from 20-day old rats/mice where the first spermatogenesis round has just started and not many germ cells are yet present. However, the properties of Sertoli cells from 20-day old rats/mice and those from adult animals are unlikely to be the same, since testosterone/FSH/LH levels at the two ages are significantly different.

Objective: To obtain a pure population of adult mouse Sertoli cells.

Methods: The Sertoli + germ cell mixture of collagenase/trypsin/hyaluronidase digested sfts was plated onto culture dishes at optimum concentrations and germ cells were gently washed out from Sertoli cells selectively adherent to the substratum. Sertoli cells in culture were identified by their specific markers and ability to form tight junctions and phagocytose apoptotic germ cells. Lipidomic analyses were performed by LC-MS/MS.

Results: By Day 5 in culture, Sertoli cells constituted >85% of adherent cells, as revealed by their marker-Wilms tumor protein and ability to form tight junctions. The average yield of our adult Sertoli cell isolation was 1 million cells/mouse. These adult Sertoli cells in primary culture could be sub-cultured even after cryopreservation and they secreted “marker” proteins (e.g., clusterin) like those in vivo. They also phagocytosed caboxyfluorescein succinimidyl ester-labelled apoptotic germ cells. <span style="mso-spacerun:

Peri-ovulatory putrescine supplementation in aged mice promotes histone deacetylation in the oocytes and improves egg quality

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Background: Site-specific histone acetylation is a major mechanism in activating transcription (Grunstein, 1997). Immature oocytes are highly acetylated in multiple lysine residues of both histone 3 and histone 4 (Gu et al., 2010), consistent with very active transcription in stockpiling maternal mrnas (Andreu-Vieyra et al., 2006). It is first demonstrated in the mouse that during oocyte maturation, there is a complete removal of most of these histone acetylation marks (Kim et al., 2003; Ma and Schultz, 2013). Inhibition of histone deacetylation, during oocyte maturation causes aneuploidy and reduces egg's developmental potentials. Furthermore, these studies suggest that histone deacetylation may be defective in aged oocytes, contributing to decline in egg quality and increased incidence of egg aneuploidies, in mice (Akiyama et al., 2006) and in humans (van, I et al., 2011). Interestingly, age-related deficiency of ornithine decarboxylase in the ovaries and the resulting peri-ovulatory putrescine deficiency caused similar phenotype: increase of egg aneuploidy and decrease of eggs' developmental potential.

Objective: In this study, we tested the hypothesis that peri-ovulatory putrescine supplementation promotes histone deacetylation during oocyte maturation in aged mice.

Methods: The oocytes derived from aged mice were cultured in vitro with or without putrescine supplementation, and histone decetylation of the mature eggs was analyzed after maturation. Besides, aged mice were supplemented in vivo with putrescine in drinking water during ovulation, and then the eggs were collected from the oviducts for histone deacetylation analysis.

Results: So far our results indicated that putrescine supplementation during in vitro maturation of aged mouse oocytes significantly improved histone decetylation of the mature eggs. Further, putrescine supplementation of mouse drinking water during ovulation significantly improved histone deacetylation of the ovulated eggs in aged mice.

Conclusions: Peri-ovulatory putrescine supplementation in aged mice promotes histone deacetylation in the oocytes and improves egg quality. Future work includes mechanism of putrescine action in promoting histone decetylation and how histone deacetylation during oocyte maturation reduces egg aneuploidy and improves egg's developmental potential.

Impact of early and late intervention with PBI-4050 in mice with diabetic kidney disease and implications for podocyte dysfunction

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Background: Currently in phase II clinical trial in Type 2 Diabetic/metabolic syndrome patients, PBI-4050 displays anti-inflammatory and anti-fibrotic effects and ameliorates kidney function in various models of acute or chronic disease via a novel mechanism of action. Here, we hypothesized that PBI-4050 is renoprotective in part through mitigation of podocyte injury.
Objective: The objective of this study was to determine whether PBI-4050 decreases renal injury in a mouse model of type-1 diabetes with concomitant hypertension, and to assess whether podocytes may directly benefit from treatment with this drug.

Methods: We used the streptozotocin type-1 diabetes model in mice overexpressing human prorenin, yielding a Hypertensive/ Diabetic phenotype (HD). After 4 or 12 weeks of HD, PBI-4050 was administered (200 mg/kg; P.O.) for 4 weeks and urine albumin-to-creatinine ratios, glomerular filtration rate and renal fibrosis were assessed. In parallel, conditionally immortalized human podocytes (hpod) were exposed to diabetic (glucose, tgfβ1) or pro-inflammatory (lipopolysaccharide) stimuli with or without PBI-4050 (100 mm). RNA expression was measured by qpcr while microparticle production, as a marker of cell stress, was also measured using Zetaview nanoparticle analyzer.

Results: In HD mice, PBI-4050 had no effect on bodyweight or glycaemia yet early intervention reduced hypertrophy of the kidneys and heart. GFR decline was prevented in the late intervention group while SBP decreased in both studies. In addition, ACR and glomerular scarring were also reduced in both early and late treatment groups. In vitro studies revealed that PBI-4050 decreased several glucose and LPS-dependent pro-fibrotic (α-SMA, Col-I, Col-IV) and proinflammatory (IL-6, IL-8, Rantes, ICAM) genes, in addition to curbing stress-induced microparticle production.

Conclusions: Early and late intervention with PBI-4050 decreased diabetic kidney injury in mice and protected hpods from injurious stimuli. These novel data indicate that the podocyte may directly benefit from PBI-4050 therapy.

Electrocardiogram Signal Quality Analysis to Reduce False Alarms in Myocardial Ischemia Monitoring

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Background: The probability of myocardial ischemia, reduction in blood flow to the heart muscle, is increased following a non-cardiac surgery, which can lead to an increase in the incidence of post-operative heart attacks, cardiac death, and increase in hospital length of stay among patients. β-Blockers can be administered to reduce the probability of experiencing a cardiac event post a non-cardiac surgery; however, they have side effects including stroke, bradycardia, hypotension, morbidity, and mortality. Myocardial ischemia causes a deviation of ST segment in the electrocardiogram (ECG), which can enable targeted administration of β-Blockers rather than prophylactic administration; however, attempts to utilize ECG as a diagnostic tool in real-time were hindered by a large number of false alarms due to noise and error in the quantification of ST segment deviation.

Objective: The objective of this research project is to develop a system to gate false alarms using the signal quality of the ECG and quality of the ST segment deviation estimate.

Methods: The system was tested using ECG records from Physionet's Long-Term ST Database (LTSTDB) that were contaminated with motion artifact noise from Physionet's MIT-BIH Noise Stress Test Database (NSTDB). Results: The system based on signal quality and ST segment trend estimation gated 100% of noise-induced alarms (86% of all false alarms) attaining a recall and precision of 0.72 and 0.73, respectively, marking an increase of 0.42 in precision and a decrease of 0.05 in recall from the commercial bedside monitor baseline performance (precision – 0.31, recall - 0.78). The signal quality analysis approach to gate contaminated data was extended to an ECG biometrics system.

Conclusion: The developed system has proven to reduce false alarms in myocardial ischemia monitoring; thereby, improving the automated monitoring of patients which has the potential to reduce post-operative cardiac complications.

Cesarean sections for abnormal fetal heart tracings: setting indicators of appropriateness based on neonatal outcomes

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Background: Up to 32% of primary caesarean sections (C-section) are performed for suspected fetal distress. Fetal distress is often suspected solely on atypical/abnormal cardiotocography, which is subject to inter-individual variability in interpretation and is not a specific marker of fetal well-being. Many C-sections done for this indication may not therefore be appropriate.

Objective: The objectives were: (1) Determine the proportion of primary C-sections done for atypical/abnormal cardiotocography; (2) Determine neonatal outcomes in infants delivered by C-section for this indication compared to those done for labour dystocia; (3) Determine the proportion of "appropriate" C-sections done for abnormal/atypical cardiotocography and identify effects of clinical and sociodemographic factors.

Methods: With OHREB approval, a retrospective cohort study was conducted using data from Ontario's BORN (Better Outcomes Registry & Network)/Niday Perinatal) database from 2006-2014. Primary outcomes included rates of primary C-section for fetal distress and their neonatal outcomes. Neonatal outcomes included arterial pH, Apgar scores, need for resuscitation, and NICU admission. A C-section for abnormal/atypical cardiotocography was considered "appropriate" if one or more of the following outcomes occurred: 1-minute Apgar<3, 5-minute Apgar<7, arterial pH<7.20, resuscitation required, or NICU admission required. Student's t-test, chi-square test, and logistic regression analyses were performed.

Results: Between 2006-2014, 146,676 primary C-sections were performed in Ontario. Twenty percent were performed for atypical/abnormal fetal heart tracings with a significant upward trend between 2006 and 2015 (16.9% vs. 21.7%, p<0.001). Compared with women undergoing C-section for labour dystocia, there were significant differences between the groups with regards to maternal age, parity, induction of labour, and level of care (all p<0.001). Overall, the odds of requiring newborn resuscitation (OR_{adj} 1.48, CI 1.41-1.54) and admission to NICU (OR_{adj} 2.21, CI 2.08-2.34) were significantly higher when the indication was atypical/abnormal cardiotocography. However, less than half (42.3%) of these neonates had at least one of the criteria used to determine "appropriateness" of C-section. The rate of "appropriate" C-sections differed significantly by size of center (p<0.001), induction of labour (p=0.0027), oxytocin use (p<0.001), level of care (p<0.0001), and type of fetal surveillance (p=0.004).

Conclusion: There is a significant upward trend in primary cesarean section rates; many are done for abnormal fetal cardiotocography. In the absence of objective measures of intrauterine fetal well-being, C-sections may be performed for fetal distress when they are not required. This study highlights the rates of C-sections performed for fetal distress and the impact of clinical and sociodemographic variables. Developing indicators and understanding C-section appropriateness may guide future strategies to reduce C-section rates.

Assessing the Need for Updating Two Clinical Practice Guidelines (cpgs) on the Use of Intravenous Immune Globulin (IVIG) Using the Ottawa Signals Detection Method

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Background: Clinical practice guidelines (CPG) need to be updated as new evidence emerges over time; however, updating is resource consuming. A signal detection method can assist in determining which conditions within a CPG may be in need of updating. We report the results of a signal detection exercise to assess the need for updating two cpgs on the use of Intravenous Immune Globulin (IVIG) commissioned by the National Advisory Committee on Blood and Blood Products.
Objective: To assess the need for updating the recommendations in the two cpgs on the use of IVIG in neurologic and hematologic conditions published in 2007.

Methods: Two cpgs addressing IVIG use for 42 different medical conditions (22 neurologic, and 20 hematologic) were assessed using the Ottawa qualitative signal detection method, which incorporates literature searches and a qualitative approach to assess the presence of signals that trigger the need for updating. Experts' opinion was also sought. The literature search using MEDLINE, Embase and Cochrane library covered from 2003 (prior to the original guidelines' last search dates) onward to April 26, 2016.

Results: A total of 5,872 records were screened for eligibility via three screening levels: 1) categorizing the records into the 42 conditions (level 1); 2) screening based on title and abstract for individual conditions (level 2); and 3) screening based on full text articles for each condition (level 3). From the search, 4,162 records met the eligibility criteria at level 1, 806 at level 2, and 181 at level 3. Of the 181 included studies, the number of eligible studies per condition ranged from 1to 27. The expert response rate for the total 74 contacted experts (30 hematologic, and 44 neurologic) was 34% (34% for neurologic and 33% for hematologic conditions). A total of ten signals for seven conditions (3 neurolgoic: Multiple Motor Neuropathy, Chronic Inflamatory Demyelinating Polyradiculoneouropathy, Myasthenia Gravis, and 4 heamtologic: Aquired Hypogammaglobulinemia, Idiopathic Thrombocytopenic Purpura in Adults, Idiopathic Thrombocytopenic Purpura in Children, and Hematopoietic Stem Cell Transplantation) were identified. The recommendations and/ or findings for 17% of the 42 conditions in the two cpgs (14% of 22 neurologic conditions, and 20% of the 20 hematologic conditions) were in need of updating.

Conclusion: The recommendations and/or findings for seven (17%) of 42 conditions (22 neurologic diseases and 20 hematologic diseases) in the two cpgs on the use of IVIG were determined to be in need of updating.

A systematic review on the effectiveness of immunoglobulin prophylaxis against clinical complications of hematopoietic stem cell transplantation

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Background: Hematopoietic Stem Cell Transplantation (HSCT) is a common procedure in the treatment of hematological malignancies. However, patient risks such as infection, graft-vs-host disease, and death increase as a result of this intervention. Prophylactic polyvalent immunoglobulin (IVIG) and Cytomegalovirus-specific immunoglobulin (CMVIG) have been used with varying efficacy to reduce complications in patients undergoing HSCT.

Objective: We sought to systematically review the effectiveness of immunoglobulin prophylaxis against post-HSCT complications.

Methods: We conducted a systematic review of 27 prospective randomized controlled trials addressing clinical outcomes of IVIG or CMVIG in 3934 patients undergoing HSCT. Search databases included MEDLINE, EMBASE, and EBM Reviews from 1966 to 09/2015. Clinical endpoints included overall survival, transplant-related mortality, graft-vs-host disease, veno-occlusive disease, interstitial pneumonitis, disease relapse, CMV infection, CMV disease, severe infections, and fatal infections. Using Review Manager, we produced forest plots by calculating the risk ratio, 95% confidence intervals, and heterogeneity. We further conducted sensitivity analyses on overall survival based on total dose of immunoglobulin, Jadad scores, risk of bias scores, and publication year. Three reviewers independently extracted data on study design, population, sample size, treatments, and clinical outcomes. Study quality was also assessed via Jadad scores, risk of bias scores, and the GRADE criteria with gradeprofiler.

Results: IVIG and CMVIG produced no significant difference on overall survival compared to placebo (risk ratio (RR)=0.94, 95% confidence interval (CI) = 0.88-1.01 and RR=1.10, CI=0.99-1.24, respectively). The use of IVIG vs placebo significantly increased veno-occlusive disease (RR=3.04, CI=1.10-8.41) and disease relapse (RR=1.26, CI=1.07-1.49), but yielded a decrease in acute graft-vs-host disease (RR=0.78, CI=0.65-0.94) and CMV disease (RR=0.52, CI=0.28-0.97). In addition, a sensitivity analysis of 2-5g/kg total IVIG or CMVIG showed significantly increased survival vs placebo (RR=1.31, CI=1.02-1.68).

CONCLUSIONS: IVIG or CMVIG prophylaxis may affect secondary outcomes but did not have a significant effect on overall patient survival. However, most studies were old and the clinical endpoints of immunoglobulin treatment in hypogammaglobulinemic HSCT recipients specifically have not been studied; therefore, these findings do not support current recommendations. Given the differences in HSCT patient demographics, transplant regimens, and medical care, newer clinical trials examining the use of immunoglobulin in HSCT recipients with or without hypogammaglobinemia are warranted.

The Ottawa Criteria for Appropriate Transfusions in Hepatectomy (OCATH): using the RAND/UCLA Appropriateness Method

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Background: Hepatectomy is associated with a high prevalence of blood transfusions. A transfusion has the potential to be life-saving in the appropriate patient, but is associated with important adverse effects. Given the prevalence of transfusions, their potential for great benefit and harm, and the difficulty in conducting clinical trials, this topic is well-suited for a study of appropriateness. Appropriateness studies aim to determine the indications for which expected health benefits of an intervention exceed expected negative consequences.

Methods: This study used the RAND/UCLA Appropriateness Method. An international, multidisciplinary expert panel in hepatobiliary surgery, anesthesiology, transfusion medicine, and critical care were identified. Panelists were asked to rate a series of 468 intraoperative and postoperative scenarios for the appropriateness of a blood transfusion, using a validated, 1-9 ordinal scale. The scenarios were rated in two stages: individually, followed by an in-person moderated panel session. Median scores and level of agreement were calculated to classify each scenario as appropriate, inappropriate, or uncertain.

Results: 48% of scenarios were rated appropriate, 28% inappropriate, and 24% uncertain. Level of agreement increased significantly after the in-person session. Based on the scenario ratings, there were five key recommendations. Intraoperative: 1) It is never inappropriate to transfuse for significant bleeding or ST segment changes. 2) It is never inappropriate to transfuse for a hemoglobin value of 75 g/L or less. 3) Without major indications (excessive bleeding or ST changes), it is inappropriate to transfuse at a hemoglobin of 95 g/L, and transfusion at 85 g/L requires strong justification. Postoperative: 1) In a stable, asymptomatic patient an appropriate transfusion trigger is 70g/L (without coronary artery disease) or 80 g/L (with coronary artery disease). 2) It is appropriate to transfuse for a hemoglobin of 75 g/L or less in the immediate post-operative setting, or with a significant hemoglobin drop (>15 g/L).Factors that increased the likelihood of a transfusion being inappropriate included no history of coronary artery disease, normal hemodynamics, and good postoperative functional status. Patient age did not affect the rating significantly.

Conclusions: Based on the best available evidence and expert opinion, criteria for the appropriate use of perioperative blood transfusions in hepatectomy have been developed. These criteria provide clinical guidance for those involved in perioperative blood management. In addition, the areas of uncertainty and disagreement can inform the direction of future clinical trials.

Direct Costs of Adult Chronic Rhinosinusitis Using Four Methods of Estimation: Results of the US Medical Expenditure Panel Survey

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Background: Chronic rhinosinusitis (CRS) is an inflammatory disease that affects 2-16% of the United States (US) population. Despite its rising prevalence, there is currently limited data in the literature evaluating the economic burden of this disease.
Objective: This study aimed to determine the direct healthcare costs of CRS from the perspective of the US government.

Methods: A prevalence-based approach was employed to estimate cost of illness for CRS from the 2011 Medical Expenditure Panel Survey (MEPS) database using a 4-part model: 1) an estimated sum of all health care expenditures; 2) an attribution model for disease-specific estimation of expenditures; 3) an estimation based on a propensity score model and 4) estimated disease-specific expenditure using a linear regression-based approach. A disease prevalence of 3.5% was utilised.

Results: The mean CRS-specific annual expenditure was $5,955 [95% Confidence Interval (CI) $5,087-6,823] by method 1, compared to $5,560 (CI 95% $4,689-6,431) by method 2 and $5560 (CI 95% $4,653-6,467) by method 3. The annual expenditure as estimated by method 4 was $5,589 (CI 95% $4,986-6,192). Ambulatory expenses accounted for the largest proportion of expenditures, followed by prescription, and in-hospital expenses.

Conclusions: This study provided a range of estimates of the direct medical expenditures associated with CRS. The study demonstrated the economic burden attributable to this disease was an estimated $60.2-64.5 billion US dollars in 2011 with a wide variation in the total and incremental direct expenditures, depending on the type of estimation model utilized and the prevalence assumed.

Impact and Trends in Medical 3D Printing: Automated Systematic Analysis of All Peer-Reviewed Literature

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Three-dimensional (3D) printing offers opportunities for personalized and precision based interventions. In medicine, 3D printing has already revolutionized how we consider and treat patients within multiple clinical scenarios. While 3D printed models of patient anatomy and pathology have undergone exponential growth, no systematic assessment of the entire field has been performed and no data has been organized regarding medically relevant terminology in peer-reviewed literature. Moreover, there is little data regarding application metrics for 3D printing stratified by organ section, imaging modality, or the 3D printing hardware itself. Medical publication repositories and data mining technologies have enabled rapid large-scale analyses of entire scientific domains. Natural language processing and semantic web technologies allow medical publications to be recast as machine usable knowledge, facilitating integration between medicine and other disciplines such as chemistry, biology, and epidemiology.

The purpose of this study is to develop and apply automated assessment methods to then enable scientific basis to objectively assess the benefits of 3D printing as well as to standardize 3D printing terminology that will facilitate scientific, clinical, and regulatory communications. We further evaluate 3D printing research trends by medical discipline as well as geographic distribution of published work to date. By standardizing 3D printing in medicine and critically assessing its clinical impact, we will facilitate more detailed analyses to further define trends and focus studies, better integrating 3D printing technologies for routine use in frontline medicine.

Application and Usefulness of Outpatient Cardiac Testing among Emergency Department Patients with Syncope

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Background: 2-3% of emergency department (ED) syncope patients suffer arrhythmia/death within 30-days of disposition. Outpatient cardiac testing is a proposed method to improve arrhythmia detection and patient safety.

Objectives: We describe the frequency of outpatient referrals among ED syncope patients, the frequency of outpatient cardiac testing and the proportion of serious adverse events (SAE) among high risk and low risk ED syncope patients, as defined by the Canadian Syncope Risk Score (CSRS).

Methods: We conducted a multicenter prospective cohort study to enroll adult syncope patients across five eds. We collected baseline characteristics, medical history, disposition, CSRS value, outpatient referrals and testing results (holter, echo), and saes. Adjudicated 30-day saes included death, myocardial infarction, arrhythmia, structural heart disease, pulmonary embolism, significant hemorrhage and procedural intervention. We used descriptive analysis.

