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

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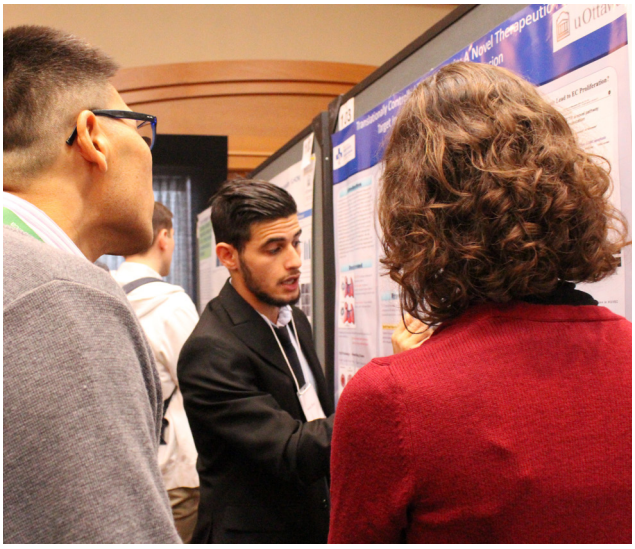
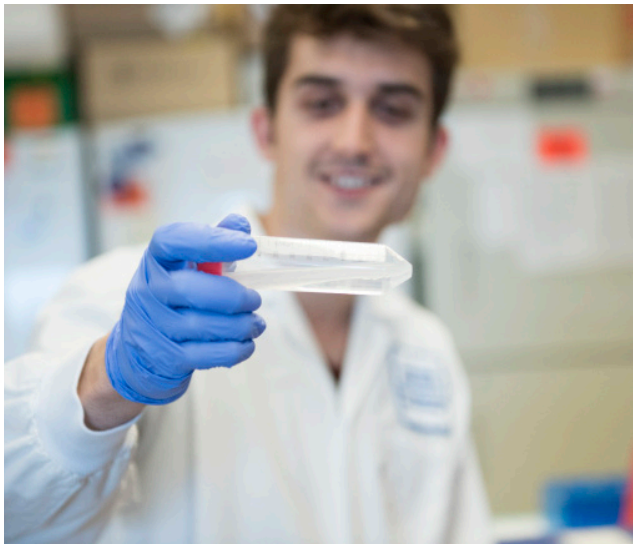
2017 RESEARCH DAY





Program and Abstracts

Thursday, November 9, 2017
7:30 a.m. – 5:00 p.m.

St. Elias Centre
750 Ridgewood Ave.
Ottawa, ON

Research Day is generously supported by:

CADTH Evidence Driven.

VWR®

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CCRM Commercializing Living Therapies

HTG Molecular

BIO-RAD

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. Angela Crawley
Dr. Ian Lorimer
Dr. William Stanford
Amelia Buchanan
Dr. Dean Fergusson
Dr. Tim Ramsay
Dr. Duncan Stewart
Dr. Ketul Chaudhary
Dr. Anouk Fortin
Emma Grigor

Volunteers

Greg Canham
Lynn Crosbie
Kathy Patterson
Melanie Genereaux
Catherine Geci
Wayne Lowe
Terri Van Gulik

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 4th 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 world-class researchers to Ottawa to give two keynote lectures.

The first will be given by Dr. John Ioannidis, one of the most cited scientists in the world. He is a leading expert in evidence-based medicine, clinical methodology and meta-research who has designed many influential clinical trials. The title of his talk today is "Meta-research: Improving research."

The second lecture will be given by Dr. Marco Marra, a world-leading expert in large-scale DNA sequencing and bioinformatics. Dr. Marra's current research activities revolve around the interplay between the cancer genome and the epigenome, with particular focus on the evolution of treatment resistant disease. The title of his talk today is "Whole genome analysis to inform cancer treatment planning."

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 speakers to our presenters, judges, moderators, 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

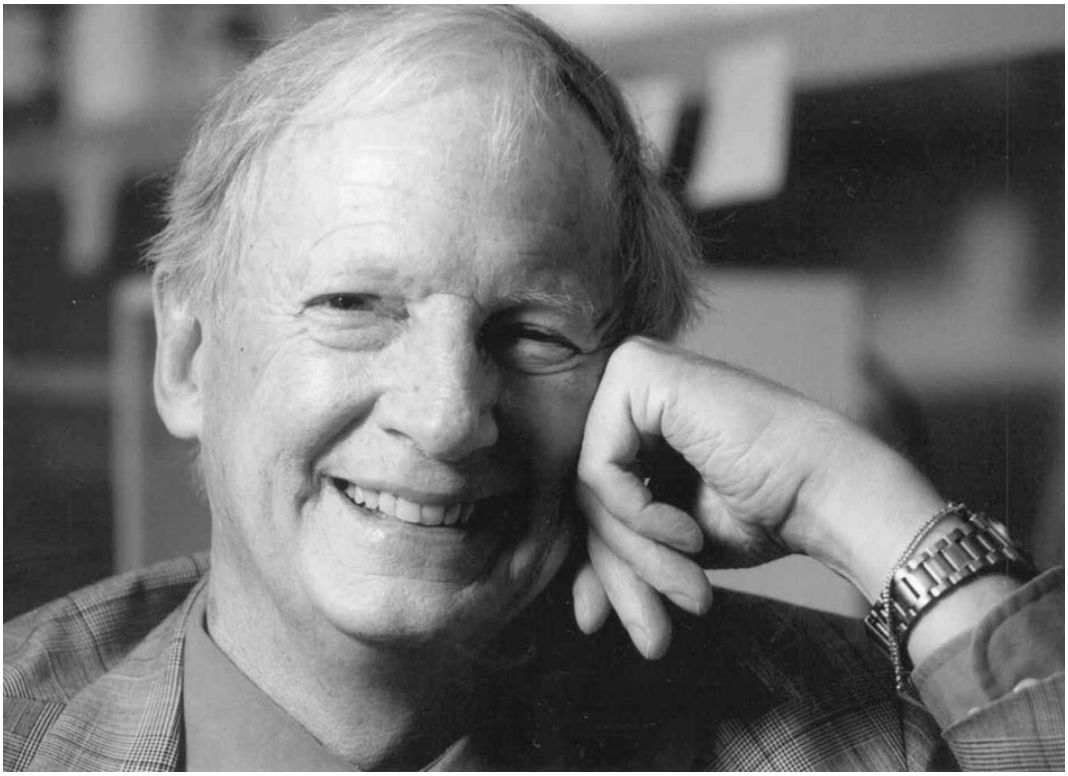
CEO & Scientific Director,
Senior Scientist in the Regenerative Medicine Program,
Ottawa Hospital Research Institute

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.

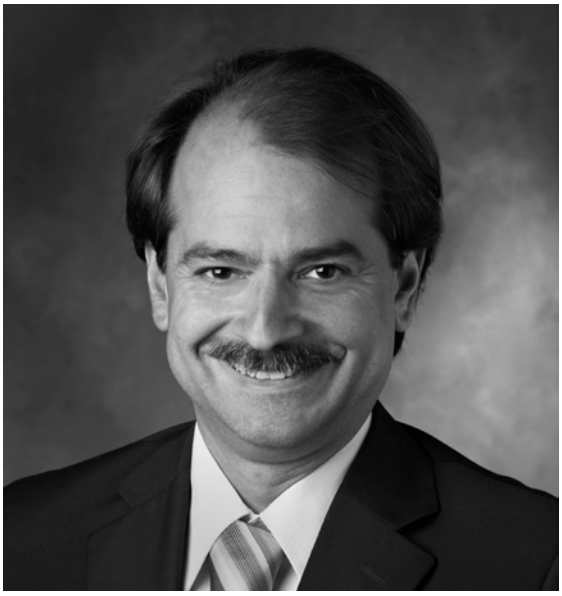
Dr. Grimes served as the Institute's CEO and Scientific Director until he retired in 1997. He recruited and mentored many of Ottawa's leading health researchers. He also practiced neurology for more than 25 years, specializing in Parkinson's disease.

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

"Meta-research: Improving research"

Dr. John P.A. Ioannidis



Dr. John Ioannidis is one of the most cited scientists in the world. He is a leading expert in evidence-based medicine, clinical methodology and meta-research who has designed many influential clinical trials. He holds the C.F. Rehnborg Chair in Disease Prevention at Stanford University, and is a Professor of Medicine, Professor of Health Research and Policy, Professor (by courtesy) of Biomedical Data Science at the School of Medicine and Professor (by courtesy) of Statistics at the School of Humanities and Sciences. He is also co-Director, Meta-Research Innovation Center at Stanford and Director of the PhD program in Epidemiology and Clinical Research.