Results: Of 4,064 enrolled patients, a total of 955 patients (23%) received an outpatient referral (mean age 57.7 years, 52.1% female). 56% of high risk syncope patients and 40% of low risk patients received outpatient referrals for cardiac testing. 4 patients (1%) who received outpatient cardiac testing suffered SAE outside the hospital. 7 patients (1.2%) who did not receive outpatient cardiac testing suffered SAE outside the hospital. 10% of high risk syncope patients who did not receive outpatient cardiac testing suffered SAE outside the hospital.

Conclusion: Outpatient cardiac testing among ED syncope patients is largely underutilized, especially among high risk patients. Better guidelines for outpatient cardiac testing are needed, as current practice is highly variable and mismatched with patient risk.

The Use of the Clinical Investigation Unit (CIU) and Liquid Chromatography Mass Spectrometry (LCMS) in Clinical Trials and Basic Research.

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Since its inception in the 1990s, the Clinical Investigation Unit (CIU) has delivered high quality research expertise to investigators at the OHRI. Seminal studies conducted in the CIU during that time in the areas of drug drug interactions (DDI), pharmacokinetic studies during pregnancy, and ABC transporters/pharmacogenomics studies will be presented.

The CIU is a 12-bed clinical research facility at the General Campus of The Ottawa Hospital that provides nurse coordinators and an REB liaison to help grant and industry funded studies and PIs with their clinical and basic research needs either in the CIU or in the PI's preferred space. The experienced staff can help researchers design and conduct all phases of clinical trials. They work closely with researchers from the earliest stages of their study design to make sure all research objectives are met. The unit also includes a pharmacokinetics laboratory that performs Liquid Chromatography Mass Spectrometry (LCMS) analysis of drugs or other small molecules in human body fluids and other solutions.

Rate of Stillbirth and Post-Delivery Death in Singleton Pregnancies by Pre-Pregnancy Body Mass Index (BMI)

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Background: In high-income countries, maternal overweight and obesity were found to be the highest ranking modifiable risk factor for stillbirth.[M1] With an increasing prevalence of overweight and obesity in the maternal population, further investigation on the timing (gestational age) of significant increase in rate of stillbirth and neonatal death is warranted.
Objective: The objective of this study was to examine the relationship of pre-pregnancy BMI to (1) the rate of stillbirth, (2) the rate of post-delivery death and, (3) the combination of stillbirth and post-delivery death by gestational age, with the goal of finding the optimal gestational age for delivery for each pre-pregnancy BMI category. Women were also stratified by parity (nulli- and multiparous).
Methods: This was a descriptive, retrospective cohort study of 268,306 singleton gestations from 2009 to 2013 obtained from the Better Outcomes Registry & Network (BORN) database from the province of Ontario.

Results: The rates of both stillbirth and post-delivery death were generally higher in underweight, overweight and obese categories relative to normal weight. In addition, rate of death was generally higher in nulliparous pregnancies. Overall, the rate of death was generally highest in Class II obesity. Notably, the rate of death from Class II to Class III obesity dropped significantly. It was not possible to detect a specific gestational age at which rate of death significantly increases in a particular BMI group, due to high amounts of missing data.

Conclusion: Similar to findings of systematic reviews, rates of death were higher in non-normal BMI classes. In may be possible that Class II obesity is a neglected group as their rates of death were much higher than the Class III group. The drop of rates of death from Class II to Class III obesity can therefore likely be explained by the more aggressive surveillance and management by delivery at earlier gestational ages in the latter group. Future goals include utilizing a larger sample to acquire adequate number of events for statistical analysis. Finally, preventative goals at aiming to reduce pre-pregnancy BMI are to be encouraged and prioritized.

Improving end-of-life care in the community: Using the RESPECT on-line prognostication tool

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Background: Older adults in the community who are frail may receive fragmented and inappropriate care due to poor prognostication, especially if they are nearing the end of life.

Objective(s): (1) To develop a mortality risk prediction model for people who may be nearing the end of life that can be used in the community setting by home care clients, as well as their informal and/or formal caregivers; (2) To develop a web-based prognostication tool – the Risk Evaluation for Support: Predictions for Elder life in the Community Tool (RESPECT) – using our final validated algorithm.

Method(s): This is an open cohort study using home care data from January 1, 2007, to January 1, 2014. The study sample included all home care clients in Ontario who received a structured health assessment, using the Resident Assessment Instrument for Home Care (RAI-HC). The derivation cohort consisted of 436 767 home care recipients with 1 137 976 assessments performed between January 1, 2007, and December 31, 2013. The temporal split sample validation cohort included 121 636 home care recipients with 171 602 assessments performed between January 1, 2013, and January 1, 2014. Proportional hazard model was used to estimate 12-month mortality risk from the time of a home care assessment. Predictors included sociodemographic factors, social support, health conditions, functional status, cognition, signs and symptoms of health decline, and health care use. Deaths (N = 244 529) were ascertained through linkage to the provincial vital statistics records. The final algorithm was implemented as an online tool (projectbiglife.ca) that can be completed by older adults and care providers in the community.

Result(s): Having an end-stage disease (Hazard ratio [HR]=3.13, standard error [SE]=1.38) and cancer (HR=2.08, SE=1.07) had the largest effect on the 1-year mortality risk of home care clients. Other predictors with hrs greater than 1.20 include male sex, limitations in activities of daily living, congestive heart failure, renal failure, symptoms of rapid health deterioration, and having 2+ hospitalizations over the last 90 days. The receiver operating curve for the final algorithm had an area of 0.74. The performance of the model improved following the inclusion of time interaction terms with variables that violated proportional hazards assumption.

Conclusion: RESPECT provides health care providers, patients, and caregivers with prognostic data that can be used to inform care, including when palliative and end-of-life care should be initiated. The online implementation of this prognostication algorithm enables its ease of use and dissemination.

Safety and efficacy of mscs for chronic stroke: a preclinical and clinical systematic review and meta-analysis

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Background: Mesenchymal stromal cells (mscs, 'adult stem cells') are known to have cytoprotective properties that could be effective to treat chronic stroke patients who have plateaued in their recovery. Objectives: Prior to considering a clinical trial we performed clinical and preclinical systematic reviews to examine the safety and efficacy of MSC therapy in stroke.

Methods: The clinical literature search of Medline, Embase and Cochrane Central Register of Controlled Trials, was conducted in January 2016. Eligibility criteria included stroke patients >18 years old, administered unmodified mscs in interventional or observational studies. The outcomes analyzed were safety (number of adverse events) and efficacy. The preclinical literature search was performed in Embase and Medline/Pubmed. Controlled comparison preclinical studies of in vivo models of stroke in which mscs were given ≥3 days after induction of stroke were included. Functional outcomes were analyzed as well as mortality. Data were expressed using either Peto's odds ratio (OR) or standard mean difference (SMD) and 95% confidence intervals (CI).

Results: Eight clinical studies met eligibility criteria (n=240 patients), including 7 interventional studies (2 randomized and 2 non-randomized controlled trials, 3 case series) and 1 observational study (cohort). 84% of patients received autologous bone marrow-derived mscs, 11% received allogeneic mscs (adipose and umbilical cord-derived) and 5% were unreported. Mscs were not associated with increased death [OR 0.45 (95%CI 0.17-1.2), n=4 studies]. The preclinical search identified 61 studies (n=1740 animals; 97% rodents). 69% of preclinical stroke models used intraluminal sutures and 55% of the models were permanent. 78% of studies used bone marrow-derived MSCS; studies used mscs from xenogenic (40%), syngeneic (23%), allogeneic (21%), or autologous (4%) sources. Mscs improved function [rotarod: SMD 1.56 (95%CI 0.87-2.25); n=12 studies, walking tasks: SMD 1.84 (95%CI 1.19-2.49); n=2]. Included clinical and preclinical data studies had mostly high or unclear risk of bias, respectively.

Conclusion: Limited clinical data suggests that mscs may be a safe treatment for chronic stroke patients. Preclinical data demonstrated that mscs may be effective however few studies used clinically relevant functional tests with an enriched rehabilitation environment. Well designed, methodologically rigorous studies of mscs in stroke will help further define the safety and efficacy of this therapy.

The Association Between Mindfulness and Mood Disturbance Among Breast Cancer Survivors with Chronic Neuropathic Pain

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Background: Chronic neuropathic pain (CNP) affects up to 50% of breast cancer survivors and is often associated with mental health disorders including depression and anxiety. There is currently no cure for CNP. However, mindfulness has been shown to be related to decreased levels of pain and psychological distress. This has not yet been explored among breast cancer survivors with CNP.

Objective: To evaluate the relationship between mindfulness and mood disturbance among breast cancer survivors with CNP.

Methods: One hundred women (Mean age = 53.2, SD = 10.6) with CNP following breast cancer treatment completed the following measures: Brief Pain Inventory – Intensity Scale (BPI; measure of pain intensity), Five Facets of Mindfulness Questionnaire (FFMQ; measure of trait mindfulness), and the Profile of Mood States (POMS-2A; measure of mood disturbance). Pearson correlations were completed to examine the relationships between variables and hierarchical regression analyses were performed to examine the unique contribution of each facet of mindfulness (observing, describing, non-judgement, non-reactivity, and acting with awareness) as a predictor of mood disturbance after controlling for pain.

Results: Initial correlation analysis revealed significant correlation between total FFMQ scores and POMS-2A ($r = -.48$, $p < .001$) as well as significant correlation between BPI and POMS-2A ($r = .35$, $p < .001$). The results of regression analyses revealed that mindfulness accounted for 28% ($p < .001$) of unique variance in mood disturbance after controlling for age and pain intensity. Of the five facets of mindfulness, acting with awareness was the only significant predictor ($\beta = -.48$, $p < .001$).

Conclusions: Consistent with previous research we found mindfulness to be associated with mood disturbance. These results build on evidence supporting the role of mindfulness in improving pain and mental health among breast cancer survivors with CNP. More specifically, certain individual facets of mindfulness may hold a larger role than others in the benefits reported. This has implications both in research and clinical practice. Future work to examine specific facet level contributions to psychological distress among breast cancer survivors with CNP is warranted.

Harnessing immune tolerance of non-inherited maternal HLA antigens in the CBS Cord Blood Bank

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Background: The genes encoding Human Leukocyte Antigens (HLA), the immunological determinant of hematopoietic stem cell (HSC) donor compatibility have diversified significantly within individual ethnic groups. As such, individuals are most likely to find a suitable HSC allograft from someone of common ancestry. Since its inception, the National Cord Bank has strived to create an ethnically diverse cord blood repository to reflect an equally diverse population. Less stringent matching requirement of cord blood will allow HLA coverage using fewer donors than would be required if using other HSC sources, however, the bank will still need to be particularly large. Emerging literature suggests additional plasticity in cord blood mediated immune reconstitution, specifically, an intrinsic permissiveness of donor T cells towards the non-inherited maternal antigens (NIMA) of the donor's mother. Leveraging the NIMA effect could potentially decrease the total number of cbus needed to cover the Canadian population, reducing costs.

Objective: Using matching simulations, this study will assess the likelihood that a Canadian bone marrow transplant (BMT) patient could find a suitable allograft within the cord blood bank, and further, if NIMA substitution at 1, 2, 3, or 4 HLA loci will improve matching probabilities.

Methods: We have obtained the cord bank registry info for over 1800 cord blood units, including 4 loci HLA typing for the cord donor and mother, as well as self-reported ethnicity data. A custom algorithm will generate hypothetical "virtual" cord phenotypes by substituting 1-4 HLA loci to create between 130,000 – 150,000 unique cord phenotypes. All cord phenotypes (real and virtual) will be subsequently matched using HLA data and self-reported ethnicity from Canadian patients undergoing a donor search with the Onematch Network at Canadian Blood Services. Match results will be grouped by ethnicity to determine the extent to which NIMA matching can improve donor options within specific groups.

Results: The algorithm to generate virtual cord phenotypes has been written and tested. Matching simulations are ongoing.

Conclusions: Simulations will assess how well our donor and recipient pool overlaps, and if NIMA substitution at 1 or more loci can improve match outcomes in particular ethnic groups.

Costs of Emergency Syncope Care in Canada

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Background: Syncope is a common Emergency Department (ED) presentation with approximately 160,000 visits annually in Canada. Lack of standardized syncope care has economic and cost implications, and the extent of resource utilization for emergency syncope care in Canada is not known. We have previously reported Emergency Medical Services (EMS) over-utilization, variations in ED management and substantial proportion (46.5%) hospitalized for cardiac monitoring. Our previous studies have proposed ways to reduce health care utilization (EMS clinical decision tool, ED risk scores, and remote cardiac monitoring).

Objective: The objectives of this study were to: 1) Estimate costs associated with care of syncope patients in Canada in the pre-hospital, ED and inpatient settings and; 2) Determine potential cost savings if proposed alternate strategies were adopted.

Methods: We conducted a prospective cohort study in Ottawa, Kingston, and Edmonton (5 eds) from 2010-2014. We enrolled adult (≥16 years) syncope patients and excluded those with prolonged loss of consciousness, mental status changes, seizure, significant trauma, or alcohol/illicit drug abuse. Demographics, medical history, mode of arrival, EMS time points, reasons for ED referrals, reasons for hospitalization and length of stay, final ED diagnosis, discharge diagnosis, and any serious adverse event (SAE) within 30 days of index visit were collected. We used descriptive and inferential statistics.

Results: The national healthcare cost estimates extrapolated based on the data from the 5 eds were $10 million in the prehospital setting, $43 million in the ED, and $191 million in the inpatient setting.

Conclusion: In conclusion, syncope is a prevalent condition that is associated with high health resource use with an estimated total annual cost of $244 million for emergency care.
Overall, it was estimated that the proposed strategies will save $70 million annually through: 1) Diversion of low-risk patients (given a high proportion of benign outcomes associated with syncope) by EMS to reduce EMS utilization and ED visits; 2) Accurate risk-stratification in the ED to reduce length of stay, ED cost and hospitalization; and 3) Deployment of remote cardiac monitoring to improve patient safety, health care efficiency and to reduce the number of patients that are hospitalized for cardiac monitoring. This is likely an underestimation of cost savings since reduction in investigations related to diversion of ED patients, reduction in ED length of stay and hospitalization from improved risk stratification were unaccounted for.

Comparing maternal serum screening markers among IVF and spontaneous conceptions in Ontario

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Background: Multiple marker prenatal screening uses maternal serum markers combined with nuchal translucency measurement to estimate risk of Down syndrome or a number of other aneuploidies. This algorithm incorporates corrections for ethnicity, smoking, diabetes and in vitro fertilization (IVF) for specific maternal serum screening markers. In Ontario, only PAPP-A, total, and μE^3 have an IVF correction factor applied.

Objective: The objective of this study was to investigate the accuracy of IVF identification on the prenatal screening record and to compare the screening markers in IVF and non-IVF pregnancies in the population of Ontario.

Methods: The Better Outcomes Registry & Network (BORN) Ontario collects comprehensive information on prenatal screening from all prenatal screening centres and information on the use of IVF from all fertility clinics in Ontario through CARTR Plus. Prenatal screening data and fertility data were merged to create a linked dataset. For each MSS marker and NT measurement, log10 transformations were performed in order to account for the skewed distributions. Linear regression models were produced for both of these scenarios. New adjustment factors were developed for each MSS marker. These adjustment factors were applied to all IVF records with prenatal screening results.

Results: When identification of the use of IVF from the prenatal screening record was compared to the gold standard for fertility treatment in Canada (the CARTR Plus database), the sensitivity was 95.8% and the specificity was 98.9%, however the positive predictive value was 68.8%. The largest differences in mean MoM for the prenatal screening Ontario (PSO) and CARTR Plus models were seen for PAPP-A, total hCG and µE3, which was appropriate given that these three MSS markers were the ones that currently have adjustment factors for IVF applied. When we compared the same population of patients, we found that the new adjustment factor for IVF improved the mean MSS marker MoMs to a greater extent than the current PSO IVF adjustment factors. Slight differences were observed among IVF and FET cycles.

Conclusions: Interestingly, the current PSO IVF adjustment factor for total hCG adjusted the mean MoM in the wrong direction. This strengthens the rationale for modifying the current MSS marker adjustment factors for IVF in the Ontario population. The majority of MSS markers would benefits from an IVF adjustment. Additionally, identification of conception method would be more appropriate for implementing correction factors if ascertained from CARTR Plus.

Relationship between Body Mass Index (BMI) and Lipophilic Persistent Organic Pollutants (L-pops) Levels in Women of the Maternal-Infant Research on Environmental Chemicals (MIREC) Study

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Background: Mechanisms through which obesity may cause congenital anomalies remain unclear. Evidence suggests that lipophilic persistent organic pollutants (L-pops) are able to reach the fetus across the placenta. Even though, data concerning the effects of these toxins in neonates is still scarce, they are known to have detrimental effects in adults. Knowledge concerning the relationship between body mass index (BMI) variations during pregnancy and L-pops serum levels is limited.
Objective: This study aimed to determine whether first trimester maternal BMI and L-pops serum levels were associated.

Methods: A cross-sectional study was performed using data from the Maternal-Infant Research on Environmental Chemicals (MIREC) Study, a prospective cohort that enrolled pregnant women from 10 different sites across Canada between 2008 and 2011. We used first trimester maternal BMI and serum levels of 41 L-pops to create unadjusted and age-adjusted linear regression models. Pairwise comparisons of L-pops levels between BMI categories were conducted when linear regression tests were determined significant.

Results: The most abundant L-pops were found to be PCB (0.494 µg/kg lipid) and DDE (0.589 µg/kg lipid), whereas the lowest PARLAR26 (0.005 µg/kg lipid) and PARLAR50 (0.005 µg/kg lipid). 19 L-pops (PBDE100, 153, 47, and 99, PCB138, 146, 153, 156, 163, 167, 170, 180, 183, 187, 194, 201, and 203, PCB, and PARLAR26) had concentration levels that significantly varied across BMI classes, and were used for pairwise comparisons between BMI classes.

Conclusion: Our findings show that several L-pops concentrations in first trimester maternal serum are associated with first trimester maternal BMI. Most associations seem to be negative. Subsequent research is needed to evaluate L-pops levels variations during pregnancy given gestational weight changes and potential teratogenic effects.

Socioeconomic status and anterior epistaxis in adult population

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Background: Many studies evaluate the various risk factors for epistaxis but there is little known about the influence of socioeconomic status.

Objective: To determine whether socioeconomic status influences the occurrence of anterior epistaxis in an adult population.

Methods: We conducted a retrospective review of emergency department (ED) visits from January 2012 to May 2014 for adult patients with a diagnosis of anterior epistaxis. Patient demographic information such as age, sex, and postal code was collected. The primary outcome was ED visits. We utilised postal code matched neighborhood-level income quintiles to represent the socioeconomic status.

Results: A total of 353 cases of anterior epistaxis were included. The mean age was 70 years and 51% of patients were male. The patients were stratified into two groups based on whether their age was equal to and above, or below 75 years. Our analysis indicated that those 75 years or older in higher income quintiles have an increased risk of anterior epistaxis compared to the subjects in the lower income quintiles ($p<0.05$). This association did not hold true for those younger than 75 years or for all age groups combined.

Conclusion: Among patients of 75 years and older, higher socioeconomic status appears to be a risk factor for the occurrence of anterior epistaxis. Below the age of 75, socioeconomic status does not appear to be a risk factor for anterior epistaxis.

The risk of venous thromboembolism in chronic kidney disease based on eGFR and albuminuria

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Background: Chronic kidney disease (CKD), defined by reductions in estimated glomerular filtration rate (eGFR) or the presence of albuminuria, is rising worldwide and is associated with a higher risk of venous thromboembolism (VTE). Whether the risk of VTE in CKD differs based on the combined or independent effects of low eGFR and/or albuminuria remains unclear.

Objective: To examine the association between decreased eGFR and increased albuminuria, and the development of VTE.

Methods: A population-based, retrospective cohort study was conducted using administrative healthcare databases for the population of the province of Ontario, Canada between 2002 and 2012. A total of 694,956 patients with a urine albumin to creatinine ratio (ACR) and eGFR measure were included. Study outcome was the time to a first VTE event. The incidence of VTE was categorized by strata of eGFR, and ACR and the eGFR X ACR interaction was examined using adjusted Cox proportional hazard models.

Results: A total of 15,683 (2.3%) VTE events occurred during the study period. The adjusted HR for VTE in patients with albuminuria were 1.2 (1.15-1.25) and 1.34 (1.24-1.45) for ACR 3-30mg/mmol and ACR >30mg/mmol respectively (using ACR < 3mg/mmol as the reference). The adjusted HR for VTE in patients with decreased eGFR were 1.12 (1.08-1.17), 1.25 (1.18-1.33), 1.31 (1.21-1.42) and 1.27 (1.11-1.45) for patients with eGFR 60-89ml/min, 45-59ml/min, 30-44ml/min and 15-29ml/min respectively (using eGFR ≥ 90ml/min as the reference). The ACR X eGFR interaction was significant (P = 0.0003). Patients with the highest level of albuminuria (>30mg/mmol) had the highest adjusted HR for VTE across the different categories of eGFR. The risk for VTE was greatest in patients with ACR >30mg/mmol and eGFR ≥90ml/min and 60-89ml/min (1.65 [1.41-1.93] and 1.63 [1.43-1.85] respectively) versus those with ACR >30mg/mmol and eGFR 45-59ml/min, 30-44ml/min and 15-29ml/min (1.52 [1.27-1.82], 1.54 [1.27-1.88] and 1.43 [1.08-1.9] respectively).

Conclusion: Increased albuminuria and decreased eGFR were independent risk factors for the development of VTE. ACR is a significant effect modifier for the association of eGFR and VTE with the highest risk for VTE seen in patients with most severe albuminuria but milder GFR impairments.

A systematic review on the perioperative interventions made for frail elderly patients undergoing surgery

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Background: Frailty, an aggregate expression of susceptibility to poor outcomes owing to age- and disease-related deficits that accumulate across multiple domains, increases in prevalence with increasing age. Older patients are the fastest growing segment of the surgical population, and often experience adverse postoperative outcomes. The presence of frailty explains much of the adverse outcome burden in our older patients, however, little is known regarding specific interventions that may improve the postoperative outcome of frail older patients.

Objective: To systematically review studies of frail surgical patients where perioperative interventions were tested to improve outcomes defined by the Institute for Healthcare Improvement's Triple Aim (health, cost, experience).

Methods: Cochrane, Medline, PubMed, CINAHL, and EMBASE databases were searched using a strategy developed with an information specialist. Trials in progress were also sought using clinicaltrials.gov. We included any experimental studies which tested interventions specifically in frail patients, or in which a frailty-specific subgroup analysis was provided. We did not limit studies to specific frailty definitions, however, a definition of the frailty measure had to be provided. Titles and abstracts were reviewed in duplicate to identify citations for full text reviews. Following duplicate full text review, data were extracted in duplicate using a form specifically designed and pilot tested for this study. The Cochrane Collaboration's Risk of Bias tools for randomized and non-randomized trials were used to assess study bias. A narrative synthesis was used to guide our analysis.

Results: We identified 2500 titles; 93 underwent full text review. Fourteen studies were included (8 RCTs and 6 non RCTs) in vascular, general, cardiac, orthopedic, and thoracic surgery populations. In three articles with low risk of bias, interventions including prehabilitation exercise, restrictive blood transfusion, and individualized care plans found either no significant differences, higher 30 day mortality in restrictive transfusion group (HR 2.4, 95% CI 1.1-5.2, p=0.03), and improved quality of life assessment at discharge (OR=0.49, 95% CI= 0.29-0.82) respectively.

Conclusions: Few studies exist which specifically investigate interventions to improve the outcomes of frail surgical patients, and those that do exist were at moderate to high risk of bias. There are/is some indication that these interventions may improve these outcomes, however, the risk of bias specifically conducted in populations of frail patients are needed to inform care directed at improving the outcomes of frail surgical patients.

Yield of Computed Tomography of the Head among Adult Emergency Department Syncope Patients: A Prospective Cohort Study

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Background: Syncope accounts for 1-3% of Emergency Department (ED) visits of which 7-23% suffer serious outcomes (e.g. Death, arrhythmias, hemorrhage) in the ED or within 30 days of the visit. Computed tomography (CT) scan of the head is performed among 29.3% - 86.9% patients with acute abnormalities identified only in 0-6.4%. Each CT scan costs $1,065 CAD. Presently there are no tools available to guide physicians regarding the use of CT head for management of syncope patients.

Objective: The objective of this study is to: 1) Evaluate the proportion of adult ED syncope patients who undergo CT head and its yield for acute abnormalities. 2) identify high-risk factors that can aid in detection of patients with serious intracranial conditions [e.g. Subarachnoid hemorrhage (SAH), subdural hematoma (SDH), or new/progressing space occupying lesion (SOL)]

Methods: We conducted a prospective cohort study of adult syncope patients presenting to six Canadian academic eds in 5 cities (Ottawa, ON - Kingston, ON – Calgary, AB and Edmonton, AB) over 54 months. We included adult (≥16 years) with syncope and excluded those with prolonged loss of consciousness (>5 minutes), altered mental status, significant trauma requiring admission, alcohol/drug intoxication, or witnessed seizure. 1052 patients with CT head were identified from an original cohort of 5722 ED syncope patients for which data was abstracted from all documentation available in print and electronic format.

Results: The characteristics of 1,052 patients who had CT head performed are: mean age was 63.5 (range = 16-102), and 521 (49.3%) were female, 36 (3.4%) had acute abnormalities identified (SAH, SDH, and progressive malignancy), 454 (43.2%) had chronic age-related changes and 562 (53.4%) had a normal CT head. Among those with acute abnormalities: 23 were ≥ 65 years old, 20 had a post-syncopal head injury, 4 were on warfarin, 4 had an INR ≥ 2.4, 3 had more than 2 episodes of vomiting, and 1 patient had focal neurological deficits.

Conclusion: The variance in the frequency of CT head scans ordered for adult ED syncope patients necessitates a robust clinical decision tool in order to reduce unnecessary neuroimaging. We are currently in the process of recruiting additional patients to derive the proposed tool.

HIV testing in young straight African migrant men in Ontario: Gendered barriers

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Background: HIV testing has various clinical and non-clinical benefits at the individual and population levels (Giordano, Suarez-Almazor, & Grimes, 2005; Wallace, McLellan-Lemal, Harris, Townsend, & Miller, 2011; WHO/ UNICEF/ UNAIDS, 2013; Ottawa Public Health, 2015). Despite that, certain sub-populations especially young heterosexual African migrant men from HIV-endemic countries in Canada, including Ontario, still do not access HIV testing likely due to various barriers. Much literature on barriers to HIV testing focuses on client characteristics and motivation, predominantly drawing on surveys (Deblonde, De Koker, Hamers, Fontaine, Luchters, & Temmerman, 2010; Leblanc, Flores, & Barroso, 2016). Missing are studies in context on the structural and provider-related factors preventing particularly young heterosexual African migrant men to access HIV testing. The question is: what barriers prevent young heterosexual African migrant men from HIV-endemic countries to test for HIV in Ontario? Abundant literature exists on HIV testing but this is largely about other populations, such as men who have sex with men, inject-drug users and women. Studies on young straight African men remain limited in Canada. Yet heterosexual contact is the second-most reported HIV exposure category among adults, after men who have sex with men, in Canada (Public Health Agency of Canada, 2015). Specifically lacking are studies on the experiences of HIV testing and those not being tested in young heterosexual African migrant men from HIV-endemic countries to highlight the barriers to accessing HIV testing.

Objective : Undertake a case study into the barriers preventing access to HIV testing in young heterosexual African migrant men from HIV-endemic countries in Ottawa, Ontario.

Methods: Relying on a multi-level social ecological framework (Richard, Gauvin, & Raine, 2011), first a scoping review was proposed to re-examine the non-clinical benefits of HIV testing and their implications for knowledge dissemination in affected communities. Second, semi-structured interviews to investigate: 1. HIV testing experiences in young heterosexual African migrant men and women; 2. the experiences of HIV test providers; and 3. the experiences of lay health community leaders who offer services to young members in Ottawa.

Results: Preliminary results from the scoping review: non-clinical benefits are experienced at many levels with various individual, social-demographic, cultural and legal implications for the dissemination of knowledge to improve HIV testing.

Conclusions (Preliminary): Health education and communication tailored to specific contexts and populations is required, on both clinical and non-clinical benefits, to modify behaviour and scale up sustainable HIV testing interventions at various levels.

Smartphones in the ED: Acceptability of the Ottawa Rules App amongst Emergency Department clinicians.

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Background: The Ottawa Ankle, the Ottawa Knee, and the Canadian C-spine Rule, together known as the Ottawa Rules, were developed by Dr. Ian Stiell with the goal of decreasing unnecessary diagnostic imaging in the emergency department (ED). While the rules have been validated internationally, physician uptake of the Rules is suboptimal. In 2015, The Ottawa Hospital mhealth Research Team developed a novel mobile and desktop application (app) housing The Ottawa Rules, to support the newest generation of ED clinicians using mobile health tools. The Ottawa Rules app was released for ios and Android devices May 9th 2016.

Objective: The primary objective for this study was to assess the usability and acceptability of the Ottawa Rules app amongst ED clinicians.

Methods: Medical students, nurses, physicians and residents at The Ottawa Hospital Civic and General campuses were approached to participate in the study between May 9 and August 4, 2016. Participants provided informed consent via the app, were verified by e-mail and enrolled in the 1-month study. At the end of the study period, participants were emailed a usability survey to complete within the following 30 days. The survey consisted of 23 questions measuring app usability and acceptability on a 5-point Likert scale, ranging from 0 or 'Strongly disagree' to 4 or 'Strongly agree'. This study was approved by the OHSN-REB (#20150405).

Results: As of September 20, 110 of 155 participants (70.9%) have completed the usability survey. Those who have completed the study include 19 (12.3%) physicians, 35 (22.6%) residents, 42 (27.1%) nurses and 14 (9.0%) medical students. Preliminary survey results have revealed the following:
64% indicated 'agree' or 'strongly agree' to "The app was useful in helping me accurately carry out these clinical rules"
73% indicated 'agree' or 'strongly agree' to "I would recommend the app to fellow colleagues"
82% reported using the app on a 'weekly' or 'monthly' basis
64% indicated 'agree' or 'strongly agree' to I will continue using the app
52.2% reported wanting more features, in particular, the CT Head Rules
28.3% reported wanting more interactive app features

Conclusions: While the study is still underway, preliminary results suggest that ED staff find the Ottawa Rules app to be acceptable and usable in the clinical environment. However, the inclusion of more features, including additional rules was requested. Survey feedback will be used to guide app updates and modifications to improve its uptake at TOH, and globally.

A conceptual framework for women's empowerment and nutrition to assess complex interventions

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Background: Malnutrition and poor diets are the foremost drivers of the global burden of disease. The 2013 Lancet Series on Maternal and Child Nutrition highlights the important role that women's empowerment plays in the nutritional status of women and children. Yet despite having evidence that empowerment interventions contribute to improved nutrition outcomes, we do not have a thorough understanding of the specific pathways through which empowerment influences t t he underlying and immediate causes of malnutrition.

Objectives: (1) To develop a conceptual framework for nutrition and women's empowerment that systematically incorporates empowerment concepts into the UNICEF Conceptual Framework for Malnutrition. (2) To apply the conceptual framework to the design of a systematic review of nutrition interventions targeting adolescent girls in low- and middle-income countries (LMICs) to assess the role of women's empowerment in intervention uptake and effectiveness.

Methods: We adopted the iterative approach of Andersen et al. for the development of logic models to capture complexity in systematic reviews and reviewed relevant women's empowerment frameworks based on consultation with top experts, stakeholders and researchers. Models included theories of empowerment, social determinants of health and health equity, and underlying causes of malnutrition in LMICs. We then applied our findings to the UNICEF Conceptual Framework for Malnutrition to create a revised conceptual framework that systematically incorporates empowerment concepts in the nutrition causal chain.

Results and Conclusions: We will present the finalized conceptual framework, which will be used as a basis for defining the research questions, search strategy, and classification of interventions for a Campbell systematic review (title registered). The framework provides guidance on what data are needed to explore characteristics of the intervention, participants and context that may influence program uptake and effectiveness. These methods may be useful for developing and understanding other complex programs beyond maternal and child nutrition.

Fetomaternal hemorrhage at term associated with intraplacental choriocarcinoma: case report and review of the literature, with recommendations for chemotherapy

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Objective: We describe a case of fetomaternal hemorrhage at term associated with intraplacental choriocarcinoma. A male infant was born with severe anemia and the mother and infant were followed up for 3 months following delivery. Examination of the mother during follow-up revealed evidence of metastasis.

Materials and methods: Clinical manifestations and results of pathological examination and laboratory investigation of the case were described and relevant literature was reviewed.

Results: Microscopic examination revealed a well-circumscribed lesion composed of atypical syncytiotrophoblasts and cytotrophoblasts with geographic tumor necrosis and hemorrhage. Investigations at higher magnification revealed nuclear pleomorphism and severe atypia. Immunohistochemical staining was positive for cytokeratin (CK), human chorionic gonadotropin (HCG), protein 63 (P63), nuclear-associated antigen Ki-67 (Ki-67), and cluster of differentiation 34 (CD34). Follow-up examination revealed increased beta-human chorionic gonadotropin (β-HCG) serum levels from 31,280 IU/L (7 days post-delivery) to 192,070 IU/L (50 days post-delivery), which then steadily fell to 42,468 IU/L (3 months post-delivery) without any therapeutic intervention; this was highly unusual and the responsible mechanisms remain unclear. The patient subsequently died as a result of metastasis and cerebral hemorrhage. The infant's β-HCG level fell to within the normal range without chemotherapy.

Conclusion: FMH at term associated with IC is a rare and severedisease and chemotherapy should be considered carefully in accordance with the relative conditionsof individual patients, particularly serum β-HCG.

Keywords: Fetomaternal hemorrhage, placenta, choriocarcinoma; chemotherapy; beta-human chorionic gonadotropin

An online to offline model in the management of critically ill placenta previa accrete cases in Guangzhou, China

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Online-to-offline (O2O) commerce has become a popular business model in China in recent years. However, the O2O model for medical service has rarely been reported. Because of the ability to connect care providers with patients, the O2O model seems an attractive model for medical service. Here we describe an O2O model using the social media app of “Wechat”, a popular app in China which has connected 800 millions of people on constant basis according to the latest statistics, to follow up and manage for critically ill placenta previa accrete patients. Started from January 1, 2015, every patient who were diagnosed with placenta previa accrete were registered in a “wechat” group created at our center. These patients were followed by a team of specialists during pregnancy, and were instructed for self-monitor for bleeding. If the amount bleeding exceeded 100 ml, the patients were instructed to seek emergent medical care and contact our specialist team through “Wechat” immediately. The patient could be admitted to our hospital within 5 minutes,and the operation could be started within 30 minutes. 168 placenta previa accrete patients have been registered into the system, 56 critically ill placenta previa accrete patients have been treated and no case of critically ill placenta previa accrete patient has died or with severed morbidity treated through using this model has been observed. This O2O is not only useful for consultation, but for emergent care for critically ill placenta previa accrete patients as well.

Risk of Venous Thromboembolism in Hospitalized Patients with Sickle Cell Disease

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Background: Patients with sickle cell disease (SCD) are at an increased risk of developing venous thromboembolism (VTE). However, the underlying risk of VTE complication during hospitalization is unclear in this patient population.

Objective: We sought to report the incidence of VTE and its associated risk factors in hospitalized SCD patients.

Methods: A retrospective cohort study of SCD patients requiring hospitalization was undertaken at a tertiary care center. Incidence ratios of VTE per hospitalization for different risk factors (thromboprophylaxis use, central venous catheter (CVC), past history of VTE, surgery during hospitalization) were assessed. Univariate, age adjusted and multivariate Poisson models were estimated accounting for the repeated hospitalizations per patients.

Results: A total of 101 patients with at least one hospitalization were included in the study. The mean of number of admissions per patients was 8.9. Overall, 17 out of 896 (1.9%) admissions were complicated by VTE. The incidence of VTE varied by risk factors, from 0.8% in patient without CVC to 6.7% among patients admitted with previous history of VTE. Age adjusted and multivariate Poisson models for incidence rate ratios of VTE per hospitalization among patients with SCD for different risk factors are depicted in Table 1.

Conclusion: The risk of VTE seems low in hospitalized SCD. A prior history of VTE and a hospitalization for surgery might be associated with higher risk of VTE complication. Future studies assessing these risk factors to tailor thromboprophylaxis regimens are needed.

Patient, Center and Provider Level Variation across Ontario Renal Transplant Centers

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Background: Significant variation in case mix and the structure of care in kidney transplantation has been described in the United States.

Objective: The objective of our study was to characterize patient, center and provider level differences across renal transplant centers in Ontario Canada.

Methods: This was a retrospective population based cohort study of adult (≥ 18 years) transplant recipients who received a primary solitary kidney between January 1st 2000 to December 31st 2013 at one of the six transplant centers in Ontario. We used linked administrative healthcare databases from the Institute of Clinical and Evaluative Sciences in Ontario, Canada.

Results: Our study included 5092 renal transplant recipients. The median age was 52 years, 36.9% were female and 63.7% were Caucasian. Significant differences were seen across the transplant centers in case mix (both recipient and donor factors). Transplant volume ranged between 55 and 1387 transplants over the study period across centers ($p<0.0001$). Overall, there were 186 physicians providing transplant related care to this cohort during their hospital admission; this included Nephrologists, Urologists, General surgeons, Vascular surgeons, Internists, General practitioners and fellows. The type of provider seen by patients varied significantly across centers. The average age and years since graduation of the included providers varied significantly across centers. Patient outcomes varied significantly by center and are described in the table listed below. The average length of transplant admission was 8 days. Overall there were 31 deaths that occurred in hospital posttransplant. Thirty-seven percent of recipients required dialysis in hospital posttransplant,and 14% of recipients required a stay in the special care unit.

Conclusion: Our study found significant heterogeneity across Ontario renal transplant centers at the patient, center and provider level. Future studies are needed linking center and provider level differences to graft and patient survival rates.

Evaluation of post-operative transfusion procedures in post-cystectomy patients at The Ottawa Hospital (TOH).

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Background: While vital in the treatment of significant blood loss during and after surgery, blood transfusion remains associated with significant risks and should only be utilized under proper indications. Clinical and laboratory findings should direct the decision to transfuse and also determine risk for preventable adverse outcomes, such as transfusion associated circulatory overload (TACO). Cystectomy is a surgery associated with high risk for both operative and post-operative transfusion. Consequently, this patient population was reviewed to determine how frequently appropriate pre-transfusion assessments are performed.

Objective: To assess the frequency and quality of recorded clinical and laboratory assessments around blood product transfusions in patients who underwent cystectomy surgery.

Methods: We conducted a retrospective review of 178 patients who underwent cystectomy at The Ottawa Hospital (TOH) from March, 2008 to May, 2016 using the patients' electronic medical records. A complete peri-transfusion assessment was defined as presence of transfusion start and end times, pre-transfusion lab testing, clinical history and physical exam, as well as vital signs assessed before and 15 minutes into transfusion. TACO risk factors were defined using accepted criteria to include acute MI, LV dysfunction, renal dysfunction, diuretic held, and age above 70.

Results: Eighty patients (44.9%) in the cohort received transfusions post-operatively. Of these episodes, only 16.3% had complete transfusion assessment recorded. Despite TACO risk factors being present in 80% of all post-operative episodes, post-transfusion fluid balance was monitored in only 52.6% of these cases. Transfusion related complications were reported in only 1.25% of all transfusion cases.

Conclusion: Only a small minority of this patient cohort received appropriate peri-transfusion assessments, suggesting that many transfusions may have occurred without clinical indications present and that strategies to minimize preventable transfusion reactions were not employed. The low rate of adverse outcomes reported is not a reliable indicator, as evidence suggests an under-reporting of adverse reactions in general, specifically those associated with fluid overload.

Modifying existing protocols at TOH surrounding transfusion ordering may result in fewer unnecessary transfusions, with a resultant decrease in associated risks. Additionally, programs for continuing transfusion education for all healthcare providers in indicated to improve understanding of the importance of safe transfusion use.

Identification of Risk Factors in the Pre-Hospital Setting for Short Term Severe Adverse Events among Syncope Patients

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Background: Among patients transported to the Emergency Department (ED) by Emergency Medical Services (EMS) for syncope, the majority (>80%) do not suffer any severe adverse events (SAE) within 30-days. Moreover >50% of patients will be diagnosed with vasovagal syncope, indicating a potential over-utilization of EMS resources in Canada.

Objective: The overall objective of our study is to develop a robust clinical decision tool to identify low-risk syncope patients for potential diversion from the ED.

Methods: Study Design: A prospective cohort study at 3 cities (5 eds) from February 2012 to February 2013. Inclusion criteria: Adult (≥16 years) syncope patients transported by EMS to the ED.
Exclusion criteria: Patients with prolonged loss of consciousness (LOC), change in mental status from baseline, LOC due to obvious seizure, alcohol/illicit drug use, and patients not transported to the ED by EMS. Data collected: Patient demographics, clinical variables obtained by EMS, EMS interventions, concerning symptoms, EMS ECG variables, and 30-day SAE defined as being either cardiac outcomes (arrhythmias, myocardial infarction, aortic dissection, serious structural heart disease), non-cardiac outcomes (pulmonary embolism, severe pulmonary artery hypertension, subarachnoid hemorrhage, significant hemorrhage), death, or procedural interventions. Analysis: Univariate analysis will be used to determine the strength of association between clinical variables and SAE. Multivariable analysis will be used to derive the final model.