KEYNOTE LECTURE

"Whole genome analysis to inform cancer treatment planning"

Dr. Marco A. Marra

Dr. Marco Marra is the University of British Colombia Canada Research Chair in Genome Science, and a member of the Order of British Columbia. In 2004, he received a Terry Fox Young Investigator Award and BC Biotech's Innovation and Achievement Award (together with the entire GSC staff) for sequencing the SARS coronavirus genome. Dr. Marra's contributions to genome science led to an honorary Doctor of Science degree from Simon Fraser University in 2004 and an honorary Doctor of Laws degree from the University of Calgary in 2005. Dr. Marra's current research activities revolve around the interplay between the cancer genome and the epigenome, with particular focus on the evolution of treatment resistant disease.

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. Amy Hsu and Dr. Sailendra Nath Sarma for their excellent job running the summer student program this year.

Winners of the Dr. Goodman Cohen Summer Student Award

Senior Award

Allyson Banville (supervised by Rebecca Auer)
"Enhancing the Immunogenicity of an Infected Cell Vaccine (ICV)"

Junior Award

Anna Munro (supervised by David Allan)
"Obstetrical factors associated with increased mobilization of hematopoietic progenitor cells in umbilical cord blood"

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
Amelia Aitken
Khalid Al-Zahrani
Arwa Alrehaili
Leonard Angka
Allyson Banville
Katherine Baxter
Marie-Claude Bourgeois-Daigneault
Caroline Brun
Lauren Carter
Ketul Chaudhary
Zhaoyi Chen
Ashley Chen
Ryan Clarkin
David Cook
Sarah Cummings
Lisa Currie
Kristin Danko
Annemarie Dedek
Marc-Olivier Deguise
Anahita Dehmoobad Sharifabadi
Daniel El Kodsi
Peter Feige
Laura Goodwin
Emma Grigor
Maria Hurskainen
Mahsa Jessri
Danny Jomaa
Faizan Khan
Sarwat Khan
Kaitlin Kharas
Samantha Kornfeld
Christopher Lavergne
Cameron Leafloor
Shannon Leduc
Marissa Lithopoulos
Anisha Lynch-Godrei
Marisa Market
Leonardo Martin Calderon
Curtis McCloskey
Matt McDonald
Leslie Nash
Adrian Pelin
Iris Perelman
Daniel Ramirez
Morten Ritso
Galaxia Rodriguez
John Saber
Bratati Saha
Reza Salehi
Teslin Sandstrom
Bojan Shutinoski
Dylan Siriwardena
Phillip Staibano
Marie-Ève Wedge
Vignan Yogendrakumar

OHRI RESEARCH DAY PROGRAM

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

8:15 AM **OPENING REMARKS** (Fraser Scott, Duncan Stewart, Jack Kitts, Sylvain Charbonneau, Bernard Jasmin)

8:30 AM **NEUROSCIENCE** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: **Vignan Yogendrakumar** and **Nathaniel Noblett**

- **Sarah Cummings** (Rashmi Kothary Group) *Chondroitin Sulfate Proteoglycans Impede Oligodendrocyte Differentiation Through Rho Kinase and Myosin II*
- **Annemarie Dedek** (Michael Hildebrand Group) *TEP61 links BDNF-mediated disinhibition to NMDAR potentiation in pathological pain processing within the rat and human spinal dorsal horn*
- **Ahmad Galuta** (Eve Tsai Group) *Comparison of Adult Human and Rat Spinal Cord Neural Stem/Progenitor Cell Proliferation and Differentiation Characteristics*
- **Alicia Duval** (Barbara Collins Group) *Incidental Effects of a Mindfulness-Based Stress Reduction (MBSR) Program on Cognition Among Breast Cancer Survivors*

9:20 AM **CLINICAL AND PRE-CLINICAL RESEARCH** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: **Samantha Kenny** and **Charles Thickstun**

- **Faizan Khan** (Marc Rodger Group) *Long-Term Risk of Recurrence After Stopping Anticoagulants for Acute Unprovoked Venous Thromboembolism: A Systematic Review and Meta-Analysis*
- **Larissa Shamseer** (David Moher Group) *Opening Pandora's box: assessing what's published in predatory journals*
- **Emma Grigor** (Manoj Lalu and Dean Fergusson Group) *Efficacy and safety of Chimeric Antigen Receptor T-cell (CAR-T) therapy in patients with hematologic and solid malignancies: A systematic review and meta-analysis*
- **Wongsakorn Kiattiburut** (Nongnuj Tanphaichitr Group) *Antimicrobial peptide LL-37 and its truncated peptides, GI-20 and GF-17, exert spermicidal effects and microbicidal impact on Neisseria gonorrhoeae and Escherichia coli, UTI-89*

10:10 AM **REFRESHMENT BREAK** (15 minutes) Sponsored by Bio-Rad

10:25 AM **POSTER VIEWING / JUDGING OF POSTERS PRESENTED BY MASTERS, PhD and MD STUDENTS** (60 minutes)

11:25 AM **DR. J. DAVID GRIMES LECTURE** (35 minutes plus 10 minutes discussion)
Sponsored by CADTH
Meta-research: Improving research
John P.A. Ioannidis, Professor of Medicine, Health Research and Policy, co-Director, Meta-Research Innovation Center and C.F. Rehnborg Chair in Disease Prevention at Stanford University
Moderator: **David Moher**

12:10 PM **BUFFET LUNCH/ POSTER SETUP** (60 minutes)

1:10 PM **CANCER STRATEGIES (50 Minutes)**
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: **Amelia Aitken** and **Adrian Pelin**

- **Leonard Angka** (Rebecca Auer Group) *Characterizing Natural Killer cell Suppressors: A closer look at surgery induced myeloid derived suppressor cells in cancer surgery patients.*
- **Oliver Varette** (Jean-Simon Diallo Group) *Exploring Novel Enhancers of Autologous Tumour Vaccine Strategies*
- **Lauren Carter** (Barbara Vanderhyden Group) *Mechanisms of Transforming Growth Factor Beta 1-Mediated Ovulatory Wound Repair*
- **Amarilis Figueiredo** (Harold Atkins Group) *A Dose Escalation Study of Total Marrow Irradiation and Autologous Stem-Cell Transplantation for Relapsed Multiple Myeloma Patients*

2:00 PM **KEYNOTE LECTURE** (35 minutes plus 10 minutes discussion)
Whole genome analysis to inform cancer treatment planning
Marco A. Marra, Director & Distinguished Scientist, Genome Sciences Centre, BC Cancer Agency, Professor & Head, Department of Medical Genetics, University of British Columbia, UBC Canada Research Chair in Genome Science
Moderator: **Fraser Scott**

2:45 PM **REFRESHMENT BREAK** (15 minutes) Sponsored by HTG Molecular Diagnostics

3:00 PM **POSTER VIEWING / JUDGING OF ALL OTHER POSTERS** (60 minutes)

4:00 PM **BIOTHERAPEUTICS** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: **Tabitha Rosembert** and **Ketul Chaudhary**

- **Matthew Spence** (Kevin Burns Group) *Biodistribution of Human Endothelial Colony Forming Cell-Derived Exosomes in Ischemia/Reperfusion Acute Kidney Injury*
- **Peter Feige** (Michael A. Rudnicki Group) *Molecular regulation of brown adipogenic lineage specification from muscle stem cells*
- **Miriel Ho** (Duncan Stewart Group) *Induced pluripotent stem cell-derived endothelial cells promote maturation of multipotent lung progenitors towards an alveolar epithelial cell phenotype*
- **Marissa Lithopoulos** (Bernard Thébaud Group) *Remote Organ Injury: Neural Progenitor Cells Are Impaired in Experimental Bronchopulmonary Dysplasia*

4:50 PM **AWARDS AND CLOSING REMARKS**
Award presentation sponsored by the Centre for Commercialization of Regenerative Medicine
Moderators: Duncan Stewart and Fraser Scott

5:05 PM **RECEPTION AND CASH BAR**

ORAL PRESENTATIONS:

NEUROSCIENCE (8:30 – 9:20)

Moderators: **Vignan Yogendrakumar** and **Nathaniel Noblett**

1-1

Chondroitin Sulfate Proteoglycans Impede Oligodendrocyte Differentiation Through Rho Kinase and Myosin II

Sarah Cummings[1,2], Samantha Kornfeld[1,2], Rashmi Kothary[1,2,3]

1. Regenerative Medicine, Ottawa Hospital Research Institute
2. Cellular and Molecular Medicine, University of Ottawa
3. Department of Medicine, University of Ottawa

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. While these factors are known to inhibit the differentiation process of the OPC, the mechanism driving this signal in the cell remains unclear.

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 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 signaling pathways of interest.