Results: 1473 patients were prospectively recruited during the one-year period across all the study sites, 67% of which were transported to the ED by EMS. Only 14.6% of these patients suffered an SAE within 30-days: 3.4% pre-hospital, 6.2% in ED, 5.0% after ED. Using univariate analysis, we identified the following predictor variables as important risk factors for SAE within 30-days of EMS evaluation: 1) Cardiac history 2) EMS interventions 3) EMS ECG abnormalities 4) Age > 75 years 5) Abnormal EMS vital signs (any systolic BP, first heart rate, first oxygen saturation, first respiratory rate).

Conclusion: We present the preliminary results of our study to develop an EMS clinical decision tool to identify low-risk syncope patients for diversion from the ED. Based on our results, predictor variables that are reliable and strongly associated with SAE will be selected for multivariable analysis by logistic regression. Once the final model is derived, we will validate the clinical decision tool.

Targeting PTP1B to treat Alzheimer's Disease

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Background: Alzheimer's disease is characterized with progressive loss of memory. The brain of Alzheimer's patients gradually loses its ability to respond to insulin, a hormone that enables the formation and consolidation of connections between nerve cells so that new memories can be formedd and recalled. Insulin resistance is a key feature of Alzheimer's disease. New studies show that patiens with anxiety and deficient memory are more likelyto progress to Alzheimer's. It has been reported that insulin resistance in the brain of an Alzheimer's mouse model is associated with elevated brain levels of tyrosine phosphatase PTP1B (Tyrosine Phosphatase 1B). Our lab found that over-activity of PTP1B impairs the brain's response to insulin and hinders the brain's production of endocannabinoids and causes anxiety phenotype in mice. Importantly, a selective PTP1B inhibitor(Trodusquemine) could restore insulin signaling and ecb production, relieving anxiety. These observations raise the possibility that PTP1B mighe be a new target for the treatment of Alzheimer's disease.

Objective: To determine whether PTP1B is a useful therapeutic target for Alzheimer's disease.

Methods : We used the injection of amyloid beta 42 (Abeta42) to induce Alzheimer's disease in wild-type and camkcre-ptp1bflx/flx mice that delete PTP1B in neurons. We tested working memory after Abeta42 injection, using Y maze and Object Recognition tests.

Results: Abeta42 injection induced a robust memory impairment in 10-month old wild-type male mice, but caused no significant memory loss in camkcre-ptp1bflx/flx mice.

Conclusions: Our results indicate that PTP1B is required to mediate the deleterious effects of Abeta42 and that PTP1B may be a new target for Alzheimer's treatment.

Efficient anti-inflammatory response requires IRF2BP2 after stroke in mice

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Background: Stroke is the second most frequent cause of death and debilitating illness worldwide. After stroke, a rapid innate immune response leadis to robust inflammatory signaling and a subsequent healing processes. In the brain, microglia are the resident immune cells that can be polarized into M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes after brain injury. Extensive studies showed members of the Interferon Regulatory Factor (IRF) family of transcription factors play a major role to control innate and adaptive immune responses regulating genes encoding IFNα, IFNβ and IFNγ, and many IFN-inducible genes. In particular, IRF2 activates M1 polarization, while IRF2 gene expression and transactivational activities are inhibited by IRF2 binding protein 2 (IRF2BP2).

Objective: In this study, we hypothesized that IRF2BP2 ablation in microglia/macrophages would affect their polarization that influences recovery from focal brain ischemia.

Methods: Using an animal model lacking IRF2BP2 in myeloid cell-lineage that includes peripheral macrophages and CNS microglia (LysM-cre, IRF2BP2 knockout mice; IRF2BP2$^{-/-}$), we analyzed phagocytosis and innate immune responses in isolated microglia as well as histological and behavioral studies at 1, 4, and 8 days after stroke to test the consequence of IRF2BP2 ablation in these cells.

Results: Our data showed IRF2BP2-deficient microglia expressed elevated inflammatory cytokines and lower anti-inflammatory markers in response to LPS and IL-4 stimulation compared to IRF2BP2$^{+/+}$ mice, respectively. Subjected to focal ischemic injury induced by photothrombosis, IRF2BP2$^{-/-}$ mice have worsened sensory-motor functional deficits shown by adhesive removal tests. Key inflammatory gene markers were elevated in the brain such as TNFα and IL1β whereas anti-inflammatory marker like Fizz1 was lower in IRF2BP2$^{-/-}$ mice. The infarct volume of IRF2BP2$^{-/-}$ mice at 4d post-stroke was significantly larger compared to IRF2BP2$^{+/+}$ mice. Although phagocytotic ability was not different in IRF2BP2$^{-/-}$ microglia, fewer M2 phagocytic cells were detected 1 day after infarction. Together, these results are consistent with delayed tissue repair and slower regression of infarction associated with fewer M2 and more M1 microglia recruited to the peri-infarct area.

Conclusions: Loss of IRF2BP2 delays activation of an M2 microglia phenotype and prolongs infarct inflammation, associated with impaired behavioral recovery. IRF2BP2 in microglia is necessary for faster recovery from stroke and exploring its beneficial therapeutic mechanism could be a promising tool during stroke recovery.

Assessing the Biocompatibility of a PCL/PEG/LDI Polyurethane for the Treatment of Spinal Cord Injury.

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Introduction: Many modern studies targeting different aspects of the neuronal inhibitory environment or the molecular pro-regenerative environment have shown positive results demonstrating that axonal regeneration in adult spinal cord is possible. Because many factors greatly influence the neuronal ability to regrow and reconnect, the possibility of manipulating the injured spinal cord environment gives hope to the final goal of creating an efficient treatment that will lead to fully functional recovery after SCI. One method of environment manipulation is the use of biomaterial implanted channel in the injured spinal cord.

Objective: Our objective is to assess biocompatibility of a polyurethane (PU) biomaterial composed of polyethylene glycol, poly-caprolactone and L-lysine diisocyanate (PEG-PCL-LDI) as an implantable channel for the treatment of spinal cord injury.

Methods: Twenty-one female Sprague Dawley rats were divided into biomaterial (channel) group (n=10) and control group (n=11). Their spinal cords were surgically fully transected at T9 and a PU membrane was placed over the transected spinal cord in the animals of the biomaterial group but not in the animals of the control group. Data about general health, functional recovery and histological morphology were collected from all the animals to compare between the groups using student's T-test and risk ratios. Slides of 20 µm parasagittal spinal cord sections were stained with Luxol Fast Blue and haematoxylin and eosin (LFB&HE) to assess histopathological changes. Immunohistochemistry staining will be done for ED-1 (macrophages), NF-200 (neurofilament) and GFAP (astrocytes) to assess biocompatibility.

Results: Our preliminary results show that the general health data (urinary tract infection, autophagy, weight loss and death) and functional recovery (2, 4 and 8 weeks) results was not significantly different between the control and biomaterial groups. Histological (LFB&HE) and macrophages immunostaining (ED1) assessment showed inflammatory tissue around the biomaterial in 2 weeks but not in 4 or 8 weeks after implantation. The NF200 immunostaining showed axons growing from the caudal stump (marked with GFAP immunostaining) of the spinal cord into the transected area in the biomaterial group suggesting sensory neurons regenerating. No NF200 immunostained neurofilament was found in the control group.

Conclusion: Preliminary results show that the biomaterial assessed had no significant statistical difference in general health and functional recovery in the rats. Further analyses of immunohistochemistry staining will help understand whether the proposed PU biomaterial is biocompatible or not.

Investigating the role of Parkin in oxidative stress pathways of recessive Parkinson disease

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Background: Recessively inherited loss-of-function mutations in the Parkin gene are associated with familial cases of early-onset Parkinson disease (PD) (Kitada et al., 1998). Currently, Parkin's function is thought to be an E3 ubiquitin ligase (Shimura et al., 2000), and a regulator of "mitophagy" (Narendra et al., 2010). However, new evidence has surfaced regarding Parkin's protective effects against oxidative stress and mitochondrial dysfunction (Henchcliffe & Beal, 2008).

Hypothesis: Parkin's role is to protect vulnerable neurons against increased oxidative stress, independently of its E3 ligase activity. I hypothesize that it does this in two specific ways: 1) by acting to neutralize reactive oxygen species (ROS) produced by mitochondria, which are critical to providing energy to neurons, and they are particularly vulnerable to ROS damage; and 2) by regulating calcium signaling which is a critical component of healthy mitochondria function (and sensitive to ROS production). My project focuses on an exciting new model of how parkin protects neurons, which will combine genetic mouse models that lack parkin expression, as well as increased oxidative stress paradigms.

Summary: I will investigate Parkin-dependent changes and dissect the molecular pathways that underlie them with the goal to bring closer cause-directed therapeutics for PD.

Comparison of Adult Human and Rat Spinal Cord Neural Stem/Progenitor Cell Proliferation and Differentiation Characteristics

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Background: Spinal cord injury (SCI) can result in permanent paralysis, with no successful treatment for humans. Neural stem and progenitor cells (nspcs) exist within and around the central canal of the mammalian spinal cord, and can be modulated towards more therapeutically beneficial fates to restore synaptic connectivity following SCI, as demonstrated with animal models. However, it remains unclear whether human spinal cord nspcs possess such regenerative potentials and can also be modulated towards therapeutically beneficial fates.

Objectives: To compare and distinguish the intrinsic stem/progenitor behavior of endogenous populations in the spinal cord of adult human and rats, which is one of the most widely studied SCI animal models.
Methods: Using an in vitro neurosphere assay, we cultured primary- and secondary-derived nspcs from human and rat spinal cord and assessed their proliferation and differentiation characteristics. Nspcs were cultured as an adherent mono-layer for 2 or 3 weeks with epidermal growth factor (EGF, 20 ng/ml) and basic fibroblast (bfgf, 20 ng/ml) to assess proliferation. To assess differentiation potential, human and rat adherent nspcs were cultured for 2 or 3 weeks with the following factors: fetal bovine serum (FBS, 1%), retinoic acid (RA, 500 ng/ml), or bone-morphogenic factor-4 (BMP-4, 100 ng/ml). Treated cultures had brdu administered 24 hours prior to fixation, and were then stained against cell specific markers for neural progenitors (β-III tubulin), astrocytes (Glial fibrillary acidic protein), oligodendrocytes (2',3'-Cyclic-nucleotide 3'-phosphodiesterase), stem (Sox2), progenitor (nestin), proliferating (brdu), or apoptotic (Tunnel) cells. Nspcs were then counter stained with Hoechst and quantified, as a % of immune-positive cells, by taking fluorescent images at 20x.

Results: Rat primary-derived nspcs (n=5) demonstrated an increased proliferative index (% $brdu^{+}$), within a 24 hour time frame, after 2 weeks in proliferating conditions. Whereas rat primary-derived nspcs differentiated predominately into astrocytes (8.3% in FBS), human cells are more dedicated towards neural lineages, with only a minor affinity for glial specification. Furthermore, BMP-4 did not induce astrocyte differentiation in either rat ($P>0.05$) or human primary-derived nspcs, but RA treatment increased neural differentiation of rat primary-derived nspcs ($n=4$, $p<0.01$).

Conclusion: Human and rat nspcs differ in their proliferative index and differentiation profiles in response to exogenous factor administration. Improved understanding of these differences will be required to improve the translational potential of this promising therapeutic strategy.
Acknowledgements: Since thanks to Dr. Mahmoud Bedawy[3], Dr. Hussam Jabri[3], Dr. Mohammad Alshardan[3], Dr. Michael Taccone[3], Dr. Carolyn Lai[3], and Dr. Jessica Rabski[3]

Refining the Mouse Photothrombosis Model, a Step towards Investigating Dopamine D1-class Receptors in Stroke Recovery

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Background and Objective: Mice and rats are nocturnal animals, yet many preclinical studies on stroke recovery induce ischemia, perform behavioural testing and deliver therapies during the animal's inactive period. The risk of ischemic stroke is higher in the mornings in humans, corresponding to circadian increases in blood pressure, activity levels and a myriad of other factors. The impact of dark-period stroke induction on animal models of stroke has not been fully examined. The objective of this study is to determine if there are any differences in the outcomes of photothrombotic strokes induced during a mouse's active (dark) period or inactive (light) period, with an aim to better model the majority of human ischemic strokes.

Methods: Our laboratory performed an exploratory preclinical experimental study in conjunction with the University of Ottawa Vivarium and University of Ottawa Behavioural Core. 32 male C57BL/6 mice, ten weeks of age at the time of surgery, were subjected to photothrombosis stroke induction. Prior to photothrombosis mice were housed for a two week acclimation period in either Light-Dark(LD) 12h:12h or reverse LD 12h:12h housing and underwent training and prestroke measurements on 3 motor-behavioural tasks. After stroke, lesions were confirmed with Magnetic Resonance Imaging. Animals underwent testing on the three behavioural tasks at 2-4 days post stroke and 20-22 days post stroke. At study completion brains were collected and infarct size was determined.

Results: Results from our 3 behavioural tests suggest that mice who underwent stroke induction and testing during their dark period tend to have a more sustained motor deficit following stroke, with less spontaneous recovery evident at three weeks post-stroke. In addition dark-period mice's pre-stroke scores tend to show more proficiency on some of our motor tasks, and they tend to learn those tasks faster. Results relating to our ongoing, pre-clinical stroke recovery study, employing dihydrexidine, a dopamine D1-class receptor agonist, will also be discussed.

Conclusions: Our data points to a potential difference in stroke outcome when stroke is induced during the animal's dark period. This result suggests that using reverse light-dark housing conditions can lead to a better rodent model of human stroke.

BAR-1 is involved in properly positioning a subset of motor neurons along the AP axis

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Background: Wnt signalling plays an important role in the positioning of ventral cord motor neurons in C. Elegans. DD motor neurons are gabaergic inhibitory neurons that are evenly spaced in a stereotypical pattern along the anterior-posterior axis of the ventral nervous cord (VNC). Mutations in vang-1/VANG and prkl-1/PRKL, members of the non-canonical Planar Cell Polarity (PCP) pathway, have shown to cause defects in the positioning of DD motor neurons by delaying cell intercalation. We thus observed various members of the canonical Wnt signalling pathway. However, mutations in upstream Wnt signalling genes, such as Wnts, Frizzleds and Dishevelleds, only show a mild positioning defect in which the positions of DD2 have slightly shifted towards DD1.

Methods: Various markers which allow the expression of flourescent proteins to be expressed in the neurons of the worms allows us to easily observe the phenotypes of the mutations at different growth stages.

Results: Our investigations in downstream Wnt genes have shown that BAR-1/β-catenin plays a significant role in the positioning of DD1 and DD2. In mutations of bar-1/β-catenin, both DD1 and DD2 localize at DD1's location, near the base of the pharynx on the ventral cord, in a side-by-side or an occasional staggered arrangement. However, bar-1 does not usually affect the positioning of the other DD neurons, DD3–DD6. PRY-1/Axin is a negative regulator of the Wnt signalling pathway as a part of a destruction complex involved with degrading BAR-1/β-catenin. Interestingly, pry-1/Axin's phenotype shows DD1shifting towards DD2's position as well as mimicking bar-1/β-catenin's DD1 and DD2 touching phenotype.

Conclusions: These findings suggest that canonical Wnt signalling is possibly involved with intercalating DD1 and DD2 while the PCP pathway intercalates DD3–DD6. The positional defects caused by Wnt signalling are potentially due to intercalation defects during VNC formation or mysregulation of mechanisms to tile neurons along the AP axis. We are currently looking at rosette structures and their ability to resolve itself during cell intercalation in various mutant embryos to determine whether they cause delays in cell intercalation. Preliminary observations of cell intercalation in bar-1/β-catenin embryos show a stable rosette structure. However, when the worm elongates into the 1.5-fold stage and the 2-fold stage, DD1 and DD2 remain close together. This evidence suggests the idea that tiling mysregulation might involve cell adhesion defects as well as defects in cell intercalation.

Clinical Prediction of Symptomatic Vasospasm in Aneurysmal Subarachnoid Hemorrhage

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Background: Symptomatic vasospasm, a feared complication in aneurysmal subarachnoid hemorrhage (asah), is neurological deterioration typically associated with abnormal intracranial arterial narrowing. It is the leading cause of preventable mortality and morbidity in asah accounting for 23% of deaths, 11% of poor outcomes, and persistent neurological deficit in 37% of survivors. Timely diagnosis of symptomatic vasospasm can be difficult, as the degree of vessel-caliber reduction does not necessarily correlate with development of neurological symptoms. A tool ready for clinical use to improve diagnostic accuracy of symptomatic vasospasm over thickness of asah, as measured by the Modified Fisher Grade, remains to be developed.

Objective: To derive a clinically-applicable decision rule to predict the development of symptomatic vasospasm in asah.

Methods: Data from 463 asah patients presenting from 2002 to 2011 were analyzed using logistic regression and recursive partitioning to identify clinical, radiological, and laboratory features that predicted symptomatic vasospasm. This was defined by expert-defined clinical criteria for neurological deficit with corresponding vessel narrowing detected by transcranial doppler, CT angiography, MR angiography, or catheter cerebral angriography, after exclusion of competing diagnoses.

Results: Angiographic vasospasm was present in 57.7% of patients with an incidence of symptomatic vasospasm of 21.0%. On multivariate logistic regression analysis, significant predictors of symptomatic vasospasm included age 40-59 years, thick amount of hemorrhage on CT (Modified Fisher Grade 3 and 4), and anterior circulation aneurysms. The resulting scoring system out performed the Modified Fisher Grade alone as demonstrated by the areas under the receiver operator characteristics curves of 0.73 and 0.66 respectively. Recursive partitioning modelling further incorporated poor neurological grade and aneurysm treatment modality achieving a sensitivity of 100% [95% confidence interval (CI): 96.2-100%] and specificity of 9.6% [95% CI: 7.0-13.1%].

Conclusions: Clinical decision rules derived by logistic regression and recursive partitioning can reliably predict the development of symptomatic vasospasm. They exhibit increased accuracy over the Modified Fisher Grade alone and may serve as useful clinical tools to individualize vasospasm risk once prospectively validated in other neurosurgical centres.

A Role for Disco Interacting Protein 2 (dip-2) in Maintaining Neuronal Morphology in Caenorhabditis elegans

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Background: Maintenance of neuronal morphology is a complicated process involving multiple factors, including DNA acetylation, methylation, mitochondrial health and multiple signalling pathways. Many of these factors change in an age dependant manner; though the extent of which they play a part in aging is poorly understood. Recently, the structure of mechanosensory neurons in Caenorhabditis elegans have been observed to change in aged worms, or in mutants of the insulin pathway and adhesion genes. Herein we describe the gene, disco interacting protein 2 (dip-2), which displays morphological changes similar to those in aged worms.

Objective: The objective of the study was to characterize the morphology of dip-2 mutants and examine a possible relationship between dip-2 and age.

Methods: Using C. Elegans as a model of the nervous system, a number of genetic crosses were performed to examine the role of dip-2 in neuronal morphology. Worm populations were synchronised and scored at the appropriate days of adulthood at 25^{o}c.

Results: Severe defects were observed in the mechanosensory neurons of dip-2 mutant worms. These included branching, the forming of waves and beads in the neuron process, as well as the formation of ectopic sprouts from the cell soma. Expression of the ectopic sprouting defects increased from day 1 to day 5 of adulthood in dip-2 null mutants, and significantly differed from wildtype. These defects could be modulated by the insulin signalling pathway, showing suppression in daf-2/insulin receptor and age-1/PI3K backgrounds dependant on daf-16/FOXO. Finally, the defect could be significantly rescued by expression of dip-2 in the mechanosensory neurons.

Conclusions: The results of the study indicated that DIP2 plays a part in maintaining neuronal maintenance in aged worms and possibly interacts with the insulin signalling pathway. The data obtained provided novel insights into a potentially significant protein involved with neuronal structure; and provided the groundwork for future research.

Physiopathology of autism during postnatal development: role of the brain vasculature.

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Autism (or autism spectrum disorders, "ASD") is characterized by impairments in communication, social interactions and motor skills, and is increasingly viewed as a developmental disorder with a genetic origin. Certain mutations have been identified as possible causes for autism, the most common of which is a loss of DNA at a precise location in the genome. These mutations are associated with symptoms such as (but not limited to) delays in the maturation of neurons and altered brain size.
Brain development and function both heavily depend on a steady supply of oxygen and nutrients through the blood stream. As such, key vascular features ensure proper brain maturation: the establishment of vascular networks, the maintenance of brain vessel permeability, and the regulation of blood flow in these vessels. Early life impairments in one or several of these vascular features almost automatically lead to neuro-developmental defects. Despite the wealth of knowledge about the underlying neuronal phenomena in autism, very few studies have investigated the contribution of the brain vasculature to the disease. A recent postmortem study using brains of young autistic patients pointed out an impairment in angiogenesis, a process through which new vessels are formed. In such brains, angiogenesis persisted after an age when it is supposed to decrease dramatically. Other studies suggested a possible link between autism and global reductions in cerebral blood flow that might lead to brain hypoperfusion. But to date, despite this scientific context, involvement of the brain vasculature to the onset and/or progression of autism remains elusive. As such, a detailed analysis of the brain vascular deficits related to autism is needed.
To address this gap of knowledge, we are thoroughly examining the brain vasculature in a genetically engineered mouse model that possesses a mutation specifically linked to autism (mutation known as the "16p11.2 deletion"). We combine a wild range of approaches including physiology, anatomy, imaging, cellular and molecular techniques to decipher the cerebrovascular underpinnings of autism. The research program will provide novel insight into the involvement of brain blood vessels in ASD, and into how these blood vessels control critical features of brain organization and function in brain regions that have been involved in ASD.