Results: We have validated the impact of CSPGs on oligodendrocyte maturation, such that exposure to CSPGs dramatically impedes morphological complexity of the cell, 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 CSPGs in oligodendrocytes. Inhibition of GSK-3ß signaling does not rectify the morphological deficits, nor does GSK-3ß phosphorylation status differ upon CSPG exposure. Contrastingly, pharmacological inhibition of Rho kinase improved the morphological perturbations of oligodendrocyte differentiation in the presence of CSPGs. Inhibition of non-muscle myosin II (NMII) with Blebbistatin treatment improved branching and process extension to a similar extent. Recently, it has become clear that oligodendrocytes are mechanosensitive, and the differentiation process relies on a permissive mechanical environment. Interestingly, the transmission of mechanical cues in the oligodendrocyte relies on Rho kinase/NMII signaling. Ongoing studies will explore the possibility that CSPG-mediated inhibition of oligodendrocyte differentiation occurs, in part, as a result of an altered mechanical environment.

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

1-2

STEP61 links BDNF-mediated disinhibition to NMDAR potentiation in pathological pain processing within the rat and human spinal dorsal horn

Annemarie Dedek[1], J. Xu[2], C. M. Kandegedara[1], A. Silver[1], E. C. Tsai[3,4,5], P. J. Lombroso[2], M. E. Hildebrand[1]

1. Department of Neuroscience, Carleton University
2. The Child Study Center, Yale University School of Medicine, CT USA
3. Neurosciences, Ottawa Health Research Institute
4. Department of Neuroscience, Faculty of Medicine, University of Ottawa
5. Division of Neurosurgery, Department of Surgery, The Ottawa Hospital

The spinal dorsal horn is an essential network for both physiological and pathological pain processing. We have recently shown that in nerve-injured rats, BDNF-mediated disinhibition gates the potentiation of GluN2B-containing NMDA receptors through Fyn kinase activation at lamina I dorsal horn synapses (*Hildebrand et al, Cell Reports, 2016*). We aim to explore whether loss of an associated phosphatase, $STEP_{61}$ (*Xu et al, J Neurochem, 2015*), mediates this pathological coupling in lamina I neurons in rodents, including following chronic inflammation. We paired patch-clamp electrophysiological recordings with pharmacology, behavior, and biochemical approaches to explore mechanisms of lamina I NMDAR dysregulation. An *ex vivo* BDNF model of spinal pathology and an *in vivo* injection of Freund's adjuvant into the hindpaw was used to model pathological pain. In all models, we observed a decrease in $STEP_{61}$ and an increase in pGluN2B and pFyn at lamina I synapses. Downregulation of $STEP_{61}$ was both necessary and sufficient to prime subsequent phosphorylation and potentiation of synaptic NMDARs by BDNF. Importantly, we also showed that inflammatory pain hypersensitivity was reversed by attenuating disinhibition using IP injected acetazolamide, and paired this with biochemical analysis to investigate lamina I synaptic signalling. Our results suggest that $STEP_{61}$ is the molecular brake that is lost to drive potentiation of NMDAR responses following BDNF-mediated disinhibition at lamina I synapses. Thus, $STEP_{61}$ modulation may be a useful pharmaceutical target for treating pathological pain.

1-3

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

Ahmad Galuta[1,2], Catherine Smith[2], Diana Ghinda[2,3], Mahmoud Bedawy[3], Hussam Jabri[3], Mohammad Alshardan[3], Michael Taccone[3], Carolyn Lai[3], Jessica Rabski[3], Suzan Chen[2], Eve C. Tsai[1,2,3]

1. Neurosciences, Faulty of Medicine, University of Ottawa
2. Neurosciences, Ottawa Hospital Research Institute
3. Division of Neurosurgery, Department of Surgery, The Ottawa Hospital

Background: The spinal cord harbors neural stem and progenitor cells (NSPCs) that are recruited following traumatic injury. In mammals, spinal cord NSPCs do not effectively replace lost cells but can be modulated in animal models to promote regeneration and restore spinal cord functioning. However, it is unclear how efficiently adult human SC NSPCs can be modulated towards similarly beneficial fates.

Objective: To identify species specific differences in the proliferation and differentiation behaviour of adult human and rat NSPCs which are cultured and assessed identically for their response to environmental signals using an in vitro assay.

Methods: Primary- and secondary-derived NSPCs (pd- and sdNSPCs) of adult human and rat SC were cultured using the neurosphere assay. Spontaneous differentiation was induced by serum supplementation (1%), while directed differentiation was induced by administration of signaling factors: RA, PDGF-AA, and BMP4 to promote neuronal, oligodendrial, and astroglial differentiation, respectively. Proliferation rate was assessed by treating NSPCs with mitogens and quantifying BrdU incorporation within the last 24 hour period of culture. Pd- and sdNSPCs were treated for 7 or 14 days, fixed, and then characterized for differentiation (β-III tubulin, GFAP, O4), stemness (Sox2), proliferation (BrdU), and death (TUNEL) using immunocytochemistry.

Results: Upon spontaneous differentiation, rat pdNSPCs favored a glial phenotype (74.6±6.7%) consisting largely of astrocytes (71.0±4.2%) while human NSPCs predominately formed neurons (68.5±16.9% for pdNSPCs; 57.9±14.6% for sdNPCs) with little gliogenesis (<2%). Neuronal differentiation of human and rat NSPCs could be enhanced (>2 fold) with RA treatment, while BMP4 only induced human NSPCs to form more astrocytes. Finally, the proliferation rate of rat pdNSPCs (2.3±0.8) is greater compared to humans, but no differences in BrdU incorporation among rat and human sdNSPCs.

Conclusion: This study provides the first direct comparison of the proliferation and differentiation profiles of adult human and rat spinal cord NSPCs. Human NSPCs possess distinct proliferation qualities, tend to differentiate more into neurons and respond differently to exogenous factor stimulation than rat NSPCs. This information is important to successfully translate therapeutic strategies based on rat NSPC studies to humans.

1-4

Incidental Effects of a Mindfulness-Based Stress Reduction (MBSR) Program on Cognition Among Breast Cancer (BC) Survivors

Alicia Duval[1], Barbara Collins[1,2,3], Eve-Ling Khoo[1], Heather Romanow[1], Patricia Poulin[1,2,3]

[1]Ottawa Hospital Research Institute
[2]Carleton University
[3]The Ottawa Hospital

Background: Many BC patients report adverse cognitive effects of cancer and cancer treatment. In many cases, these cognitive changes are long-lasting and of sufficient severity to affect function and quality of life. Some studies have found that mindfulness training is of benefit in mitigating these cognitive symptoms.

Objective: This study was conducted to determine if an MBSR intervention for neuropathic pain would have incidental beneficial effects on cognition in BC survivors.

Methods: The study was conducted as an optional add-on to a randomized controlled trial examining the effectiveness of an MBSR program in reducing neuropathic pain in BC survivors. Participants were randomized to an MBSR group or a waitlist control group. Cognitive assessment was conducted prior to, 2 weeks following, and 3 months following the intervention in the MBSR participants and at equivalent time intervals in the controls. Cognition was measured objectively with CNS-Vital Signs (CNS-VS), a 30-minute computerized test that assesses attention, memory, executive function, and processing speed. Cognition was also measured subjectively with the Functional Assessment of Cancer Therapy-Cognitive Function (FACT-Cog; Version 3) and the Prospective-Retrospective Memory Questionnaire (PRMQ). Data were analyzed using hierarchical linear modeling.

Results: There were 36 participants in the MBSR group and 37 in the control group. There was no change across sessions in CNS-VS or in FACT-Cog scores and no group difference in the trajectory of these scores across sessions. However, there was a group-by-time interaction on the PRMQ (p = .048), such that the MBSR patients showed a reduction in cognitive complaints after the intervention that was not evident in the controls. Further analyses showed that this was driven by a change in the prospective memory subscale of the PRMQ.

Conclusions: These results suggest that MBSR training benefits prospective memory in BC patients, even when memory is not the target of the intervention. There was no effect of MBSR on objective cognitive test scores or on subjective measures of retrospective memory. *Prospective memory* refers to executing a previously formulated intention at the appropriate moment ("remembering to remember") whereas *retrospective memory* refers to recalling past events or facts. Qualitative studies have found that BC patients report more prospective than retrospective memory failures, however, traditional neuropsychological tests do not include prospective memory measures. This might account for the well-documented discrepancy between subjective and objective cognitive measures in BC patients.

CLINICAL AND PRE-CLINICAL RESEARCH (9:20 – 10:10)

Moderators: **Samantha Kenny** and **Charles Thickstun**

2-1

Long-Term Risk of Recurrence After Stopping Anticoagulants for Acute Unprovoked Venous Thromboembolism: A Systematic Review and Meta-Analysis

Faizan Khan[1, 2], Alvi Rahman[1], Marc Carrier[1, 2, 3], Clive Kearon[5], Jeffrey I. Weitz[5], Sam Schulman[5], Francis Couturaud[6], Paolo Prandoni[7], Sabine Eichinger[8], Paul A. Kyrle[8], Cecilia Becattini[9], Giancarlo Agnelli[9], Harry R. Büller[10], Timothy A. Brighton[11], Gualtiero Palareti[12], Laurent Pinede[13], Mary Cushman[14], Elham Sabri[2], Brian Hutton[1,2], George A. Wells[1,4], Marc A. Rodger[1, 2, 3]; for the MARVELOUS Collaborators

1. School of Epidemiology and Public Health, University of Ottawa, Ottawa, Ontario, Canada
2. Clinical Epidemiology Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada
3. Ottawa Blood Disease Centre, Department of Medicine, The Ottawa Hospital, Ottawa, Ontario, Canada
4. Cardiovascular Research Methods Centre, University of Ottawa Heart Institute, Ottawa, Ontario, Canada
5. Department of Medicine, McMaster University, Hamilton, Ontario, Canada
6. Department of Internal Medicine and Chest Diseases, Brest University Hospital, Brest, France
7. Department of Cardiovascular Sciences, Vascular Medicine Unit, University of Padua, Padua, Italy
8. Department of Medicine I, Medical University of Vienna, Vienna, Austria.
9. Internal and Cardiovascular Medicine, Stroke Unit, University of Perugia, Perugia, Italy.
10. Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands.
11. Department of Haematology, Prince of Wales Hospital, Sydney, Australia.
12. University of Bologna, Bologna, Italy.
13. Department of Internal Medicine, Infirmerie Protestante, Caluire - Lyon, France
14. Department of Medicine, University of Vermont College of Medicine, Burlington, USA

Background: The optimal duration of anticoagulant therapy (AT) for patients with a first unprovoked venous thromboembolism (VTE), remains a crucial clinical dilemma. Guidelines recommend indefinite duration of AT for all unprovoked VTE patients with non-high bleeding risks, but this is based on weak evidence. Deciding to stop anticoagulants after the initial 3-6 months of treatment or to continue anticoagulation indefinitely, is primarily governed by the long-term risk of recurrent VTE when AT is discontinued. This risk however, is uncertain, hindering decision making.

Objective: We aimed to establish the absolute, long-term risk of recurrence at 1, 2, 5, 10 and 20 years after stopping anticoagulation in patients with a first unprovoked VTE.

Methods: We conducted a systematic review and meta-analysis of randomized controlled trials (RCT) and prospective observational studies (OBS) involving unprovoked VTE patients who had completed at least 3 months of initial AT; and who were followed-up for the standardized time intervals of 1, 2, 5, 10, and 20 years (± 3 months) after stopping anticoagulation. The primary outcome of the rate of recurrent VTE was calculated for each observational study and each applicable arm of the RCTs, from the total number of recurrent events and the corresponding number of patient-years of follow-up. We used a random-effects model to pool study results.

Results: Of the 896 records identified, we deemed 18 studies to be eligible for our analysis, and contacted the primary investigator of each study for required additional data. Based on currently available data obtained from 15 studies involving 5,559 patients, the absolute rate of recurrent VTE was 9.65 events per 100 patient-years (95% CI, 8.28-11.11) within the first year, 8.69 events per 100 patient-years (95% CI, 7.15-10.38) within the first 2 years, 6.50 events per 100 patient-years (95% CI, 5.26-7.86) within the first 5 years, and 5.74 events per 100 patient-years (95% CI, 4.64-6.94) within the first year 10 years after discontinuing anticoagulants.

Conclusions: To the best of our knowledge, this is the first, large meta-analysis to provide precise estimates of the long-term risk of recurrence after a first unprovoked VTE. Our study demonstrates that the absolute rate of recurrent VTE peaks in the first year after stopping anticoagulants, and then continuously decreases over time. These results are practice-defining as they fill a knowledge gap in the decision-making process regarding the optimal duration of anticoagulation, and emphasize an individualized, patient-centered approach for treatment of unprovoked VTE.

2-2

Opening Pandora's box: assessing what's published in predatory journals

David Moher[1,2,†, ††, *] , **Larissa Shamseer**[1,2,††], Kelly D Cobey[1,2,3,††], Manoj M Lalu[1,2,4,5], James Galipeau[1], Marc T Avey[1,2], The STudy Reporting In Predatory journals (STRIP) Group[‡]

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Background: Entities that have become known as 'predatory' journals and publishers are disrupting the world of scholarly open access publishing, yet little is known about the papers they publish.

Objective: In this research, we set out to: 1) Characterize what biomedical research is being published in predatory journals, and, 2) Determine the completeness of reporting of the most common clinical study designs.

Methods: We examined a selected cross-section of 1907 human and animal biomedical studies from journals appearing on Beall's Lists of 'potential, possible, or probable predatory scholarly open-access' journals and publishers, recording their study designs, extracting their epidemiological characteristics, and assessing their reporting quality.

Results: More than two million humans and over eight thousand animals were included in the predatory publications we evaluated. Only 40% of studies report having ethics approval (n=724). Of the 17% of articles reporting their funding source (n=323), the US National Institutes of Health was most frequently named (n=41 studies). Corresponding authors were most often from India (511/1907, 27%), followed by the US (288/1907, 15%). Overall, however, the majority of corresponding authors (n=1067, 57%) were based in higher income countries. The reporting quality of work reported in our sample was poor and worse than contemporaneous samples from the legitimate literature; many studies were missing key methodological details and findings.

Conclusions: Our findings indicate that predatory publishing is a problem affecting biomedical researchers globally, not limited to those in lower income countries as current narratives suggest. These entities also raise important ethical concerns since the research they publish is not consistently indexed in scientifically curated biomedical databases. The result is an enormous waste of research. Not only have millions of patients have contributed time and effort with the hope of benefiting others, studies published in predatory journals are difficult or nearly impossible to detect, limiting their future usefulness. We estimate that data from at least 52 million humans and animals are tucked away in predatory journal publications. Funders and academic institutions need to develop explicit policies to drive grantees and prospective authors away from these entities.

2-3

Efficacy and safety of Chimeric Antigen Receptor T-cell (CAR-T) therapy in patients with hematologic and solid malignancies: A systematic review and meta-analysis

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Background: Patients with relapsed or refractory malignancies have a poor prognosis. Immunotherapy with Chimeric Antigen Receptor T (CAR-T) cells redirects a patient's immune cells against a tumor antigen. CAR-T cell therapy has demonstrated promise in treating patients with several hematologic malignancies, including acute B cell lymphoblastic leukemia and B cell lymphomas. CAR-T cell therapy for patients with other solid tumors is also being tested. Safety is an important consideration in CAR-T cell therapy given the potential for serious adverse events, including death.

Objective: We reviewed controlled and uncontrolled interventional studies of CAR-T cell therapy to examine the safety and efficacy of this treatment in patients with relapsed or refractory hematological malignancies.

Methods: We searched MEDLINE, including In-Process and Epub Ahead of Print, EMBASE, and the Cochrane Central Register of Controlled Trials. Studies were screened by title, abstract, and full-text independently and in duplicate. Studies that investigated CAR-T of any chimeric antigen receptor construct targeting antigens in patients with hematologic malignancies were included. Outcomes extracted included complete response rate (primary outcome), overall response rate, overall survival, relapse, and adverse events. A meta-analysis was performed to synthesize the prevalence of outcomes reported as proportions with 95% confidence intervals. The potential for bias within included studies was assessed using a modified Institute of Health Economics tool. Heterogeneity of effect sizes was determined using the Cochrane I2 statistic.

Results: 1259 studies were screened and 35 single-arm interventional studies were identified (n= 374 patients) that treated patients with hematologic malignancies (such as lymphoma, leukemia, and myeloma). Studies used both CD19 constructs (n= 30 studies) and non-CD19 constructs (n = 5 studies). Among the 35 studies, 32 (91%) reported our primary outcome of complete response. Meta-analysis demonstrated that 42% of patients who received CD19 CAR-T experienced a complete response [95% CI: 32%, 52%; I2 52%]. Among studies that investigated non-CD19 CAR-T, 22% of patients achieved complete response [95% CI: 6%, 55%; I2 47%]. Within study biases were demonstrated to be minimal based on the results from the modified IHE risk of bias tool. However, nearly all studies lacked an independent assessor, 32 (91%), which could create detection biases.

Conclusions: The present review demonstrated that CD19 CAR-T constructs are effective in patients with hematologic malignancy. However, limitations of single arm interventional studies must be considered when interpreting the results. The high incidence of toxicities and variable effectiveness suggests that further refinement of this therapy is warranted.