A new mechanism underlying Schizophrenia

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Background: Schizophrenia (SZ) is a debilitating mental disorder affecting young adults. Current treatments targeting dopamine remedy psychosis but not other SZ symptoms (early cognitive decline, social withdral, anxiety/depression, impaired pre-pulse inhibition (PPI) of the acoustic startle response). This study seeks to understand the mechanisms underlying SZ. Emerging evidence suggests hypofunction of glutamate receptors (mglur5, NMDAR) and endocannabinoid (ecb) production in SZ-implicated neural circuitry that eventually disrupts homeostasis of the dopaminergic system. Previously, we reported that an LMO4 PTP1B cascade governs mglur5 function and ecb signaling (Neuron, 2015). Given the involvement of mglur5 hypofunciton in SZ pathology, we tested whether LMO4KO mice with mglur5 hypofunction due to elevated PTP1B activity also display SZ-like phenotypes.

Objectives: (1) to determine whether LMO4KO mice display SZ-like phenotypes. (2) to determine the effect of PTP1B activity on synaptic transmission in brain regions implicated in SZ; (3) to test whether blocking PTP1B with genetic ablation or systemic treatment with PTP1B inhibitor ameliorates SZ symptoms.

Methods: Electrophysiological, behavioural and biochemical experiments are performed to achieve the goals.

Results: We found that mice lacking LMO4 with hyperactivity of PTP1B display SZ phenotypes (novelty-induced hyperlocomotion, and cognitive, social & PPI deficits, as well as anxiety). These phenotypes can be improved with pharmacological and genetic inhibition of PTP1B. On-going work is to examine the underlying mechanism of PTP1B's effect in other well-established SZ animal models. The outcome of this study will bring into a new solution of ameliorating SZ symptoms not remedied by current antipsychotics.

Conclusions: 1) LMO4 neuronal knockout mice show robust schizophrenia like behaviors that can be rescued by pharmacological or genetic inhibition of PTP1B. 2) PTP1B inhibitor treatment also reverses hyperlocomotion, and cognitive function impairment in ketamine induced mouse model of Schizophrenia. Our data suggest that PTP1B is a new therapeutic target for schizophrenia.

Lrrk2 Alleles Modify Host Responses to Microbial Infections

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Objective: The mechanisms by which allelic changes in LRRK2 modulate the susceptibility to Parkinson disease, Crohn's disease and leprosy remain unknown. We hypothesized that leucine-rich repeat kinase-2 (LRRK2) plays a key role in the innate immune response of mammals. We sought to test this hypothesis using infectious paradigms in vivo using genotypically modified mice exposed to virulent pathogens.

Background: We and others have found that LRRK2 protein is highly abundant in immune cells and organs (1). Recent studies also suggested that endogenous Lrrk2 modifies inflammation in rodent brain following exposure to bacterial mimics and elevated □synuclein expression. Methods: We employed viral and bacterial infection paradigms using genotypically modified mice and primary macrophages with no endogenous Lrrk2, carrying wild-type Lrrk2, or expressing mutant G2019S knock-in Lrrk2.

Results: First, we inoculated newborn mice with a respiratory-enteric-orphan virus ('reovirus', serotype-3) applied to the nose pad. During the ensuing encephalitis, we detected more reovirus protein in Lrrk2-deficient mice than heterozygous and wild-type (WT) animals. The odds ratio for death from encephalitis in Lrrk2-deficient mice was 3.45 (p=0.002). In a bacterial sepsis model, we inoculated adult animals i.v. With Salmonella typhimurium; there, Lrrk2 deficiency led to more colony forming units in solid organs of adult mice (p=0.05). In contrast, the Parkinson's-linked Lrrk2 G2019S mutant lowered S. Typhimurium burden and reovirus titres in acutely infected organs (including brain) from knock-in mice. Unexpectedly, despite lower viral burden fewer G2019S Lrrk2 mice survived encephalitis. The odds ratio for death from encephalitis in all G2019S Lrrk2 animals was 1.46 and showed a female sex bias (p=0.056). Furthermore, we identified Lrrk2 dependency in STAT1 phosphorylation at Ser727 (pser727), a major signaling component in response to viral and bacterial infections. There, reovirus infected macrophages and brains expressing G2019S Lrrk2 showed significantly higher pser727-positive STAT1 levels versus wild-type controls, whereas Lrrk2 KO cells and brains revealed significantly less pser727-positive STAT1 ($p<0.05$).

Conclusions: We identified a systemic, anti-microbial effect for Lrrk2. Paradoxically, both Lrrk2 deficiency and the Parkinson's-linked G2019S mutant both worsened the outcome of a viral brain infection that was naturally acquired; the former reduced the host's innate immune responses, the G2019S mutation augmented it. We speculate that the risk association of LRRK2 alleles with three human diseases may be in part explained through altered regulation of host immune responses after exposure to virulent microbes. Infectious xenobiotics rather than microbial mimics may better inform us as to LRRK2’s function in vivo.

The role of Parkin in mitigating oxidative stress in Parkinson's disease

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Background: Parkinson's disease (PD) is a progressive neurodegenerative disease that affects >110,000 Canadians and is the second most prevalent neurodegenerative disorder after Alzheimer's disease. Early-onset, recessive PD is associated with >100 distinct mutations in the DJ-1, PINK1 and Parkin encoding genes, which are collectively linked to mitochondrial dysfunction and oxidative stress (OS). To date, the suggested roles for Parkin in E3 ligase activity and mitophagy have not yet been convincingly validated in vivo; however, signs of OS have been reported in unbiased studies of humans and animal models of the disease.

Objective: Containing a large number of cysteine residues, which are susceptible to oxidation, we propose that Parkin is able to protect cells from OS by regulating redox changes in cells and accomplishes this by altering the redox state of its own thiol groups. Therefore, our objective was to determine whether and, if so, how Parkin is able to protect cells from OS.

Methods: We tested Parkin's ability to directly and indirectly mitigate OS (H_2O_2). 1) Direct mitigation: The antioxidant activity of recombinant Parkin was determined using a chemiluminescence assay and its ability to directly reduce intracellular reactive oxygen species (ROS) was measured using dichlorodihydrofluorescein-diacetate (DCFH-DA) in HEK293 cells treated with H_2O_2. 2) Indirect mitigation: Parkin's ability to protect redox homeostasis in HEK293 cells from OS was determined by measuring reduced, oxidized and total glutathione levels before and after treatment with H_2O_2.

Results: 1) In the chemiluminescence assay, we found Parkin to confer stronger antioxidant activity than equimolar amounts of glutathione, bovine serum albumin and DJ-1 (considered by the field to be a PD-linked protein with anti-oxidant activity). Preliminary results from the DCFH-DA experiments indicates that Parkin is able to reduce intracellular ROS following cellular exposure to 2 mm H_2O_2. 2) Overexpression of Parkin inhibits oxidation of glutathione when cells are treated with 1 mm H_2O_2.

Conclusions: Parkin has a similarly potent antioxidant profile in vitro as glutathione. Our cellular experiments suggest that it may also play a role in direct mitigation of OS. These findings, coupled with further investigations in the context of dopamine-linked OS, will help decipher how the loss of Parkin underlies pathogenesis in recessive PD.

Epigenetic regulation of myogenic stem and progenitor cells

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While the mechanisms of the epigenetic regulation of cell type specification and identity are gaining clarity, and genome wide epigenetic profiling reveal the epigenetic trends of the earliest stages of embryonic stem cell differentiation, specific epigenetic events leading to specific lineage and fate decisions remain to be clearly delectated for many tissues and cell types. Here, using a Pax7-creer conditional MLL1 knockout, we find that, in vivo, the H3K4 methyltransferase MLL1 is necessary for maintenance and proliferation of muscle stem and progenitor cells and is necessary for muscle regeneration. Induced knockout of MLL1 in cultured myoblasts derived from conditional MLL1 knockout mice reveal an essential role for effective expression of the myogenic specification gene Pax7. When MLL1 expression is lost in myoblasts, expression of the myogenic regulatory factor Myf5 is lost conditionally with Pax7, however myogenic identity is partly retained as myod expression is maintained, as is the capability for induced myogenic differentiation. Many developmentally important genes are epigenetically regulated via bivalent domains with repressive H3K27 trimethylation and permissive H3K4 trimethylation, which is then resolved during gene activation. Myf5 is not repressed during early development by H3K27 trimethylation but rather by DNA methylation. We have found that DNA methylation at Myf5 is not removed until the transition from satellite stem cells to committed myogenic progenitor satellite cells. The loss of DNA methylation at Myf5 during myogenic commitment seems to be a important stem to progenitor cell demarcation as Myf5 DNA remains unmethylated after its expression is lost during terminal differentiation. Interestingly in MLL1 KO myoblasts, methylation at Myf5 is re-established indicating that MLL1 KO may lead to partial loss of myogenic commitment. Further, continuous ex-vivo expansion in conditions repressing myogenic differentiation also leads to re-establishment of DNA methylation at Myf5 indicating that myoblasts are amenable to ex-vivo reprogramming towards a myogenic stem cell state.

Mesenchymal stromal cells in Bronchopulmonary dysplasia: Systematic review and Meta-analysis of preclinical studies.

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Background: Preclinical evidence suggests that mesenchymal stromal cells (MSC) improve neonatal lung structure, function and inflammation in experimental models of Bronchopulmonary dysplasia (BPD). To date, there has been no systematic review and meta-analysis on the therapeutic potential of MSC in experimental BPD.

Design/Methods: We searched MEDLINE, Embase, Pubmed, Web of Science and conference proceedings for controlled comparative studies of preclinical neonatal animal models that received mscs or cell free MSC-derived conditioned media. Study selection was according to PRISMA guidelines, data analysis by random effects models and 'Risk of bias' by using modified Cochrane Risk of Bias tool for animal studies.

Results: Out of 990 citations, 26 met inclusion criteria. All used neonatal rodents exposed to hyperoxia to model BPD. Seventeen studies reported on the primary outcome, lung alveolarization.
Mscs had a significantly large treatment effect on alveolarization [Standardized mean difference (SMD) of -1.330, 95% Confidence interval (CI) (-1.724, -0.94)], irrespective of timing of treatment and assessment, source, dose or route of administration. Mscs also had a significantly large effect on lung inflammation [TNF-α(SMD-1.26, 95% CI (-1.94,-0.58), TGF-β (SMD -1.54, 95% (-2.54, -0.54), IL-6 (SMD -2.28, 95% CI (-3.54,-1.02) and IL-1β (SMD -3.17, 95% CI (-4.47,-1.87)], pulmonary hypertension [SMD -1.18, 95% CI (-2.09, -0.27)], lung fibrosis [SMD -2.54, 95% CI (-3.95, -1.13)], lung angiogenesis [SMD -1.53, 95% CI (-1.94, -1.16)], lung apoptosis [SMD -6.86, 95% CI (-11.06, -2.67)], and oxidative stress [SMD -5.82, 95% CI (-7.44, -4.2)] and survival [Odd Ratio 0.88, 95% CI (-0.33, 2.37), P<0.001)] Similarly, MSC-derived conditioned media significantly improved alveolarization [SMD of -2.07, 95% CI (-2.74, -1.33)], pulmonary hypertension [SMD -0.96, 95% CI (-1.6,-0.32)], lung angiogenesis [SMD -3.17, 95% CI (-4.72, -1.62)] and pulmonary artery remodelling [SMD -2.16, 95% CI (-3.98, -0.33)]. None of the studies met all the criteria for low risk of bias across 11 domains. In general, we found high heterogeneity and incomplete reporting in the primary studies with potential publication bias for our primary outcome.

Conclusions: MSC therapy in preclinical hyperoxic models of BPD in rodents significantly improved lung injury. Although this may be true in rodents, there is a need to explore this effect in larger animal models/other species. Overall, we noted incomplete reporting in the primary studies suggesting a need to implement reporting standards such as the ARRIVE guidelines. For this reason, we hope that this review would help highlight methodological flaws and bring more rigour in the design of new studies, both experimental and clinical.

Hedgehog signaling regulates satellite cell function.

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Background: The regenerative capacity of skeletal muscle relies on a subpopulation of muscle stem cells, termed satellite stem cells. Tracking the activation of Myf5 with Myf5-Cre:ROSA26-YFP mice revealed that YFP-negative satellite stem cells can perform symmetric cell divisions, which give rise to two identical daughter cells to self-renew and expand the satellite cell pool, or asymmetric divisions, which generate one self-renewing stem cell and one YFP-positive committed cell that express Myf5 and will progress through the myogenic lineage. However, the behavior and the myogenic fate of the YFP-negative satellite stem cell population are still unclear.

Objective: We propose to investigate the differentiation potential of satellite stem cells. Methods: Myf5-Cre:ROSA-YFP mice are used to sort both YFP-negative and YFP-positive satellite cells and compare their molecular profiles and phenotypes during proliferation and differentiation in vitro. EDL myofibers are also isolated to analyze satellite cell divisions and differentiation.

Results: By immunostaining on cultured myofibers, we discovered that satellite stem cells can progress through the myogenic lineage without expressing Myf5 but other myogenic markers of differentiation, such as myod and myogenin. Moreover, we showed that freshly sorted YFP-negative cells are able to differentiate in vitro. Cellular and molecular characterization of the differentiation potential will be presented. Our preliminary data support the hypothesis that multiple myogenic lineages coexist. Expression analysis by RNA-sequencing revealed that Sonic hedgehog (Shh) signaling genes are differentially expressed in YFP-negative stem cells and YFP-positive committed cells. Shh is a key regulator of myogenesis since the signaling pathway triggered by Shh leads to the activation of Gli transcription factors and expression of Myf5. We showed that activation of Shh signaling enhances asymmetric satellite cell divisions while its inhibition leads to satellite stem cell expansion.

Conclusion: Therefore, we proposed that Shh signaling pathway could regulate the satellite stem cell fate decision to give rise to two distinct types of committed cells.

Mitochondrial energetics and quality control as determinants of mesenchymal stromal cell regenerative activity

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Background: Increased interest on the role of mitochondria in determining stem cell regenerative potential has been triggered by the recognition of novel roles for mitochondria in intra- and intercellular signalling. Mitochondria play a particularly relevant role in mediating the broad protective effects observed following mesenchymal stromal cells (mscs) transplant. Recent work demonstrated direct transfer of functional mitochondria between mscs and target cells is essential in the rescue or improvement of host cell mitochondrial function (bioenergetics) and overall cellular function. Eukaryotic cells have developed dynamic mechanisms of organelle quality control (QC), which for mitochondria is assured by the coordinated action of biogenesis, fusion/fission, autophagy and mitophagy. Failure of cells, in particular mscs, to maintain competent mitochondria can result in accumulation of dysfunctional mitochondria, compromising their ability to promote tissue repair. Thus, we hypothesized that mitochondrial QC and bioenergetics are targets for the enhancement of MSC reparative potential leading to improved cell therapy benefits.

Methods: Bone marrow-derived human mscs (hmscs) were obtained from the Ottawa Hospital Cell Manufacturing Facility and characterized according to the ISCT for surface markers and trilineage differentiation. Hmscs were culture and expanded in Nutristem MSC XF complete growth media (21% O_2), cryopreserved in passage 3 and used for all experiments, after thawing, in passage 5. Mitochondrial networks within hmscs were imaged using epifluorescence microscopy after either immunolabeling of mitochondrial proteins (pyruvate dehydrogenase, PDH, and transporter of the outer membrane 20, TOM20) or transduction of hmscs with PDH-GFP or PDH-RFP.

Results: Preliminary observations of live mscs in culture by phase contrast microscopy revealed the existence of membrane projections forming connections between cells. Upon cell fixation and mitochondrial labelling we were able to determine that these projections form microtubes (1-2 µm in diameter) between two cells and contain mitochondria. Live imaging indicates that these nanotubes are transient in nature, existing for less than 60 min.

Conclusions: On-going experiments aim to confirm these preliminary observations and characterize mitochondria respiration and glycolytic flow using Seahorse XF technology, in conjunction with membrane potential measurements using TMRE. Live cell fluorescent microscopy will be used to determine the directionality of mitochondria flow between cells and the dynamics of tube formation. Next, we will evaluate the impact of inhibition of mitochondrial QC (mitophagy and fission) on MSC biology and repair potential. Finally, we will assess whether approaches to promote transfer of mitochondria from mscs to host cells will improve MSC biological function and repair potential.

Modifier mutations associated with hyper-responsiveness to VEGFR2 inhibition resulting in severe pulmonary arterial hypertension in a sub-strain of Sprague-Dawley rats

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Introduction: Our lab has identified a unique sub-strain of SD rats that is hyper-responsive (HR) to SU5416 (SU) alone and develops severe progressive PAH in response to single injection of SU, even in absence of chronic hypoxia (CH).

Hypothesis: We hypothesized that the HR phenotype in response to SU is conferred by genetic determinants that potentiate the response to VEFR2 blockade.

Methods and Results: Male and female rats were injected with SU (20mg/kg, sc) or vehicle (control). Right ventricular systolic pressure (RVSP) was measured at 7 weeks after SU injection. In absence of CH, 72% (13 of 18) male SD rats demonstrated HR-phenotype and developed severe PAH in response to SU with mean RVSP of 97±18 mmhg; whereas only 27% (7 of 26) of the female rats showed HR-phenotype. Furthermore, crossing non-responsive male and female rats from the HR colony markedly decreased the proportion of animals exhibited the HR phenotype in the F1-generation (HR 15% and 0%, male vs. Female, respectively), consistent with the presence of modifier genes. Using whole genome-wide exome sequencing approach, we identified a number of mutations unique to the HR SD colony. Bioinformatics analysis of the data identified several candidate genes exhibiting mutations tightly associated with the HR phenotype, including the hypoxia inducible factor 1α (HIF1α), SP110, cingulin-1 (CGNL1) and pituitary tumor-transforming gene 1 (PTTG1). Interestingly, an INDEL mutation in HIF1α was found in all SD rats (80% homozygous and 20% heterozygous), both in responders and nonresponders. However, mutations in PTTG1 and CGNL1 genes were present only in responder rats but absent in the non-responder rats from the HR colony. In addition, HR rats demonstrated exacerbated hemodynamic response to CH as compared to the non-HR SD rats (RVSP: 72±5 mmhg vs. 49±4 mmhg) consistent with increased hypoxia signaling and a gain-of-function HIF1α mutation. We also observed significantly higher HIF1α and BNIP3 (a downstream target of HIF1α) expression in the lungs of HR SD rats.

Conclusion: A previously unrecognized mutation in HIF1α in SD rats is associated with increased susceptibility to severe PAH, which requires the presence of mutations in additional unique modifier genes. These findings may provide insight into possible genetic determinants influencing the penetrance of the PAH phenotype in humans harboring disease-causing mutations.

Funding: Canadian Vascular Network, Heart and Stroke Foundation of Canada, Training Program in Regenerative Medicine and Canadian Institutes of Health Research, Entelligence Young Investigator Award.

Identification of Potential Targets of Satellite Stem Cell Division using a Novel In-niche High Content Analysis Platform

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Background : Skeletal muscle satellite stem cells in adult muscle facilitate postnatal growth and regeneration. Satellite cells (scs) express Pax7 and heterogeneously express Myf5: Myf5- are considered satellite stem cells, whereas Myf5+ are committed progenitors. During regeneration, scs may reenter the cell cycle from quiescence to undergo both asymmetric and symmetric division, giving rise to stem cells (self-renewal), and myoblasts (myogenic progenitor cells). In the context of Duchenne Muscular Dystrophy (DMD), deficiencies in stem cell divisions due to a loss in cell polarity result in hyperplasia of the SC population and increased mitotic errors that lead to decreased regeneration potential. Similarly, transplantation experiments of aged muscle stem cells demonstrate a decline in stem cell self-renewal and reduced capability for muscle regeneration. Evidently, SC function is critical for muscle repair and maintenance. However, the molecular mechanisms required for self-renewal and maintenance of the stem cell pool are not yet well characterized. There are likely additional cell polarity pathways that play a role in regulating asymmetric division and symmetric division, and these pathways may be perturbed in mdx mice. We hypothesize that the restoration of SC asymmetric division and cell polarity pathways will restore muscle regeneration capacity in mdx mice.