2-4

Antimicrobial peptide LL-37 and its truncated peptides, GI-20 and GF-17, exert spermicidal effects and microbicidal impact on Neisseria gonorrhoeae and Escherichia coli, UTI-89

Wongsakorn Kiattiburut [1], Ruina Zhi [1, 2], Seunggee G. Lee [1], Yan Wang [1], Alexander C. Foo [3], Duane Hickling [1, 4], Jeffrey W. Keillor [3], Natalie K. Goto [3], Weihua Li [2], Wayne Conlan [5], Guangshun Wang [6] and Nongnuj Tanphaichitr [1, 2, 6, 7]

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Background: Antimicrobial peptide LL-37 can kill various sexually transmitted and urinary tract infection microbes. Also, our work indicates its spermicidal effects. Microbicidal/spermicidal activities of LL-37 may link to its alpha helical structure. While LL-37 could be developed into a vaginal spermicide/microbicide, the cost of its synthesis is a challenge. Truncated peptides with equivalent spermicidal/microbicidal activities to LL-37 may therefore be used instead of LL-37. In fact, truncated LL-37 peptides, GI-20 and GF-17, with sequences encompassing the alpha helix region, have similar microbicidal activities to LL-37.

Objective: Determine whether GI-20/GF-17: 1. have LL-37-equivalent spermicidal activity with dependence on their helical structure, and 2. are antimicrobials to *N. gonorrhoeae* and uropathogenic *E. coli*, UTI-89, but not to vaginal commensal *Lactobacillus* crispatus.

Methods: Sperm motility, intactness of the plasma membrane and acrosome, and ability to fertilize eggs *in vitro* were assessed as described by Srakaew et al. (2014). Sperm with or without peptide were transcervically injected into naturally cycling mice, and % *in vivo* fertilization was calculated from the number of [2-cell embryos] / [2-cell embryos+unfertilized eggs]. d8-GF-17, a mutant GF-17 peptide, with reduced helicity was also evaluated for sperm fertilizing ability. Antimicrobial effects (MIC/MBC) of each peptide were determined following standard methods.

Results: GI-20/GF-17 induced immotility, plasma membrane damage and premature acrosome reaction dose-dependently on sperm. These deleterious effects were reaching the plateau at 10.8 µM of GI-20/GF-17 for human sperm and 3.6 µM for mouse sperm. At 3.6 µM, peptide pretreated mouse sperm did not fertilize eggs both *in vitro* and *in vivo*. These results indicated the equivalent spermicidal efficacy among GI-20/GF-17 and LL-37. In contrast, d8-GF-17 acted minimally as a spermicide, results indicating the importance of helicity in spermicidal activities. At 3.6-10.8 µM, all three peptides exerted bactericidal effects to UTI-89, and bacteriostatic activity on *N. gonorrhoeae*, but only LL-37 and GI-20 could kill *N. gonorrhoeae*. However, LL-37 had no antimicrobial effects on *L. crispatus*, whereas GI-20 and GF-17 induced a bacteriostatic and bactericidal action, respectively.

Conclusion: The spermicidal efficacy of GI-20/GF-17 was the same as LL-37 and likely dependent on their helical structure. All three peptides were effective antimicrobials against *N. gonorrhoeae* and *E. coli*, UTI-89. With lower costs to synthesize GI-20/GF-17, these two peptides are attractive to be developed into vaginal spermicides/microbicides. Although GI-20/GF-17 showed some adverse effects on *L. crispatus*, vaginal commensal bacteria, their vaginal application can be implemented together with lactobacilli probiotics.

CANCER STRATEGIES (1:10 – 2:00)

Moderators: **Amelia Aitken** and **Adrian Pelin**

3-1

Characterizing Natural Killer cell Suppressors: A closer look at surgery induced myeloid derived suppressor cells in cancer surgery patients.

Leonard Angka[1,2], Michael A. Kennedy[1], Manahil Sadiq[1], Ahwon Jeong[1], Christiano Tanese de Souza[1], Laura Kuhlmann[3], Thomas Kislinger[3], Rebecca C. Auer[1,4].

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4. Department of Surgery, The Ottawa Hospital

Background: Surgery, while required for curative tumour resection, is accompanied by adverse physiological changes, including a prolonged period of immunosuppression. Specifically, Natural Killer (NK) cells display markedly reduced cytotoxicity and IFNγ cytokine secretion following stimulation and this has been linked to the development of metastases. Previously, we have shown, in a murine model of surgical stress, that myeloid derived suppressor cells (MDSCs) accumulate following surgery with enhanced ability to suppress NK cell cytotoxicity (NKC) and facilitate metastases formation. We hypothesized that MDSCs would also accumulate in patients following cancer surgery with unique phenotypic characteristics.

Objective: Identify therapeutic targets restricted to the suppressive MDSC population that accumulates post-operatively in cancer surgery patients.

Methods: Blood was donated by patients (n=18) at the Ottawa Hospital before surgery and on post-operation day (POD) 1. Peripheral blood mononuclear cells were isolated by density centrifugation and all experiments were performed immediately following isolation. Immunophenotyping was done via flow cytometry and cell surface proteomics using a glycocapture approach. Giemsa-wright stain was used to assess MDSC morphology. Using a flow cytometry based killing assay, the capacity of isolated MDSCs to suppress NKC of human NK92-MI cells against K562 leukemia was determined *ex vivo*.

Results: A unique population of immature myeloid cells expressing $CD33^{+}CD14^{+}CD15^{lo/-}Lin^{-}$ and $HLA\text{-}DR^{lo}$ consistently expand ~2.4 fold ($p=0.0001$; n=18) on POD1 irrespective of the cancer or surgical procedure. Giemsa-wright staining further reveals their distinct immature morphology (promonocytic/promyelocytic) in comparison to mature monocytes and neutrophils. Importantly, in a co-culture assay these cells suppress NKC when isolated from patients postoperatively, as compared to an equal number of cells isolated from the same patient at preoperatively. Moreover, equal numbers of neutrophils isolated from matched patients were unable to suppress NKC. There is a high level of expression of known inhibitors of immune cell function including IL-4Rα and VISTA restricted to these suppressive cells compared to their neutrophilic counterparts. Finally, preliminary surface proteomics of $CD33^{+}$ cells have revealed potential surface marker targets which are detectable only on POD1 which require further validation by flow cytometry.

Conclusion: We have identified an MDSC phenotype that consistently and robustly expands following surgical stress which has been incompletely characterized in literature. Further characterization of these cells will deepen our knowledge of how these MDSCs suppress the immune system in order to devise a targeted perioperative strategy to improve cure rates following cancer surgery.

3-2

Exploring Novel Enhancers of Autologous Tumour Vaccine Strategies

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Background: Colorectal cancer was the second leading cause of cancer-related deaths in Canada in 2016, thus highlighting the continuing need for novel therapeutic options. Autologous whole cell tumours vaccines are immunotherapies consisting of a patient's own tumour cells often rendered more immunogenic through processes such as irradiation. Infected cell vaccines are further prepared by infecting these autologous preparations with oncolytic virus (OV), which are a class of multi-modal biotherapeutics that be effectively combined with viral sensitizing (VSes) compounds to potentiate their anti-tumour activity. Autologous vaccines are useful immunotherapies as they present the host immune system with essentially every putative tumour antigen for targeting and can harness the immunostimulatory properties of OV infection to combat malignancies that may be refractory to direct virotherapy.

Objective: To develop and optimize a highly efficacious autologous vaccine strategy for the treatment of colorectal cancers. Furthermore, we will be investigating the ability of additional immunogenic factors such as VSe compounds to further enhance the performance of our autologous vaccine platform.

Methods: Autologous and infected cell vaccines were prepared by γ-irradiating CT26 wild-type murine colon cancer cells and subsequently infecting with oncolytic virus. These vaccine preparations were then investigated using our syngeneic murine tumour models and as well as for factors of immunogenicity such as the strength and quality of the induced anti-tumour immune response.

Results: In this context we have interestingly demonstrated that a heterologous combination of irradiated and infected cell vaccine doses can improve the resulting anti-tumour cytotoxic T cell response, as well as improve survival in our tumour challenge models. Furthermore, preliminary results indicate a potential for VSes to improve vaccine efficacy and this will be further investigated.

Conclusion: The combination of immune-modulating VSes and our heterologous vaccine strategy represent an interesting platform for immunotherapy of colorectal cancers, as well as a potentially wide range of tumour types. Moving forward, we will be continuing to optimize both the vaccination regimen and combination with VSes. It is hoped that this will lead to a consistent and highly effective immunotherapy option for the treatment of cancer.

3-3

Mechanisms of Transforming Growth Factor Beta 1-Mediated Ovulatory Wound Repair

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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. Wound repair in other systems requires re-epithelialization, and maintenance of a stem cell population, both thought to be regulated by an epithelial-to-mesenchymal transition (EMT). We have previously shown the mouse OSE (mOSE) contains a population of cells exhibiting stem cell characteristics (stemness). Treating OSE cells with Transforming Growth Factor Beta 1 (TGFB1)—a factor found in the follicular fluid and known to induce an EMT—enhances their stemness. We hypothesize that TGFB1 in the follicular fluid aids the ovulatory wound repair process by increasing OSE stemness and regulating the re-epithelialization by inducing an EMT.