Objective: 1) Develop a high content analysis platform to screen well-characterized small molecule libraries, which will be used as tractable markers for SC division 2) Identify and characterize novel pathways and mechanisms that control stem cell fate 3) Establish a potential therapeutic target for muscle regeneration in DMD

Methods: Our screening platform combines traditional fiber culture on FDB fibers with bioimaging and bioinformatics, using the ratio of SC divisions as a read-out. Libraries of well-characterized small molecule compounds will be screened to identify potential targets that affect SC division. These targets will be analyzed with in-vitro techniques such as sirna knockdowns, proximity ligation assays and chromatin immunoprecipitation assays to determine molecular interactions. The effects of identified targets will then be validated by in-vivo regeneration studies such as cardiotoxin regeneration, fiber transplantation and force generation experiments.

Results: Our screening platform identified two targets, KDR and pdgfrb, to affect stem cell division. Inhibitors of KDR and pdgfrb induced a >1.5 fold increase in symmetric division. RNA-seq and microarray data indicate that both KDR and pdgfrb are differentially expressed in quiescent and activated scs. KDR and pdgfrb expression is also downregulated in mdx scs.

Conclusions: KDR and pdgfrb may play a role in SC division, and these pathways may be perturbed in the DMD context.

Mechanisms Underlying Oligodendrocyte Inhibition in the Multiple Sclerosis Lesion

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Background: In multiple sclerosis, oligodendrocyte precursor cells (OPCs) migrate to lesion sites to repair myelin. Throughout disease progression, the ability to repair such damage diminishes considerably. This reduced regenerative capacity is thought to be a consequence of lesion-associated inhibitory factors, including chondroitin sulfate proteoglycans (CSPGs), which perturb OPC maturation into myelinating oligodendrocytes.

Objectives: The current study aims to characterize the oligodendrocyte response to CSPG exposure, as well as explore the molecular pathways involved in CSPG-mediated inhibition of oligodendrocyte differentiation.

Methods: We investigated potential signaling proteins and receptors that have been previously implicated in CSPG-mediated inhibition of neuronal morphology to determine if the same pathways are involved in the CSPG- oligodendrocyte interaction. A primary oligodendrocyte cell culture system was used for all experiments. Characterization of morphological and molecular differentiation was performed following manipulation of the receptors and signaling pathways of interest.

Results: We have validated the impact of CSPGs on oligodendrocyte maturation in our system, such that exposure to CSPGs dramatically impedes morphological complexity of the oligodendrocyte, while molecular maturation remains unaltered. Interestingly, CSPG-mediated inhibition of neuron development depends greatly on both GSK-3ß and RhoA activity. Here we show that GSK-3ß signaling is likely not crucial in mediating the effects of CSPG exposure on oligodendrocyte morphology. Inhibition of GSK-3ß signaling does not rectify the morphology of primary oligodendrocytes on CSPGs, nor does GSK-3ß phosphorylation status differ upon CSPG exposure. Contrastingly, pharmacological inhibition of Rho kinase improved some of the morphological perturbations of oligodendrocyte differentiation in the presence of CSPGs. Ongoing studies aim to identify the receptors activated in the oligodendrocyte to mediate CSPG signaling.

Conclusion: This study reveals specific targets involved in CSPG-mediated inhibition of oligodendrocyte growth (RhoA).It also highlights previously unappreciated important differences between oligodendrocyte and neuronal responses to the same inhibitory cue (GSK-3ß). Further investigation of these signaling mechanisms will be necessary to provide a better understanding of the lesion microenvironment contribution to pathophysiology in multiple sclerosis.

Expression of p14 Fusion Associated Small Transmembrane Protein in an Oncolytic Adenovirus for Improved Vector Efficiency

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Background: Adenovirus (Ad) vectors are the most commonly used delivery system in gene therapy applications. The ability of Ad to efficiently infect many different cell types with a relatively large cloning capacity, without integration into the host genome makes them an excellent candidate for anti-cancer gene therapies. One of the main drawbacks of Ad viral vectors is their relatively poor ability to spread through a tumor mass following intratumoral injection. A particular class of fusion-associated small transmembrane (FAST) proteins have been investigated for their potential anti-cancer properties. Conditionally replicative Ad viral vectors (crad) contain mutations to facilitate replication in cancer cells or block replication in non-cancer cells. Over-expression of FAST proteins in a conditionally replicative Ad viral vector system (cradfast) could increase virus spread in the tumor through cell-cell fusion, as well as amplify virus oncolytic activity.

Objective:
1-Produce therapeutic amounts of Ad virus expressing the FAST protein
2-Examine the potential for FAST-expressing Ad as an oncolytic agent in tissue culture models of cancer
3-Conduct in-vivo experiments with cradfast intratumoral injections in xenograft and syngeneic mouse models of cancer

Methods: Protein expression was examined to validate the cradfast construct. Following this, cradfast was serially cultured to increase yield. Cell culture assays were performed to characterize the relative ability of cradfast to kill tumor cells vs crad. Finally, CD1-nude mice bearing subcutaneous A549 tumors were treated with either intratumoral cradfast, crad, or PBS.

Results: Previous work with replication defective Ad expressing FAST protein showed promise in cell culture models of cancer, but was unable to translate to efficient tumor suppression when applied to a mouse model. We are currently exploring a cancer targeting replication competent oncolytic adenovirus expressing the FAST protein. In-vitro characterization has shown promise in initiation of cell death in A549 and 4T1 models of human and mouse cancer. Finally, 20 days post-intratumoral injection in xenograft model, both cradfast and crad injected tumors are significantly smaller than PBS treated tumors, while cradfast tumors had smaller volumes on average than crad injected tumors, in-vivo trials are ongoing.

Conclusions: Our conditionally replicating Ad expressing the fusion inducing FAST protein (cradfast) kills cancer cells at lower MOI and with higher efficiency than control viruses in-vitro. In-vivo, it appears to have halted tumor growth in our mouse xenograft model of cancer at day 20 post injection. Monitoring of this trial continues, and syngeneic mouse trials are to begin shortly.

Molecular regulation of satellite cell fate switching

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Background: The prevalence of obesity and its related disorders presents a growing obstacle for the medical community worldwide. Where white adipose is the main reservoir for excess energy, brown adipose has the ability to convert excess energy to heat, and in turn poses a promising therapy for obesity. Brown adipose and muscle are derived from a common progenitor and adult muscle stem cells (satellite cells) remain permissive to brown adipogenic signaling. A key switch involved in the myogenic commitment of satellite cells involves the muscle enriched microrna-133 (mir-133), that blocks brown adipogenic signaling. Antagonizing mir-133 during muscle regeneration leads to de novo brown adipocyte generation, promotes energy expenditure and impedes diet-induced obesity. Screening regulators of mir-133 uncovered the tumour suppressor p53 as a potential regulator of mir-133.

Objective: To study how p53 affects fate choices in satellite cells and identify pathways involved in promoting brown adipogenesis.

Results: Using a luciferase based assay to screen small molecule inhibitors of mir-133, we uncovered the involvement of p53 in lineage reinforcement within satellite cells. We found Pifithrin-a (a p53 transactivation inhibitor) to be a potent inhibitor of mir-133 expression in mouse and human myoblasts and to markedly stimulate brown adipose determination in C2C12 myoblasts and satellite cells. We characterized the effects of satellite cell-specific p53 genetic depletion on induction of brown adipocytes where we discovered satellite cells lacking p53 result in precocious brown adipose formation within regenerating skeletal muscles. Transient inhibition of p53 in regenerating fibers through Pifithrin-a likewise results in brown adipose formation as well as an increase in mitochondrial biogenesis. Mechanistically, we uncovered that p53 inhibition leads to a deficit in mir-133 processing suggesting that p53 promotes myogenesis in part through promoting microrna processing. These results suggest cyclic Pifithrin-a and other transient p53 inhibitors may hold potential as anti-obesity compounds.

Conclusion: The p53 axis poses a novel pathway regulating satellite cell fate choices through microrna processing where pharmacological inhibition may pose a potential as a treatment for obesity.

Role of exosomes in wnt7a secretion: a potential therapeutic approach for wnt7a systemic treatment in duchenne muscular dystrophy

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Background: Duchenne Muscular Dystrophy (DMD) is a devastating genetic muscular disorder of childhood manifested by progressive debilitating muscle wasting and ultimately death around the second decade of life. To date, no successful therapies exist to correct the etiology and development of DMD. Our laboratory has established that Wnt7a/Fzd7 signaling represents an important intrinsic mechanism for stimulating regeneration of skeletal muscle. Injection of Wnt7a protein into the muscle of mdx mice, a mouse model of DMD, results in increased numbers of muscle stem cells, larger myofiber caliber, together with restoration of force generation to normal levels. These studies provide a solid rationale to further develop Wnt7a as a treatment for DMD. However a significant limitation of Wnt7a, given its highly hydrophobic nature, is that it cannot be delivered systemically via the circulation to treat all muscles.

Objective: Our goal is to design a carrier that will successfully allow Wnt7a to be systemically delivered and will consequently be a more efficient method of treatment. We hypothesize that introducing Wnt7a into endogenous carriers along with muscle stem cells-specific targeting peptides, will allow specific delivery of Wnt7a in the extracellular space directly to muscle stem cells.

Methods: We will use exosomes as a carrier for Wnt7a. Exosomes are membrane nanovesicles of endocytic origin that are secreted to the extracellular microenvironment from diverse cells types to target distal cells. Exosomes contain proteins, mirna or lipid contents from their cell of origin, making them ideal carriers. To address this question we have transfected cells from human origin with different plasmids containing full length or truncated versions of Wnt7a. We subsequently isolated the exosomes from the conditional media and analyzed them for presence of Wnt7a.

Results: We have found that Wnt7a is released through exosomes from human cells after DNA transfection. However, exosomal secretion of Wnt7a is completely impaired upon deletion of the N-terminal region of Wnt7a. Results have also shown that these exosomes of human origin can be taken up by murine primary myoblasts.

Conclusions: Our data thus far supports our hypothesis, demonstrating that it is possible to deliver Wnt7a trough exosomes. Moreover, exosomes of human origin can be taken up by cells of different species, opening new avenues on the field of xenotransplants and regenerative medicine towards an effective treatment for DMD.

WN1316, a Novel Anti-oxidant Compound for the Treatment of Leber's Hereditary Optic Neuropathy

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Background: Leber's Hereditary Optic Neuropathy (LHON) is a mitochondrial genetic neurodegenerative disorder causing irreversible blindness in the early decades of life and for which there is no cure to date. Oxidative stress is a main pathway in LHON pathology resulting in specific loss of retinal ganglion cells (rgcs) and their axons. WN1316 is a novel small molecular compound with potent anti-oxidant and neuroprotective properties.

Objectives: We will test the efficacy of WN1316 as a treatment for LHON in an in vivo mouse model of the disease.

Methods: A mouse model of LHON was generated by intravitreal injections of adeno-associated virus expressing the mutant form of the human mitochondrial ND4 (mnd4) gene. Mice were treated daily for 5 months with 100µg/kg WN1316 by oral gavage. RGC function was assessed by electroretinography using the Scotopic Threshold Response (STR) test, followed by funduscopy every two months. Morphological analysis of optic nerves was performed using electron microscopy (EM) and toluidine blue staining. Optic nerve cross-sectional areas and axon counts were determined using Image J software.

Results: Administration of WN1316 was well tolerated by test mice and did not cause any overt signs of toxicity or lethality. STR analysis suggested a trend towards improvement in RGC function upon treatment with WN1316. Analysis of optic nerve sections did not show any differences from controls with respect to optic nerve diameter, area or axon counts. However, EM analysis revealed a delay in disease progression as indicated by decreased apoptosis, reduced nuclear fragmentation and better preservation of cellular components in the WN1316 group compared with controls.

Conclusion: WN1316 appears to be a safe therapeutic agent in mice, and potentially delays disease progression in our in vivo mouse model of LHON. These initial studies suggest that WN1316 may be effective in the treatment of neurodegeneration, but it is important to perform long-term studies to more accurately assess its efficacy as a therapeutic option for LHON.

Translationally Controlled Tumor Protein: A Novel Therapeutic Target in Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a complicated disease for which the fundamental molecular mechanisms are only partially understood. Existing therapies which chiefly focus on vasomotor changes, have limited therapeutic effects. There is increasing interest in the association between the increased pulmonary vascular resistanceand complex lung arterial remodelling which result in occlusive "plexiform" lesions, a hallmark of this disease. Recent studies have shown that endothelial cell (EC) apoptosis may be a central trigger for PAH, resulting in the emergence of growth-dysregulated and apoptosis-resistant ecs that contribute to the formation of complex neoplastic-like vascular lesions. However, the mechanism which links EC apoptosis to dysregulated growth is not yet known. Previous studies in our lab have identified increased expression of a protein previously implicated in transformation of neoplastic cells in cancer, translationally controlled tumour protein (TCTP/TPT1), in blood outgrowth ecs from patients with PAH. Moreover, TCTP expression was found to be elevated in the lungs of patients with PAH, tightly localized to complex arterial lesions, and was detected in obliterative intimal lesions of an experimental rat model of severe PAH induced by treated with a VEGFR2 antagonist, SU5416.As well, TCTP has recently been described to be released in exosomes from apoptosing ecs, and mediate pro-survival signalling in adjacent vascular cells. Therefore, we hypothesize that TCT represents a molecular link between EC apoptosis and the subsequent emergence of growth dysregulated cells, contributing to the development of plexiform lesions in PAH. To test thishypothesis, the effect of TCTP overexpression on EC growth and survival will be studied both in vitro and in vivo. We anticipate that forced overexpression of TCTP will induce a neoplastic-like transformation in ecs in vitro and that these growth-dysregulated ecs will induce the formation of complex lung arterial lesions in vivo, leading to obliteration of arterioles and ultimately PAH.

Driving Human Induced Pluripotent Stem Cells Towards a Lung Cell Fate

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Background: Lung transplantation is often used to treat severe respiratory disorders. There is an increasing prevalence of these diseases worldwide, and shortages in donor-compatible lungs coupled with poor graft survival highlight an urgent demand for organ replacement therapies. Induced pluripotent stem cells (hips) are capable of multi-lineage differentiation with the advantage of circumventing immune rejection as they may be derived from the patients themselves. Driving hips to produce specific cells for tissue repair heralds an exciting forefront in regenerative medicine.

Objective: This study generated de novo lung progenitors from hips that further differentiated to produce vital components of mature lung epithelium. Methods: Activin-A and bone morphogenetic protein-4 (BMP-4) induced definitive endoderm (DE) formation prior to patterning of the anterior foregut endoderm (AFE) with fibroblast growth factor-2 (FGF-2) and sonic hedgehog (SHH). Epithelial morphogens (FGF-7, FGF-10, FGF-18 and BMP-4) specified lung progenitor differentiation and expansion of immature lung cells (ilcs). Epithelium polarization and maturation followed 4-weeks of culture at the air-liquid interface. Morphological changes and gene expression profiles (qpcr) were evaluated throughout major differentiation stages (n=4/stage).

Results: The transition from a compact and well-defined hips colony to a growing uniform monolayer of cells displaying tight cell-cell contact post-DE and AFE differentiation was observed. Pluripotency genes (OCT4, SOX2, NANOG) were downregulated by ≥40% ($^{\#}p<0.01$); and positive endodermal markers (CXCR4, SOX17, FOXA2) concomitantly upregulated by ≥4.5-fold ($^{\#}p<0.01$) relative to hips. Lung endoderm fate was defined by NKX2.1 and increased SOX9 expression ($^{*}p<0.05$), and accompanied by decreased expression of thyroid (TG) and pharyngeal endoderm (FOXG1) genes. Compared to ilcs, epithelial-associated markers: CFTR (chloride channel), SOX2 (regulate branching morphogenesis and airway epithelium differentiation), FOXJ1 (ciliogenesis) were significantly augmented by ≥1.7-fold ($^{\#}p<0.01$) in mature epithelium; with mucin (MUC5AC) and surfactant protein (SFTPC) expressed only in the latter.

Conclusions: These results collectively demonstrated efficient generation of functional lung progenitors from hips that yielded integral elements of mature lung epithelium downstream. Novel regeneration platforms that integrate hips-derived lung progenitors or their more differentiated progenies with revascularized lung matrices may offer a promising therapeutic strategy.

Progressive multiple sclerosis - the role of microrna mir-145-5p in remyelination failure

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Background: Progressive multiple sclerosis (pms) is a debilitating disease in which demyelinated lesions form in the central nervous system (CNS). Normally, demyelination leads to recruitment of oligodendrocyte progenitor cells (opcs), which differentiate into mature oligodendrocytes (ols) that regenerate lost myelin. However in pms, recruited opcs fail to differentiate and remyelinate, ultimately leading to neurodegeneration. One characteristic of pms lesions is abnormally high expression of microrna mir-145-5p. In opcs, mir-145-5p is also expressed at high levels but is strongly downregulated as they begin to differentiate. Importantly, mir-145-5p is predicted to target myelin gene regulatory factor (MYRF), a transcription factor critical for OL differentiation and myelination through its activation of myelin proteins such as MAG, PLP1 and MBP. The downregulation of mir-145-5p likely plays a key role as opcs transition to ols, and thus its high levels may be a factor in the OL differentiation block observed in pms lesions.

Objective: We aimed to determine if mir-145-5p does directly target MYRF and how altering normal expression of mir-145-5p affects OL maturation, in order to better understand how its upregulation may contribute to the inhibitory microenvironment of the pms lesion.

Methods: Targeting of MYRF by mir-145-5p was assessed by dual luciferase assay in HEK 293T cells. Lentiviral vectors were used to overexpress mir-145-5p in both immortalized and primary ols, and to knock-down mir-145-5p in primary ols. Transduced cells were assessed for both morphological and molecular changes by immunofluorescence. Molecular changes were further assessed by qrt-PCR and Western blot.

Results: MYRF is directly targeted by mir-145-5p at two distinct binding sites. Differentiating ols overexpressing mir-145-5p show significant defects in branching and myelin protein expression, including MYRF and its downstream targets MAG, PLP1 and MBP. Conversely, mir-145-5p knock-down ols display enhanced differentiation evidenced by increased expression of MYRF, MAG, PLP1 and MBP, and greater myelin membrane area.

Conclusions: Taken together, these data suggest that mir-145-5p is able to regulate OL differentiation through direct targeting of MYRF, leading subsequently to changes in expression of MYRF downstream targets. Overexpression of mir-145-5p, as is observed in pms lesions, severely stunts OL differentiation, while knock-down of mir-145-5p enhances differentiation. This research suggests that the overabundance of mir-145-5p in the lesion microenvironment may be a factor in the OL differentiation block observed in pms, and that targeting mir-145-5p might serve as a therapeutic strategy to help overcome this block and promote remyelination.

The Snf2h Chromatin Remodeling Protein is Essential for Retinal Structure and Function

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Background: Chromatin remodeling proteins are critical for cellular integrity and function in the CNS, including the maintenance and activity of retinal neurons. Snf2h is an ATP-dependent nucleosome remodeler that functions within multi-protein complexes involved in the regulation of many nuclear processes. We have previously shown that Snf2h is essential in the brain for neurodevelopment, behaviour, and survival.

Objective: Here we describe the generation and characterization of conditional knockout mice targeting Snf2h deletion in the retina to deduce the role of this protein in the visual system.
Methods: The Chx10-GFP/Cre-IRES-AP mouse driver line was crossed with Snf2h$^{+/-}$ and floxed Snf2h transgenic lines to generate retina-specific Snf2h conditional knockout mice. Adult mice were analyzed for retinal function by electroretinography followed by funduscopy and post-mortem analysis of retinal histology. Immunohistochemistry was performed at embryonic and early postnatal stages to determine the impact of Snf2h loss on retinal development. Cell proliferation was assessed by brdu incorporation and Ki67 immunoreactivity. TUNEL assays and activated caspase-3 immunoreactivity were used to examine cell death in the mutant and control retinas. The impact of a voluntary exercise regime on retinal function in the mutants and control mice was tested by placing a running wheel in the cage for several weeks, followed by electroretinography.

Results: The loss of Snf2h in the retina causes a dramatic impairment in visual signal transduction, virtually eliminating the scotopic a-wave, b-wave, ON/OFF responses, and oscillatory potentials. This functional deficit is accompanied by a degenerative phenotype, however much of the retina remains intact. The cell loss does not appear to be due to a retinal progenitor proliferation defect but as a consequence of cell death beginning at developmental stages. The introduction of a running wheel in the cages of conditional knockout mice leads to some restoration of electrophysiological function, suggesting that the severe visual impairments are not permanent but treatable.

Conclusions: Snf2h is an essential protein for the proper development, integrity and function of the retina. Nevertheless, its deficiency can be at least partially overcome by a physiological response to voluntary physical exercise. Characterization of the retinal molecular pathways in which Snf2h participates, and those demonstrating plasticity in its absence, may reveal novel targets for therapeutic intervention of retinal dysfunction.