Objective: To elucidate the mechanisms by which TGFB1 mediates EMT and induces stemness to facilitate ovulatory wound repair.

Methods and Results: TGFB1 treatment of mOSE cells induces an EMT that is characterized by increased *Snai1* and decreased *Cdh1* expression, and increased cell migration. Overexpression of *Snai1* in mOSE cells similarly increased migration and sphere formation. A TGFB signaling targets PCR array identified an 8-fold increase in *Ptgs2* in mOSE cells treated with TGFB1. Constitutive *Ptgs2* expression did not promote an EMT or stemness, but enhanced cell survival under common wound repair stressors such as hypoxia and reactive oxygen species. Since BRCA1 regulates stemness in mammary epithelium, we explored the possible role of BRCA1 in modulating OSE stemness. TGFB1 treatment decreased *Brca1* expression and deletion of *Brca1* increased expression of stem cell markers and sphere formation, suggesting that the increase in stemness induced by TGFB1 is mediated by repression of *Brca1* expression. These key gene expression changes and increased sphere formation in response to TGFB1 treatment are replicated in human OSE cells, suggesting the results in mouse cells can be translated to human cells.

Conclusions: These data suggest that TGFB1 promotes ovulatory wound repair by inducing an EMT and stemness through the upregulation of *Snai1*. The stemness phenotype results, at least in part, from the TGFB1-mediated repression of *Brca1*. The induction of pathways that promote cell survival, while simultaneously increasing stemness characteristics and repressing *Brca1* could render OSE cells more susceptible to transformation during ovulatory wound repair. This work provides novel insights as to why ovulation is the primary non-hereditary risk factor for ovarian cancer.

3-4

A Dose Escalation Study of Total Marrow Irradiation and Autologous Stem-Cell Transplantation for Relapsed Multiple Myeloma Patients

Amarilis Figueiredo[1], Rajiv Samant[2,3], Harold Atkins[1,3,4,5], Jason Tay[6], Medical Physicists, Grizel Anstee[4,5], Peter Cross[2], Lynn Montgomery[2], Lee Gerig[2], Dave Wilkins[2], David Allan[4,5], Christopher Bredeson[4,5], Lothar Huebsch[4,5], Natasha Kekre[1,3,4,5], Andrea Kew[3,4,5], Arleigh McCurdy.[1,3,4,5]

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Background

Local irradiation results in long-term control and even cure of solitary plasmacytomas, but traditional total body irradiation (TBI) elicits excessive normal tissue toxicity limiting dose escalation for multiple myeloma patients. Intensity modulated radiation, a more targeted form of irradiation to the entire marrow (total marrow irradiation or TMI), delivers up to 70% less radiation to adjacent normal tissues compared to standard TBI approaches. We postulate that TMI will allow delivery of higher doses of radiation to the marrow which might improve outcome of patients with myeloma.

Objectives

Primary outcomes are to determine safety and maximum tolerated dose (MTD) of TMI. A secondary outcome was time to next treatment (TTNT).

Methods

This is an ongoing dose-escalation study of TMI as sole conditioning for salvage autologous hematopoietic stem-cell transplant (TMI-SCT) in radiation-naïve myeloma patients failing a first-line melphalan-aHSCT (Mel-SCT). Measurable myeloma and stored $CD34^{+}$cells ($\geq 2.5 \times 10^{6}$/kg) were study prerequisite. TMI doses were given twice daily starting with 14Gy, then one fraction of 2Gy was added to successive cohorts of three patients. Acute and long-term toxicity were graded using Bearman scale and LENT-SOMA respectively.

Results

Fourteen patients were transplanted between January-2010 and May-2017. Three patients received 14Gy, 16Gy, 18Gy and 20Gy. One patient received 22Gy and one patient scheduled for 22Gy received 19.8Gy because of early mucositis. Median age at TMI was 59.5 years and median time between Mel-SCT and TMI-SCT was 2.9 years. Increase in TMI dose was associated with mucositis worsening ($p<0.01$), but not with narcotics usage, or fever. Xerostomia was the most common long-term toxicity observed, and it was graded LENT-SOMA≥2 in 63.6%, 38.5% and 25% of the patients at D100, 6 months and 12 months post-TMI respectively. There was no difference in length of hospitalization, neutrophil engraftment, and incidence of acute toxicity between TMI-SCT and Mel-SCT.

Median TTNT was 1.6 year after second-line TMI-SCT and 2.0 years after first-line Mel-SCT ($p=0.21$). Patients' response status was deepened after TMI-SCT and it was similar to responses achieved after Mel-SCT.

Conclusion

When compared to a well-established conditioning, TMI was safe and disease control was encouraging. MTD was not reached. Further increase in TMI dose and longer follow-up are needed for better investigation.

BIOTHERAPEUTICS (4:00 – 4:50)

Moderators: **Tabitha Rosembert** and **Ketul Chaudhary**

4-1

Biodistribution of Human Endothelial Colony Forming Cell-Derived Exosomes in Ischemia/Reperfusion Acute Kidney Injury

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3. Division of Hematology, Department of Medicine, Ottawa Hospital Research Institute, University of Ottawa

Background

Acute kidney injury (AKI) is a highly prevalent clinical disorder with significant mortality and no current treatment. Patients who recover from AKI are at increased risk for chronic kidney disease. We have previously shown that infusion of human cord blood endothelial colony-forming cell (ECFC)-derived exosomes exerts protective effects in a mouse model of ischemia/reperfusion (IR) AKI (Viñas et al. Kidney Int 2016). ECFC exosomes are enriched with miR-486-5p, which protects against endothelial cell apoptosis. The in vivo localization of these exosomes and the possible targeting mechanisms represent a crucial step in determining their therapeutic potential in AKI.

Objective

The aims of the present study were to i) investigate the biodistribution of ECFC exosomes in vivo and the transfer of miR-486-5p to tissues in AKI, and ii) study potential targeting mechanisms, including the role of the chemokine receptor type 4 (CXCR4)/stromal-derived factor (SDF)-1α axis.

Methods

ECFC-derived exosomes were isolated by differential centrifugation and characterized using nanoparticle tracking analysis, electron microscopy, and immunoblot. Kidney IR injury was induced in FVB mice by bilateral renal vascular clamping (30 min), followed by reperfusion, and animals were studied after 30 min, 4 hrs, or 24 hrs. The IVIS Optical Imaging system was used to measure organ biodistribution of DiR-labeled ECFC exosomes, and tissue levels of miR-486-5p were quantified by qPCR. The role of the chemokine SDF-1α in exosome targeting was studied in cultured human umbilical vein endothelial cells (HUVECs) with a blocking antibody to SDF-1α.

Results

In mice, i.v. injection of exosomes at the time of reperfusion increased kidney miR-486-5p levels after 30 min, 4 hrs, and 24 hrs of reperfusion ($p<0.01$ vs AKI alone, $n=3$) with no significant change in miR-486-5p levels in liver, spleen, heart and lungs. Optical imaging showed selective homing of exosomes to kidneys after 30 min ($p<0.01$ vs sham, $n=4$) and 4 hrs of reperfusion ($p<0.05$ vs sham, $n=4$), but not at 24 hrs. By western blot exosomes expressed CXCR4 and incubation of HUVECs with a neutralizing antibody to SDF-1α completely prevented the uptake of labeled exosomes, while non-specific immunoglobulin had no effect on uptake ($p<0.001$ vs control, $n=3$).

Conclusions

These data indicate that ECFC exosomes home to the kidneys post-AKI, and this may involve interaction of CXCR4 with endothelial cell SDF-1α. The selective targeting of exosomes enriched with miR-486-5p to ischemic kidneys may represent a key advantage for the potential treatment of human AKI.

4-2

Molecular regulation of brown adipogenic lineage specification from muscle stem cells

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Brown adipose converts excess energy to heat, and thus plays a central role in thermoregulation. The stimulation of brown adipogenesis has been suggested to be a potential treatment for obesity. During embryonic development, brown adipose and skeletal muscle progenitors are derived from a Pax7-expressing muscle stem cell. In adult muscle, Pax7+ muscle stem cells (satellite cells) similarly give rise to both lineages. We discovered a role for microRNA-133 (miR-133) as a lineage switch in muscle stem cells controlling the switch between myogenic versus brown adipogenic specification. Antagonizing miR-133 during muscle regeneration leads to de novo brown adipocyte generation from muscle stem cells, 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. We found Pifithrin-□ (p53 transactivation inhibitor) to be a potent inhibitor of miR-133 expression in mouse and human myoblasts and to stimulate brown adipose determination in primary 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-□ 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-□ and other transient p53 inhibitors may hold potential as anti-obesity compounds.