Investigating the Role of TLQP-21 Neuropeptide in Myelination

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Background: Previously we demonstrated that in an animal model of cerebellar ataxia, exercise enhanced VGF (no acronym) expression, increased myelination and prolonged survival. Importantly, VGF adenovirus could mediate this effect without exercise suggesting that it might be a critical factor regulating myelination. VGF transcripts are abundant in undifferentiated oligodendrocyte precursor cells (opcs) and found within brain lesions of MS patients suggesting that VGF peptides represent a potential therapeutic target to promote remyelination in MS patients.

Objective: The objective of this study was to determine if opcs and oligodendrocytes (ols) express complement C3a receptor 1 (c3ar), the VGF receptor and whether they are responsive to VGF neuropeptides (TLQP-21).

Methodology: Primary opcs were isolated from the cortex of P0 wild-type C57BL/6 mice and allowed to differentiate into ols over 6 days in vitro (DIV). To test the responsiveness to VGF, TLQP-21 neuropeptide was included in the culture medium. OL differentiation was assessed using stage-specific markers (myelin-associated glycoprotein (MAG) for mature ols, and myelin basic protein (MBP) for myelin producing ols) and analyzed by immunohistochemistry, quantitative PCR, and western blot for differences.

Results: We demonstrate that maturing ols express c3ar suggesting that they are able to respond to VGF. Cultures containing TLQP-21 extend their processes and express MAG earlier, 1-2 DIV. By 3DIV, the addition of TLQP-21 significantly increased the number of myelin producing ols ($p<0.05$, 30.66%±10.59). However, after 4DIV the treated and control cultures showed no significant difference in membrane area or MBP expression by western blot.

Conclusion: Preliminary results suggest that TLQP-21 neuropeptide treatment may accelerate the differentiation process of ols. Further studies will aim to confirm these results and define the pathways involved.

Remote Organ Injury: Neural Progenitor Cell Function is Impaired in a Novel Triple Hit Model of Neonatal Chronic Lung Disease

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Background: The most common complication of prematurity is bronchopulmonary dysplasia (BPD), a chronic lung disease due to ventilator and oxygen (O_2) therapy for acute respiratory failure. BPD is characterized by an arrest in lung growth and commonly associated with antenatal and postnatal inflammation. BPD is an independent risk factor for adverse neurodevelopmental outcomes. The mechanisms explaining these outcomes remain unclear. Neural progenitor cells (npcs)—cells crucial for brain development, residing adjacent to damaged neonatal brain regions—have yet to be examined in BPD.

Hypothesis: NPC function is impaired in a BPD mouse model.

Objective: Characterize NPC functional damage in a BPD model to explain the link between BPD and adverse neurodevelopment.

Methods: C57 Black 6 mouse pups were exposed to 4 conditions: 1) room air + saline (control group); 2) room air + lipopolysaccharide (LPS, an inflammatory stimulus); 3) 85% O_2 + saline (to mimic postnatal oxygen exposure); 4) 85% O_2 + LPS (severe triple hit model of BPD, i.e., hyperoxia + ante- and postnatal inflammation). Saline or LPS was injected intraperitoneally at embryonic day 17 and postnatal (P) day 5. Pups were exposed to oxygen conditions from P0-P14. Lungs and brains were harvested at P14. To assess lung damage and confirm a BPD-like phenotype, the mean linear intercept (MLI) of lung sections was measured. Npcs were isolated from the subventricular zone. NPC self-renewal was examined using primary and secondary neurosphere assays. Spontaneous differentiation was investigated through immunocytochemistry.

Results: The MLI scores of both the hyperoxia-exposed and hyperoxia + LPS-exposed (triple hit) mice were significantly larger than those of control mice. Further, npcs isolated from 85% O_2 + saline-exposed mice formed significantly fewer primary neurospheres than those isolated from the room air + saline-exposed group. The number of secondary neurospheres formed by npcs isolated from groups 2-4, was significantly lower compared to the control group.

Conclusions: The considerably larger MLI scores of the 85% O_2 + saline and 85% O_2 + LPS-exposed mice compared to control mice, indicates a dramatic simplification of the alveoli, and a robust BPD-like phenotype. Reduction in secondary neurosphere formation of npcs isolated from groups 2-4, signifies decreased self-renewal ability of npcs in a BPD mouse model, and ultimately, impairment in their functional ability. These results provide insight into possible mechanisms of BPD-associated brain damage. This study will help to develop future treatments to improve and save the lives of preterm infants.

Identification of Novel proteins that interact with the Adenovirus genome

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Background and Objective: Adenovirus (Ad) is a common human pathogen that typically causes minor illness, but can lead to severe disease in certain at-risk populations such as pediatric or geriatric patients. Within the infecting virion, Ad DNA is tightly condensed by the virus-encoded protein VII (pvii), and this protein must be removed and replaced by cellular histones before efficient expression of virus-encoded genes can occur. Indeed, in the nucleus of the infected cells, Ad adopts a nucleoprotein structure similar to host cell genomic DNA, although the mechanism that drives this assembly of viral DNA into chromatin is not well understood. Our objective is to identify cellular proteins that interact with pvii and facilitate this transition in Ad chromatin structure.

Methods: A recombinant Ad containing a Strep-tag on pvii was used to infect HEK293 cells. Eighteen hours later, pvii and its associated proteins were purified using a one-step Streptactin magnetic system. SDS-Page, Western analysis and staining methods were utilized to visualize these proteins. Mass spectrophotometry (MS) was used to identify proteins, and Scaffold4/Uniprot bioinformatics analysis was used to characterize their normal function in the cell.

Results: From the MS results, 22 proteins were found to interact with pvii. Of these proteins, SET (TAF1B) has been shown previously to interact with pvii during the early phase of infection, validating our approach. The interaction of pvii with SET was confirmed by standard co-immunoprecipitation analysis. Another protein, protein arginine methyltransferase 1 (PRMT1) is known to interact with other viral proteins during the late phase of infection but has not been described as an interacting partner with pvii. Three other proteins that were identified in our assay, 14-3-3 protein epsilon, 40S ribosomal protein SA and PCNA, could also play a role on pvii regulation. Further analysis would be needed to determine the relationship between pvii and these binding partners

Conclusion: We have developed a system to identify cellular proteins that interact with Ad pvii, and used this system to identify several novel cellular proteins that interact with pvii. We are currently validating these novel interacting proteins and determining their role in regulating the nucleoprotein structure of Ad in the infected cell nucleus.

The role of neuronal dystonin in intracellular trafficking

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Background: Recently it was discovered that the cause of a newly identified lethal sensory neuropathy (HSAN-VI) is a mutation in the human dystonin gene. This gene encodes many isoforms, such as the three major neuronal isoforms: dystonin-a1, -a2, and -a3. These proteins have both actin and tubulin binding domains, which permit them to act as cytoskeletal-linker proteins with various roles, including intracellular transport. Mutations in the mouse dystonin gene result in a lethal sensory neuropathy called dystonia musculorum (dt). Our recent findings of a dysregulation in the autophagic pathway, along with the classic pathology of axonal swellings in dt sensory neuron axons are consistent with a disruption in intracellular trafficking.

Objective: Assess the impact of dystonin loss-of-function on two necessary components for efficient trafficking: 1) microtubule stability, and 2) molecular motors.

Methods: We use qpcr and immunoblot techniques to asses levels of motor proteins, post-transcriptional modifications to microtubules, and flux through the endo-lysosomal pathway.

Results: We report a discrepancy in microtubule stability between two dt mouse models. The more severe dt^{27j} mouse (lacking all 3 isoforms) does exhibit reduced levels of tubulin acetylation, however the dt^{tg4} mouse (lacking only dystonin-a1, and -a2) does not show these same defects. Analysis by qpcr suggests the possibility of the dystonin-a3 isoform having a compensatory effect in dt^{tg4} sensory neurons. We also observed no major differences in the overall levels of retrograde or anterograde motor proteins between wild type and dt mice. Evaluation of the endo-lysosomal pathway revealed no difference in levels of early or late endosomes, however there was a significant increase in lysosomes in dt dorsal root ganglia as indicated by higher levels of LAMP1.

Conclusion: The loss of tubulin acetylation observed in dt^{27j} sensory neurons likely has a dual effect on reducing microtubule stability, and in impeding recruitment of motor proteins. This suggests that microtubule instability is not central to disease pathogenesis, but likey contributes to increased severity of disease. Also, the increased expression of dystonin-a3 in dt^{tg4} sensory neurons might indicate a compensatory role for this isoform. Finally, although total levels of motor proteins were not affected, their recruitment to vesicles might be. The increase in lysosomes could be indicative of a build up, however, further analysis of this pathway is required to validate this hypothesis.

Evaluation of the clinical efficacy of the PeTrack motion tracking system for respiratory gating in cardiac PET imaging.

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Background: Respiratory motion during cardiac PET imaging can lead to inaccurate estimates of myocardial perfusion that may impact patient management. Respiratory gating is commonly used to evaluate and potentially compensate for patient breathing motion and decrease the prevalence of unwanted image artifacts.

Objective:The purpose of this study was to compare a new gating method with conventional optical tracking when sub-optimal respiratory gating was observed.

Methods: The PeTrack (Positron emission based Tracking) algorithm was developed to determine the 3D location of a positron-emitting fiducial marker placed on the abdomen of the patient. PeTrack calculated the position of the marker throughout the scan using raw list-mode data. Respiratory triggers were generated from the motion trace of the marker in 6 rubidium-82 cardiac PET scans. The performance of respiratory gating using PeTrack was evaluated by comparing the number of respiratory triggers, patient breathing intervals and observed respiratory motion in the reconstructed PET images, with those obtained from a conventional optical motion tracking system.

Results: The PeTrack system showed reduced variability in measured breathing intervals compared to the optical system. In cases of sub-optimal trigger response of the optical system, PeTrack was able to allow visualization of respiratory motion during the scan. Respiratory-gated image reconstruction using PeTrack triggers showed comparable image quality to those obtained in the subset of patients with adequate optical system gating.

Conclusion: PeTrack was able to accurately capture patient respiratory motion for the purpose of respiratory gating in rubidium-82 cardiac PET scans. This method appears to be more robust and potentially more accurate than conventional optical motion tracking

Exploiting exosomes as biomarkers and therapy for Spinal Muscular Atrophy

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Background: Spinal muscular atrophy (SMA) is a neuromuscular disease caused by reduced levels of survival motor neuron (SMN) protein. SMA results in degeneration of motor neurons, atrophy of muscle, and death in severe forms of the disease. With no cure, or accurate prognostic marker, development of rapid and sensitive biomarkers are needed that accurately correlate with the SMA phenotype, or that can monitor the efficacy of therapeutic interventions. Serum-derived exosomes have been evaluated in numerous studies in an attempt to identify novel biomarkers for many disease states. Since exosomes naturally transit between cells, many research groups have examined whether exosomes loaded with therapeutic proteins or nucleic acids can act as effective therapies for a variety of disease states.

Objective: To evaluate exosomes as a potential biomarker for SMA, and to determine whether exosomes can be used as a therapeutic delivery vehicle to carry SMN protein to SMN-deficient cells, leading to the phenotypic correction of the disease.

Methods: Exosomes were isolated using differential centrifugation or the commercially available kit, Exoquick, and characterized using exosome markers Flotillin, Tsg101 and Alix. Particle size and concentration was determined using the Particle Metrix Zetaview system. SMN protein-loaded exosomes were produced using cell lines stably expressing a 3X Flag-tagged SMN protein, or by infection with an adenovirus vector expressing 3X Flag-tagged SMN protein.

Results: We show that SMN protein is naturally released in extracellular vesicles from all cell types examined, of both mouse and human origin. Cell lines with varying levels of SMN protein expression had exosomal SMN protein levels that correlated to their parental cell line. Fibroblasts derived from a mouse model of SMA or from patients with SMA release exosomes with SMN protein levels significantly reduced relative to their normal controls. Exosomes isolated from the serum of a mouse model of SMA also show a corresponding reduction in SMN protein compared to wildtype controls. We have also determined that exosomes can be used to deliver SMN protein to recipient cells, suggesting a novel approach to treat SMA.

Conclusions: Taken together our studies suggest that exosomes may be a useful tool as both a biomarker and therapeutic for SMA.

Preconditioning of Human Neural Stem Cells with Metformin to Promote Post-Stroke Recovery

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Background: The generation of human induced pluripotent stem cells (hipscs) from human fibroblasts has revolutionized cell therapy by providing a source of autologous cells for transplantation. Several studies have demonstrated that transplantation of hipsc-derived neural stem cells (hipsc-nscs) increases regeneration and recovery following stroke, supporting their therapeutic potential. However, major concerns for translating hipsc transplantation therapy to the clinic are efficacy and safety. Therefore, there is demand to develop an optimal strategy to enhance the engraftment and regenerative capacity of transplanted nscs. The recent published work shows that metformin, an FDA approved drug, is an optimal neuroregenerative agent by promoting proliferation and differentiation of neural stem cells and limiting tumorigenesis following transplantation.

Objective: We hypothesize that preconditioning of hipsc-nscs with metformin before transplantation into the stroke-damaged brain will improve engraftment and regenerative capabilities of hipsc-nscs, ultimately enhancing functional recovery.

Methods: Generate human neural stem cells from a human ipsc line and test the ability of metformin to enhance the proliferation and differentiation of hipsc-nscs in vitro. Furthermore, determine the extent to which metformin preconditioning improves cell survival, proliferation, differentiation and functional recovery following transplantation of hipsc-nscs into an endothelin-1 focal model of ischemia.

Results: Treatment of hipsc-nscs in vitro with metformin increases number of Ki67/brdu and B-III Tubulin positive cells following proliferation assay and differentiation assays, even after withdrawal of metformin treatment for several days.
Future experiments will determine whether preconditioning hipsc-nscs with metformin will enhance their survival and regenerative capability following transplantation into a rat stroke model. Preconditioned and naïve hipsc-nscs will be transplanted into an endothelin-1 focal model of ischemia. After 1 and 8 weeks post-transplantation, we will assess engraftment, proliferation, differentiation and functional recovery.

Conclusion: Here we show treatment of hipsc-nscs in vitro with metformin enhances the proliferation and differentiation of hipsc-nscs even after withdrawal of metformin treatment, showing its promise as a novel preconditioning strategy. These studies represent a vital step in the optimization of hipsc-based replacement therapy to enhance post-stroke recovery.

The Ottawa Bioinformatics Core Facility

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The Ottawa Bioinformatics Core is core facility of the University of Ottawa and the Ottawa Hospital Research Institute. We provide advice on bioinformatics research design, conduct bioinformatics analysis, provide data warehousing services, and provide support for grant proposals that involve bioinformatics (including conducting pilot studies, support/collaboration letters, methodological text, etc.)
Our areas of greatest expertise are:

- High-throughput sequencing,
- Microarrays,
- Genomic sequence/motif analysis
- Integration of multiple data sources
- Network analysis

We have experience with many other types of bioinformatics, and/or can refer you to other local experts, depending on your needs. We can also help to arrange for the analysis of your biological samples with local core facilities such as StemCore, the Proteomics Resource Centre, or the OHRI Mass Spectrometry Core Facility.

Caspase-dependent pathways governing cardiac hypertrophy

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Background: Cardiac hypertrophy occurs when the heart size increases to maintain cardiac output at times of stress. Interestingly, this pathological process is characterized by cell behaviours which are typically associated with apoptosis. We previously demonstrated the essential role of the intrinsic cell death pathway during cardiac hypertrophy; however, the caspase-dependent pathways and cleavage targets remain elusive. It is proposed that cardiac hypertrophy is mediated by the activation of caspase-dependent pathways which activate hypertrophic transcription/gene expression programs and induce cytoskeletal remodelling which originate in part by caspase-mediated cleavage. Here, the myocyte enhancer factor 2 (MEF2) transcription factor inhibitor histone deacetylase 3 (HDAC3) and gelsolin (GSN), an actin-binding protein, were evaluated as potential caspase cleavage substrates.

Objectives: We investigated the importance of substrate caspase-mediated cleavage in the induction and/or maintenance of cardiomyoycte hypertrophy.

Methods: In vitro cleavage assays were completed with effector caspase 3/7 and HDAC3 or GSN recombinant protein. Analysis was completed by both western blot and silver nitrate staining followed by mass spectrometry analysis of the potential caspase-dependent cleavage fragments. Additionally, HDAC3 and GSN levels were analyzed in primary rat cardiomyocytes treated with hypertrophy agonist phenylephrine compared to control serum-free media treatment. Cardiomyocytes were also transfected with luciferase reporter plasmids for hypertrophic markers and reporter activity was measured.

Results: HDAC3 cleavage was observed during early stages of hypertrophy and reduced in the presence of a caspase inhibitor. Luciferase assays demonstrated that the transcriptional activity of MEF2 is dependent on intact caspase function suggesting caspase-directed HDAC3 cleavage may serve as a novel regulatory mechanism to alleviate MEF2 suppression to engage the hypertrophy gene expression program. Caspase mediated GSN cleavage occurs at latter stages and is coincident with the cytoskeletal alterations that occur during this process. We have generated adenoviral vectors containing caspase cleavage mutants and cleaved forms of GSN and will discuss the impact of these modified substrates on the hypertrophy process.

Conclusion: Collectively, this work suggests that caspase signalling acts to engage both the transcriptional program and cytoskeletal accommodations that characterize cardiac hypertrophy. Importantly, these observations suggest that identification of inhibitors that suppress caspase activity and/or activity of its cognate substrates may offer novel therapeutic targets to limit the development of pathological hypertrophy.

Cell Division Mechanisms in Human Dystrophic Satellite-like Cells

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Background: Satellite cells are muscle progenitors capable of self-renewal and repairing damaged skeletal muscle in response to activation cues. Duchenne Muscular Dystrophy (DMD) is a muscle wasting disorder caused by mutations in the DMD gene, which encodes the dystrophin protein. Loss of dystrophin leads to sarcolemmal fragility in skeletal and cardiac muscle, as well as dysfunctions in satellite cell homeostasis in skeletal muscle. Recent breakthroughs in induced pluripotent stem cell (ipsc) myogenic differentiation have provided methods for investigating patient-specific mechanisms of muscle diseases.

Objective: To optimize DMD patient ipsc-derived satellite-like cell differentiation and transplantation into mice for investigating human dystrophic satellite cells in situ and in vitro on isolated single muscle fibers.

Methods: Lentiviral vectors will be used to reprogram DMD patient and healthy control cells to generate ipsc lines that will subsequently be chemically differentiated down the myogenic lineage to generate satellite-like cells. The gene and protein expression profile of cells undergoing myogenesis will be assessed at several stages of the differentiation protocols with Western blotting, immunofluorescence staining and qrt-PCR. These muscle progenitors will be transplanted into cardiotoxin-injured tibialis anterior (TA) muscles of immunocompromised mice and the human cells expected to repopulate the muscle satellite stem cell niche. Immunohistochemistry on TA cryosections in addition to single fiber culture from the engrafted and contralateral sham-injected muscles will be used to confirm presence and characterize the contribution of human cells within the tissue.

Results: Myogenic progenitors were generated using two methods of differentiation. The techniques were assessed and compared for their efficiency to give rise to satellite-like cells. Initial expression of paraxial mesoderm markers was followed by the onset of myogenic markers. Progenitors at various stages of differentiation were transplanted into mouse TA muscle to assess engraftment potential and contribution to newly formed muscle fibers post-injury. TA single fiber isolation was optimized for investigating satellite cell activation and proliferation, compared to conventional extensor digitorum longus-based satellite cell characterization methods.

Conclusions: The nature of muscle development and fine balance of cues maintaining satellite cell quiescence as opposed to activation and proliferation have made in vitro studies into the muscle stem cell pool a challenging task. For the same reasons robust techniques for differentiating satellite cells from ipscs had not been established until recently. This project anticipates to create human dystrophic muscle progenitors that can be used for confirming findings made in model organisms as well as testing therapeutic candidates for DMD.

The Role for Negative Elongation Factor in Regulating Muscle Regeneration

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Background: Genome-wide studies of RNA Polymerase II (RNA Pol II) binding in various cell types has revealed the importance of promoter proximal pausing as a means to regulate the expression of developmentally important genes. In this context, pausing of RNA Pol II immediately upstream of the transcription start site allows the maintenance of active transcriptional machinery on 'stand-by' for subsequent quick and efficient gene expression upon specific signaling cues. When Drosophila was used as a model organism, it was found that promoter-proximal pausing could be suppressed by knocking down Negative Elongation Factor (NELF), a key protein complex required to establish the paused polymerase during transcriptional regulation. Further gene expression meta-analysis showed that only a small subset of genes had altered expression upon NELF knockdowns, composed mainly of developmental and genes affected in light of environmental factors such as hsp70. Collectively, this work suggests that NELF-mediated promoter-proximal pausing is not a general transcriptional mechanism, but suggests instead that it acts as a checkpoint to effectively suppress expression of specific genes until a proper temporal or environmental signal becomes available to allow transcription to proceed.

Objective: Given the speed and coordination with which cellular fates of myogenic progenitor cells change during muscle regeneration, we hypothesize that NELF maintains key muscle differentiation genes involved in a paused state, awaiting cues to induce transcriptional elongation. This would allow myoblasts to proliferate and produce a sufficient population of cells needed for proper muscle repair, at which point NELF repression would be lifted to allow expression of genes required for differentiation.

Methods: To explore my hypothesis, I am using a combination of in vitro (aim 1) and in vivo (aim 2) techniques. In my first aim, I will examine the proliferation and differentiation of cultured myoblasts using comparitive analysis of chip-seq and RNA-seq to identify which genes may be directly regulated by NELF. In my second aim, I will be using sastellite cell specific conditional knockouts of NELF-B in mice to characterize the ability of satellite cells to regenerate injured muscle in the absence of the NELF complex.

Results & Conclusion: To this point I have been able to make the necessary molecular tools and mice crossings for in vitro and in vivo analysis. As such, I will now be able to start studying the implication of NELF in myogenesis.

Investigating the role of acetylation in PAX7 function

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Background: PAX7 is essential for the function of muscle satellite cells. It was previously determined that PAX7 methylation is important for its transcriptional activity and function in satellite cells. By mass spectrometry using immunoprecipitated FLAG-PAX7, we identified two lysine residues (K105 and K193) within the PAX7 protein that are acetylated; both residues are conserved between species. To test whether these modifications have an influence on PAX7 transcriptional activity, we created a set of PAX7 mutants where the acetylated lysine residues are replaced by arginine. PAX7 transcriptional activity was monitored using a luciferase reporter under the control of Myf5, a Pax7 target gene. Treatment with trichostatin A (TSA), a histone deacetylase inhibitor, increased significantly luciferase activity, but this activity was progressively lost when the mutants were introduced. This suggests that acetylation plays a role in PAX7 transcriptional activity.

Objective: We are interested in understanding if PAX7 displays other post-translational modifications important for its function.

Methods: Immunoprecipitation, sirna knockdown, Invitro acetylation Assay.

Results: In an attempt to find the PAX7 acetyltransferase, we used a candidate approach. MYST1 (also known as KAT8 and Mof) is expressed in muscle satellite cells. MYST1 is known for its interaction with Wdr5, a known PAX7 partner. MYST1 is also known for global histone H4K16 acetylation. We detected an interaction between PAX7 and MYST1 by co-immunoprecipitation in fibroblasts and in primary myoblasts. MYST1 sirna knockdown in primary myoblasts causes a reduction in a subset of PAX7 target genes such as Myf5, Perp and Gas7. Surprisingly, MYST1 does not seem to interact with Wdr5 (known Pax7 interactor) in primary myoblasts. Furthermore, PAX7 acetylation does not seem to be crucial for its interaction with known partners (Carm1, Wdr5 and Ash2L), protein stability or PAX7 localization since the K/R mutations do not affect these processes.

Conclusion: MYST1 seems to be an interesting candidate for modulating posttranslational modification on PAX7.

Investigating the role of Pax7 methylation on gene transcription and chromatin organisation.

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Background: Satellite cells exist as a heterogeneous population where the self-renewing stem cells express the transcription factor Pax7 but not Myf5 and committed progenitors or satellite myogenic cells express both Pax7 and Myf5. Induction of Myf5 expression in satellite myogenic cells requires Pax7 recruitment of a H3K4 histone methyltransferase complex to enhancer elements upstream of the Myf5 promoter, which recruits chromatin remodeling complexes that favor gene expression. Furthermore, coactivator-associated arginine methyltransferase 1 (Carm1) methylates Pax7 at four N-terminal arginine residues (R10, R13, R22 and R37) in order to regulate its transcriptional activity. Preventing this methylation diminished binding to the methyltransferase complex, and reduced Myf5 expression. Furthermore, $Carm1^{-/-}$ mice were unable to regenerate muscle tissue following injury with cardiotoxin.

Objective: To investigate the function of Pax7 methylation on its transcriptional activity in vivo independently of Carm1, and conveniently characterise the function of Pax7 methylation at all stages of differentiation. Isolation of Pax7+/Myf5+ and Pax7+/Myf5- satellite cells from this Pax7 3RK mutant as well as our existing mouse model will allow us to investigate molecular differences across both populations of satellite cells with or without Pax7 methylation.

Methods: The CRISPR/Cas9 system will be used to create a mouse model containing mutations to three N-terminal arginine residues of Pax7. The mutant mouse will be obtained by injecting mouse zygotes with the Cas9 protein, a sgrna recognizing the area of interest and a repair template containing three mutations: R10K, R13K and R22K (collectively known as 3RK) in order to obtain a genetically modified mouse. The resulting Pax7 protein will contain lysine residues instead of arginine residues, which cannot be methylated by Carm1. Furthermore, we can use the Assay for Transposase-Accessible Chromatin with high throughput sequencing (ATAC-seq) to investigate chromatin organization. We aim to use this technique on satellite cells from our Pax7 3RK mouse and the normal mouse. A reduced chromatin accessibility and increased nucleosome density around Myf5 enhancer elements in Myf5+ cells from the Pax7 3RK mouse combined with an increase in chromatin accessibility in the normal mouse will corroborate the initial observation that Pax7 methylation is important for the activation of Myf5 expression. Furthermore, we can perform ATAC-seq on cells downstream in the myogenic lineage to determine the effect of Pax7 methylation on differentiation.

Conclusion: This research will allow us to better understand the fundamental role of Pax7 methylation implicated in satellite cell function through their activation and differentiation.

Identification of cellular targets and small molecules inhibiting adenovirus replication

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Background: The human adenovirus (Ad) causes minor illnesses in most patients, but can lead to severe disease and death in pediatric and geriatric populations, and in immunocompromised individuals. No approved antiviral therapy currently exists for the treatment of severe Ad-induced diseases.

Within the first few hours of infection, the Ad DNA associates with cellular proteins including histones and adopts a nucleoprotein structure similar to the host DNA. Assembly of the viral genome into this repeating nucleosome-like structure is required for efficient expression of virus-encoded genes. Consequently, one approach to treating Ad-induced disease may be to prevent the viral DNA from transitioning to this transcriptionally active state, allowing time for non-cytolytic elimination of the virus genome from infected cells.

Objective: To identify novel small-molecule inhibitors of Ad replication and determine their effects on Ad DNA chromatinization and other cellular processes.

Methods: We have produced a wildtype-like Ad construct encoding the red fluorescent protein (RFP) gene under “late” transcription regulation (Ad-late/RFP). RFP from the Ad-late/RFP is only expressed following viral DNA replication, and therefore, can be used to effectively monitor Ad replication. Using the Ad-late/RFP, we designed an efficient method for screening small-molecule libraries to identify potential inhibitors of Ad replication. Any compound that significantly reduces RFP expression compared to untreated controls is further evaluated.

Results: Following validation of the Ad-late/RFP construct and the small-molecule screening procedure, a preliminary screen of few compounds identified the pan-histone deacetylase (HDAC) inhibitor vorinostat as a potential inhibitor of Ad replication. Further studies revealed that vorinostat significantly delays the onset of viral gene expression and replication, and that these effects are likely mediated through the inhibition of HDAC2 activity. In addition, the Prestwick Chemical Library was screened and several FDA-approved compounds were identified to reduce RFP expression by more than 50% compared to untreated, infected cells. We are currently working to elucidate the underlying molecular mechanism of vorinostat's transient inhibition, evaluating the comopound in vivo, and validating the positive hits from the screen.

Conclusion: The costs associated with Ad-induced disease are significant in terms of medical expenses, lost work hours and loss of life in some populations. Identification of new compounds to combat Ad infections will lead to decreased disease pathogenesis and higher survival rates. In addition, investigation of the mechanism by which these compounds impact Ad replication will provide novel insights on virus-cell interactions, as well as enhance our knowledge of the Ad infection process.

Mapping Erythropoiesis with Bleeding-Edge Quantitative Proteomics

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The differentiation of hematopoietic stem cells (hscs) to erythroid cells (red blood cells), called erythropoiesis, is a process guided by stage-specific transcription factors (tfs). These tfs control the expression of genes that drive erythropoiesis. Gene Regulatory Networks (grns) are a useful tool to study the dynamics of this process. However, grns are typically based on RNA measurements and it is not clear that RNA levels are a good proxy of protein levels. In this talk, we present a GRN model of erythropoiesis, based on RNA-seq expression data and quantitative proteomics data (itraq and SRM), to study quantitatively the expression of genes regulating erythroid cell differentiation.

Alveolar Epithelial Cell Therapy Rescues The Lung Phenotype in a Mouse Model of Surfactant protein C Deficiency

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Background: Pulmonary surfactant protein C (SP-C) is synthesized by type II Alveolar epithelial cells (AEC-II) as a precursor and is processed to form the functional secreted protein. Mutations in the SP-C gene (Sftpc) are inherited as an autosomal dominant trait and lead to a misfolded protein and subsequent cellular stress in AEC-II. Sftpc mutations are linked to respiratory distress in term newborn infants, hereditary forms of idiopathic interstitial pneumonitis in older children, and chronic pulmonary fibrosis and emphysema in adults. Histological features due to SP-C deficiency include alveolar remodeling, airspace loss, fibrosis, cellular infiltrates and epithelial cell dysplasia in conducting and peripheral airways. The application of fresh AEC-II therapy and human induced pluripotent stem cell (hipscs)-derived aecs-II to rescue a lung genetic disease has never been investigated.
Objectives: In this study, we hypothesized that AEC-II and hipsc-derived AEC-II therapy can rescue the phenotypic and pathological consequences of Sftpc gene knock-out in SP-C deficient mice ($SPC^{-/-}$).

Methods: Wild type AEC-II were isolated from 4-6 weeks old 129J mice by enzymatic digestion followed by Ficoll-purification and non-plastic adherence selection. We also established a highly efficient method to differentiate hipscs into a homogenous population of AEC-II. Both cell types were labelled by a membrane-specific fluorescent dye and were intratrachealy administered as single cells to one year old $SPC^{-/-}$ mice. Cyclosporin A was administered to mice that received human cells to prevent immune rejection. Mice were treated with two doses of AEC-II during a period of 12 days. Age matched $SPC^{-/-}$ and 129J mice that did not receive cells were controls.

Results: Freshly isolated AEC-II engrafted into the distal lung, improved lung function and structure and attenuated cell infiltration in $SPC^{-/-}$ mice. Aecs therapy increased the exercise capacity and improved lung mechanical properties. Our results also showed that hipsc-derived AEC-II were retained in the distal lung, engrafted into alveolar structure and attenuated lung structural injuries.

Conclusions: Patient-specific AEC-II therapy exerts short-term therapeutic benefit in this experimental model and may offer new therapeutic options for lung genetic disorders that affect alveolar epithelial cells.

PAX7 function in muscle satellite cells is regulated by acetylation and by interaction with MYST1, SIRT1 and SIRT2

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Background: The understanding of muscle stem cell (satellite cell) biology is crucial for the development of stem cell-based therapies in the treatment of muscular dystrophy. The transcription factor PAX7 is a master regulator of satellite cell function, as $Pax7^{-/-}$ mice are deprived of satellite cells.

Objective: The goal of this project is to identify upstream regulators of the PAX7 protein, and to determine their function in satellite cells.

Methods and Results: By mass spectrometry, we determined that PAX7 is acetylated on two conserved lysine residues. Acetylation is important for PAX7 transcriptional activity, as treatment with deacetylase inhibitors significantly increases the expression of a PAX7-responsive Myf5 -111kb luciferase reporter. Mutation of the lysine residues into arginine results in a decrease in Myf5 reporter activity. Acetylation is not required for PAX7 nuclear localization and does not impact PAX7 protein stability or its capacity to mediate protein-protein interactions. Instead, acetylation seems to be necessary for PAX7 recruitment to chromatin. We aimed at identifying the acetyltransferase and the deacetylase involved in PAX7 regulation. By immunoprecipitation in myoblasts, we detected an interaction between PAX7 and the acetyltransferase MYST1, as well as with the deacetylases SIRT1 and SIRT2. Decreasing MYST1 levels by RNA interference leads to a decrease in the expression of Myf5. Inversely, sirna treatment against Sirt1 increases the expression of a subset of Pax7 target genes. SIRT1 is specifically expressed in committed satellite cells and not in satellite stem cells, suggesting that PAX7 activity might be fine tuned in satellite cell sub-populations.

Conclusion: MYST1, SIRT1 and SIRT2 appear as strong candidates for the regulation of PAX7 acetylation. Their effect on PAX7 biological function in satellite cells remains to be determined

Periostin Induced Pancreatic Regeneration

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Background : Periostin (Postn) has been identified as being highly induced in the mouse pancreas following partial pancreatectomy. Postn is a 90kda secreted protein implicated in tooth, bone and heart development. In addition, Postn has been shown to promote tissue repair of the skin, heart and spinal cord. In the pancreas Postn is known to be expressed by Pancreatic Stellate Cells.

Objective : To determine if Periostin can induce pancreatic regeneration in the mouse and also within human islets.

Methods: Investigation of pancreatic regeneration in the absence of Periostin by performing partial pancreatectomies in Postn-mutant mice. Delivering Postn into the mouse pancreas by direct and intraperitoneal injection to determine if pancreatic regeneration can be induced by Postn. Injection of Postn into the common bile duct to induce regeneration throughout the pancreas and improve the glucose tolerance of streptozotocin treated mice. Treatment of human islets with Postn to improve β-cell function as determined by performing a glucose stimulated insulin secretion (GSIS) assay.

Results: We found that adult Postn-mutant mice had reduced β-cell regeneration following partial pancreatectomy. Furthermore, directly injecting Postn into the mouse pancreas activated pancreatic stellate cells to develop a mesenchymal stroma facilitating pancreatic regeneration in a localized area. Furthermore, intraperitoneal injection of Postn resulted in increased insulin staining and long-term glucoregulatory benefits with no adverse effects found in other tissues. Injection of Postn into the common bile duct increased delivery throughout the pancreas and significantly improved the glucose tolerance of streptozotocin treated mice. Moreover, treatment of human islets with Postn increased insulin staining and improved β-cell function.

Conclusions: Postn has the potential to reduce the number of human islets required to reverse hyperglycemia in diabetes. Taken together, Postn is novel molecule capable of potentiating pancreatic β-cell regeneration which requires further investigation as a candidate therapeutic for the treatment of diabetes.

Mir-126 high expressing CD-146+ pbmcs yield L-epcs and is differentially expressed in patients with cardiovascular pathology.

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Background & objectives: Endothelial progenitor cells (epcs) have been the center of attention in the field of regenerative medicine for its potential in the therapy of cardiovascular pathologies including pulmonary arterial hypertension (PAH). Two types of epcs have been described. The first, early-EPC, are cells that provide therapeutic effect through pro-angiogenic factors. The second, late outgrowth endothelial progenitor cells (L-epcs), are highly proliferative cells capable of incorporating into the vascular network. Unlike early-epcs, which are derived from the mononuclear cell fraction of peripheral blood and arise in culture within one week, L-epcs will appear in culture only after two to three weeks when kept under endothelial selective media.
Although the true nature of L-EPC precursors is still under investigation, recent studies suggest that late outgrowth endothelial cells rise from the fraction of pbmcs expressing the surface markers CD-34 and CD-146. Here we set to further understand the phenotype of these precursor cells with the goal of improving selection for L-EPC formation. Furthermore, we set to characterize the phenotype of L-epcs derived from healthy individuals and patients with cardiovascular pathology in terms of metabolism, proliferation and angiogenic activity.

Results: Following a genome-wide microrna array, we have identified mir-126 to have significantly higher expression levels in L-epcs when compared to early-epcs. Furthermore, we have shown that the population of L-EPC precursor cells (CD-146 positive) consistently shows higher levels of mir-126, when compare to the CD-146 negative fraction. We are currently using an in vivo mirna labeling system to further demonstrate that the CD-146 positive fraction derived from pbmcs can be further divided into mir-126 positive or negative, where only the positive fraction is capable of giving rise to L-epcs.
We have also observed that mir-126 levels are down regulated in patients with cardiovascular pathology and that L-epcs from these patients show abnormal proliferative and angiogenic properties and we are currently investigating the metabolic differences in these cells.

Conclusion: Improving selection for L-epcs may results in a more robust L-EPC formation in shorter time. This has a tremendous implication for the use of L-epcs as a therapeutic target in the field of regenerative medicine. Furthermore, by characterizing the phenotype of L-epcs in patients with cardiovascular pathology offers an avenue to better understand the progression of the disease and to appropriately target these cells for therapeutic purposes.

Epigenetic Regulation of the Hypoxic Response: Role of Sirtuin 1 in Chronic Hypoxia Induced Pulmonary Hypertension

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Background: Pulmonary Hypertension (PH) is caused by remodelling of pulmonary arterioles leading to increased pulmonary vascular resistance, right ventricle hypertrophy and eventually heart failure. Sirtuin-1 (Sirt1) is an NAD^+-dependent deacetylase strongly implicated in maintenance of endothelium homeostasis and metabolic balance. Chronic Hypoxia (CH) exposure (10% Oxygen for 3 weeks) is a common model for induction of PH in rodents. Resveratrol is a natural phenol that has been proposed to play a protective role in the vasculature by activation of Sirt1.

Objective: To understand the role of the deacetylase Sirt1 in chronic hypoxia induced Pulmonary Hypertension and the hypoxic response.

Methods: Mice lacking Sirt1 catalytic activity ($sirt1^{y/Y}$, H355Y) and their wild type (WT) littermates were exposed to chronic hypoxia (CH; 10% O_2) for 21 days with or without Resveratrol in food at 30mg/kg/day.

Results: Absence of Sirt1 activity led to exaggerated increase in right ventricle systolic pressure (RVSP) at 3 weeks (42±2 $sirt1^{y/Y}$ vs. 30±1 WT; n=27/group, $p< 0.001$) as well as significant right ventricular hypertrophy, assessed by the RV/LV+S weight ratio (0.56±0.01 $sirt1^{y/Y}$ vs. 0.43±0.01 WT; n=27/group, $p< 0.001$) and increased hematocrit levels (71±2% $sirt1^{y/Y}$ vs. 63±1% WT; n=17/group, $p<0.001$). Erythropoietin levels were also exaggerated in the mutant mice compared to WT mice in hypoxia (1778±841 $sirt1^{y/Y}$ vs. 185±72pg/ml WT; n=11-12/group, $p<0.05$). Treatment with the Sirt1 activator, Resveratrol, did not alter the hemodynamic response to CH in WT animals (27±1; n=11), but prevented the exaggerated CH-induced increase in RVSP in the Sirt1 mutant mice (28±2; n=10, $p<0.001$ vs. CH alone). Furthermore, treatment with Resveratrol selectively reduced RV hypertrophy in the mutant mice (0.48 ±0.02; n=10, $p<0.001$ vs. CH alone) but not in WT mice (0.4±0.01; n=11). Finally, Resveratrol led to a modest, but significant decrease in hematocrit in Sirt1 mutant mice (65±2%; n=10, $p<0.05$ vs. CH alone) but not the WT littermates (60±1%; n=11).

Conclusions: Loss of Sirt1 deacetylation activity led to an exaggerated pulmonary hemodynamic response to CH, consistent with a regulatory role for Sirt1 in hypoxia sensing and/or signaling. Resveratrol can prevent the development of severe PH in mice lacking Sirt1 activity, suggesting that Resveratrol normalized the pulmonary vascular and systemic response to hypoxia through mechanisms that compensate for the lack of Sirt1 activity.

Stimulation of Muscle Stem Cell Asymmetric Division Enhances Regeneration of Dystrophin-Deficient Muscle

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Loss of dystrophin in Duchenne Muscular Dystrophy (DMD) brings about progressive and severe degeneration of skeletal muscle. Dystrophin-deficiency in muscle stem cells results in reduced rates of asymmetric division, reduced generation of progenitors, and impaired regeneration. Through a small molecule screen, we identified epidermal growth factor receptor (EGFR) and Aurora kinase A (Aurka) as regulators of muscle stem cell asymmetric division. Inhibition of EGFR results in a pronounced shift from asymmetric to symmetric divisions. Conversely, activation of EGFR signaling stimulates a 2.5-fold increase in asymmetric divisions. Phosphorylated EGFR recruits Aurka, and inhibition of Aurka blocks EGF stimulation of asymmetric division. EGF treatment marked stimulates asymmetric divisions in dystrophindeficient muscle stem cells in mdx mice, resulting in increased numbers of progenitors, enhanced regeneration, and functional recovery of muscle strength. Therefore, activating the egfrpolarity pathway stimulates the functional rescue of dystrophin-deficient satellite cells by increasing the rate of asymmetric cell division.

Some of our research trainees who hold salary awards

For a full list of trainees with salary awards, please see page 5.

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