4-3

Induced pluripotent stem cell-derived endothelial cells promote maturation of multipotent lung progenitors towards an alveolar epithelial cell phenotype

Miriel Ho[1] and Duncan Stewart[1,2]

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Background: Recellularization of lung matrices with cells derived from recipient induced pluripotent stem cells (iPSC) offers promise for creating transplantable bioengineered lung constructs. This personalized therapy is advantageous as it mitigates the burden of donor-compatible lung shortages; and circumvents potential graft immune-rejection. The critical functional unit of the lung is the air-blood barrier formed by endothelial cell-alveolar epithelial cell juxtaposition; which gases can diffuse efficiently. However, the switch from proximal lung epithelium, differentiated from PSCs, to a distal alveolar nature has proved elusive.

Objective: This study investigates the role of human iPSC-derived endothelial cells (iECs) in influencing the cell fate decision of *de novo* lung progenitors.

Methods: Human iPSCs, cultured on Matrigel, were differentiated into iECs using BIO and VEGF. VE-cadherin^{+} cells were selected by immuno-magnetic beads, expanded and then their conditioned medium (iEC-CM) was collected. Lung progenitors (LPs) were derived from human iPSCs via a definitive endoderm intermediate, followed by selection of CXCR4^{+} cells which were subsequently treated with Wnt agonist, bone morphogenetic protein-4, and fibroblast growth factors. Multipotent CPM^{+}/EpCAM^{+} LPs were cultured in media with and without 50% iEC-CM for 21 days at the air-liquid interface.

Results: Compact iPSCs colonies exhibited well-delineated borders that were gradually loss during differentiation. iECs grew in a "cobblestone" monolayer; whereas LPs displayed high cytoplasmic-to-nuclear ratio and bronchial epithelial cell morphology. QRT-PCR confirmed significant decreases in all pluripotency genes (*NANOG*, *OCT4*, *SOX2*) by ≥69% ($^{\#}p<0.01$) upon differentiation of hiPSCs into iECs or LPs. Successful EC differentiation was verified by flow cytometry studies showing ≥88% of the population expressing VE-cadherin and PECAM-1. Lung progenitor-associated markers, *NKX2.1* and *SOX9,* were highly upregulated by ≥5-fold ($^{*}p<0.05$) in the LP population compared to undifferentiated hiPSC or iECs. Notably, iEC-CM-treated LPs showed enhanced expression of alveolar type I and II pneumocytes (*AQP5*, *SFTPB*, *SFTPC*) by ≥3-fold ($^{*}p<0.05$) compared to LPs cultured in basal lung media. These changes were also accompanied by a concomitant reduction of ~50% ($^{*}p<0.05$) in *FOXJ1* and *MUC5AC*, hallmarks of proximal lung epithelial cells. This gene expression pattern is highly suggestive of an alveolar epithelial cell phenotype in contrast to proximal airway differentiation.

Conclusions: Our results support ECs instructing LP cell fate during early morphogenesis through paracrine mechanisms. Preliminary studies indicate that this effect is partially attributed to EC-exosome secretion. Incorporating ECs may represent a novel strategy to bolster generation of distal lung epithelium from hiPSC, and improve tissue graft survival post-recellularization of lung matrices.

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Remote Organ Injury: Neural Progenitor Cells Are Impaired in Experimental Bronchopulmonary Dysplasia

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Background: The most common complication of preterm birth is bronchopulmonary dysplasia (BPD), a chronic lung disease characterized by an arrest in lung growth. Oxygen supplementation, mechanical ventilation, and inflammation are all key factors leading to BPD. BPD is an independent risk factor for adverse neurodevelopment; however, the mechanism of brain injury remains unclear. Neural progenitor cells (NPCs)—cells crucial for brain development—have not yet been examined in BPD.

Objective: To determine whether NPCs are impaired in experimental BPD, leading to adverse neurodevelopment.

Methods: Neonatal mouse pups were exposed to three conditions mimicking BPD: 1) hyperoxia (80-85% oxygen) for 10-14 days; 2) postnatal inflammation and mechanical ventilation for 8 hours; or 3) given a triple-hit of i) antenatal inflammation, ii) hyperoxia (85% oxygen from P0-P14), and iii) postnatal inflammation. Age-matched controls remained in room air and did not receive exposures. Tissue was harvested from P10-P14. The mean linear intercept (MLI) was used to assess lung damage. NPC function was assessed using neurosphere and differentiation assays. *In vivo* neurogenesis was examined using confocal microscopy. Long-term follow-up at 4.5 months and 1 year included a cohort of behavioural tests to evaluate neurodevelopment.

Results: Mice from all three BPD models had significantly higher MLI scores compared to control mice. NPCs isolated from the subventricular zone (SVZ) across BPD models formed significantly fewer secondary neurospheres compared to the control group. Confocal microscopy revealed significantly fewer newborn neurons in the SVZ and dentate gyrus of hyperoxia-exposed mice. At 4.5 months, fear conditioning demonstrated that hyperoxia-exposed mice have significant learning and memory deficits. At 1 year, the Morris Water Maze behavioural test further confirmed this finding.

Conclusions: The high MLI scores of mice from all three BPD models, indicates a dramatic simplification of lung structure, and a robust BPD-like phenotype. Reduction in secondary neurosphere formation of NPCs signifies decreased self-renewal ability in experimental BPD. Fewer newborn neurons in the SVZ and dentate gyrus of hyperoxia-exposed mice, indicates an impairment in postnatal neurogenesis. Follow-up at 4.5 months and 1 year shows that hyperoxia-exposed mice have significant, long-term learning and memory impairments—key cognitive functions linked to postnatal neurogenesis. Together, these results demonstrate that NPCs are perturbed in experimental BPD, leading to long-term adverse neurodevelopment. By providing insight into possible mechanisms of BPD-associated brain damage, this study will help to develop future treatments to improve the lives of preterm infants.

Conditional Inactivation of Lats1/2 in Mouse Ovarian Surface Epithelium Results in High-Grade Serous Carcinoma

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Background: Epithelial Ovarian Cancers (EOC) have been reported to arise mainly from the epithelia of the ovarian surface (OSE) and distal fallopian tube. However, the molecular mechanisms underlying the initiation and progression of EOC derived from the OSE remains unclear. The Hippo signaling pathway controls organ size and tumorigenesis through a kinase cascade that inactivates Yes-associated protein (YAP). Unphosphorylated YAP activates genes involved in cell proliferation and survival, and YAP activation is suppressed by large tumor suppressor family (LATS1/2) members. It has been shown that LATS1 is expressed in human ovarian epithelium, but this expression is decreased in the transition to carcinoma, although the role of LATS1 during this process is unknown.

Objective: Explore the consequences of loss of LATS1/2 in OSE cells.

Methods and Results: OSE cell-specific deletion of *Lats1/2* in the ovaries was achieved by intrabursal injection of adenovirus expressing Cre-recombinase (AdCre) into *Lats1/2*(flox/flox) mice. Ovarian tumors were detected in all injected animals (n=10) after a month. Mice subjected to injection exhibited the tumor in their ovaries and 5/10 animals had ascites with metastases spread throughout the peritoneal cavity. Histologically, these tumors derived from the conditional deletion of *Lats1/2* resembled high-grade serous carcinoma (HGSC) starting from the OSE. Tumor cells were generally intermediate to large in size, with prominent nucleoli. In addition, these cells were extremely proliferative as seen by Ki67 positivity. By immunohistochemistry, the tumors were found to express high levels of YAP protein, as well as PAX8, WT1 and cytokeratin which are characteristic of the majority of HGSCs. Moreover, isolation of OSE cells from *Lats1/2*(flox/flox) mice and loss of *Lats1/2* expression by treatment of the primary cultures with AdCre led to the faster growth of OSE cells. This increased proliferation was associated with the disruption of Hippo pathway signaling, as seen by a decrease in YAP phosphorylation and an increase in YAP-TEAD transcriptional targets, including the growth factors *Ccn2*, *-1*, *-5* and the apoptosis inhibitor *Birc7* ($P<0.05$). Morphologically, the absence of LATS1/2 resulted in more elongated cells that formed a spindle-shaped structure. Assays to determine capacity for substrate-independent growth showed that OSE cells lacking LATS1/2 formed colonies in soft agar while control cells did not.
Conclusion: the data indicate that dysregulation of the Hippo signaling pathway can lead to the initiation and progression of OSE-derived ovarian cancer and loss of LATS1/2 is a potent stimulus for the growth of ovarian serous carcinoma.

WITHDRAWN

New strategies to improve oncolytic virus therapy

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Towards Diagnostic and Therapeutic Targets for Invasive Lobular Carcinoma

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Background: Invasive lobular carcinoma (ILC) is a distinct form of breast cancer responsible for ~15% of breast cancer cases. Patients with ILC are at a disadvantage as the cancer is difficult to diagnose and treat. Consequently, patients with metastatic ILC are at a worse disposition for treatment options, therefore researching the root cause of ILC metastasis could identify new therapeutic targets. MicroRNA (miRNA) are ideal candidates as biomarkers since miRNA are post-transcriptional regulators involved in numerous biological processes, and are known to be stable in plasma, serum and formalin fixed paraffin embedded archival tissues. We decided to investigate the potential of miRNA as prognostic indicators of ILC metastasis due to their regulatory abilities of many biological processes, including invasion, and propensity to being modified in cancers.

Objective: We hypothesize that differential miRNA expression will be observed in invasive and non-invasive ILC. It is further speculated that these differentially expressed miRNA participate in the regulation of pathways that modulate invasion and metastasis.

Methods: Through a PCR-based miRNA genome screen (miRNome) of metastatic vs. non-metastatic patient ILC tissue and ILC cell lines, potential miRNA have been validated for their possible role in invasion. Candidate miRNA were validated by qPCR for differences in expression between the invasive and non-invasive ILC cell lines. Through modifying their expression levels by hairpin inhibitors and mimics, and monitoring their invasive capabilities via Transwell, we will determine whether the miRNA has a role in invasion. miRNA with suspected roles in invasion will be further analyzed to determine their potential targets, which will be modulated to confirm their participation in regulating invasion.

Results: Analysis of the miRNomes generated potential miRNA that may have a role in invasion. Select miRNA were chosen for further identification of their potential role in metastasis/invasion. Of the validated miRNA we will pursue the analysis of the role of miR-3943 in regulating cell invasive properties as it has exhibited an increase in expression in the invasive compared to non-invasive ILC cell lines. We have begun modulating its expression using mimic or hairpin inhibitor miRNA in ILC cell lines and will be evaluating its role in invasion.

Conclusions: This project aims to ascertain the role of miRNA in metastatic ILC, and contribute to identifying biomarkers of metastasis to aide with early diagnosis of metastatic ILC. In addition, to determine therapeutic targets of metastasis to derive new treatment approaches for patients with metastatic ILC.

Studying the Expression and the Function of the Inhibitory PD-1 Receptor on Human Natural killer Cells

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Background: Natural killer (NK) cells are cytotoxic lymphocyte that play an important role in the immune response against tumors. In the tumor microenvironment, checkpoint receptors transduce inhibitory signals to immune cells and block their activity. We showed that cancer cells take advantage of the suppressive activity of checkpoint receptors to escape from NK cells in mouse models of cancer. Particularly, we found that mouse NK cells express and are inhibited by the checkpoint receptor PD-1.

Objective: Our hypothesis is that PD-1 receptor inhibits human NK cells from mediating cytotoxicity against tumors. The aim of this study is to understand the role of PD-1 on human NK cells.

Methods: We will analyze the expression of PD-1 and other checkpoint receptors on NK cells and T cells from cancer patients. We will dissociate tissues obtained from surgical resections of melanoma and lung cancer, or from the peripheral blood, and analyze them by flow cytometry.

Results: Thus far, we have analyzed 5 melanoma and lung cancer samples. We are still optimizing the protocol for tumor dissociation and flow cytometry analysis of immune cells infiltrating the tumor. However, our preliminary results suggest that, at least in some patients, PD-1 is expressed on NK cells within the tumors.

Conclusions: Our data suggest that PD-1 is expressed on human NK cells in the tumor and this may be responsible for the inhibition of NK cell anti-tumor activity. We will confirm these results in more tumor samples and will look at other checkpoint receptors in addition to PD-1.

Loss of the Ste20-like Kinase, SLK in ErbB2-induced mammary tumors drives a Sox10-positive subtype.

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Background: HER2/Neu/ErbB2 is an epidermal growth factor receptor whose overexpression is implicated in approximately 30% of human breast cancers. HER2 expression has been associated with highly metastatic breast cancer and its activation has been shown to mediate epithelial-to-mesenchymal transition. Herceptin in combination with chemotherapy is currently the best form of treatment for HER2-positive breast cancers, however, approximately 70% of patients develop Herceptin resistance. Therefore, identifying novel downstream effectors of HER2-mediated tumorigenesis is a critical step in the development of novel therapeutics.

Objective: Our lab has shown the Ste20-like kinase, SLK is activated downstream of HER2 and is required in part for its pro-migratory effects. We propose to further investigate the role of the Ste20-like kinase, SLK in the development and progression of ErbB2-induced mammary tumor growth.

Methods and Results: Using a HER2-overexpressing mouse line, we generated conditional SLK knockout cells in culture. We used this line to generate stable SLK knockout cells using Adenoviral GFP-Cre followed by sorting the top 30% of GFP expressing cells. SLK-null cells were smaller in size but did not display any differences in growth or motility. Using microarray analysis, we identified that SLK knockout cells have upregulated *Sox10* expression which may compensate for loss of SLK *in vitro*. Deletion of SLK *in vivo* results in a faster tumor onset and a quicker endpoint (40 days, n=25 mice per group). Hyperplastic glands that arise on SLK-null glands are larger in size and show increased nuclear levels of Sox10 by IHC. As Sox10 activates genes responsible for immune evasion, we will analyze components of the immune system by flow to validate the extent of immune infiltration. Using our *in vitro* cell culture system, we have identified that loss of SLK reduces Sox9 phosphorylation at S181 suggesting that Sox9 is less active. Sox9 and 10 have been shown to be antagonistic and is the mechanism by which *Sox10* is induced.

Conclusions: We have shown that loss of SLK significantly reduces Sox9 activity and increases levels of Sox10, which upregulates several target genes that play a role in immune evasion. We believe that this decrease in immune regulation results in the more aggressive phenotype that we observe. Targeting SLK with inhibitors may result in tumor cell evasion of the immune system, however, in combination with immune stimulants should provide a novel therapeutic strategy for treating HER2-positive cancers.

Interaction of glioblastoma with macrophages through CD47/SIRPa

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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 through CD47/SIRPα.

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 at the Ottawa Hospital and macrophages were obtained by differentiating monocytes isolated from the blood of healthy volunteers.

Results: Co-culture of macrophages and glioblastoma cells resulted in increased glioblastoma cell proliferation compared to control. Addition of anti-CD47 to co-cultures of primary glioblastoma cells blocked macrophage stimulated proliferation of glioblastoma cells. Furthermore, addition of anti-SIRPα blocked macrophage stimulated glioblastoma proliferation. Addition of a SHP-1/2 inhibitor to co-cultures, thus blocking downstream signalling of SIRPα, resulted in no change in macrophage stimulated glioblastoma proliferation suggesting the observed increase in proliferation 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 proliferation whereas addition of the PI3Kβ inhibitor resulted in a partial blockage of macrophage stimulated cell proliferation.

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

ATF3 as a key regulator of cisplatin cytotoxicity: combining other ATF3 inducing agents enhance cisplatin activity in NSCLC

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Background

Lung cancer accounts for the majority of cancer-related morbidities and mortalities, with non-small-cell lung cancer (NSCLC) representing 85% of all lung cancers. Platinum-combination chemotherapy is a commonly used treatment against NSCLC and has displayed enhanced efficacy. However, toxicities and resistance associated with this treatment have restrained the five-year survival rate of NSCLC, therefore, there is an urgent need for more effective therapeutic regimens. 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. ATF3 can be induced by a wide variety of environmental and cellular stressors, and through a number of different mechanisms, including the MAPKinase-dependent mechanisms, DNA repair pathways and Integrated Stress Response (ISR). An elevated and sustained expression of ATF3 can push the cell towards apoptosis; therefore, the hypothesis is that an enhanced and sustained induction of ATF3 through the combination of platins and other ATF3 inducers will result in a more effective therapeutic strategy against NSCLC.

Objective

The objective is to further evaluate ATF3 as a key regulator of cisplatin response by establishing ATF3 as a therapeutic target in NSCLC through the identification and combination of novel ATF3 inducers with platins, as well as evaluating its expression in human NSCLC *ex vivo* samples in order to further highlight its clinical relevance.

Methods

Commonly employed NSCLC cell lines, Calu6 and H23, were previously used to establish cisplatin-resistant cell lines, Calu6cisR1 and H23cisR1, respectively. The *in vitro* expression of ATF3 will be targeted using siRNAs. A 1200 FDA approved chemical library screen was previously performed to identify novel ATF3 inducers that enhance cisplatin cytotoxicity, such as vorinostat and topotecan. Finally, ATF3 expression will be evaluated in *ex vivo* NSCLC tumours and their adjacent normal tissue after treatment with cisplatin, vorinostat and topotecan.

Results

The role of ATF3 in cisplatin, vorinostat and topotecan cytotoxicity was confirmed using wild type and mouse embryonic fibroblasts (MEFs). Both vorinostat and topotecan induced ATF3 expression in the parental and cisplatin-resistant cell lines. Furthermore, the combination of cisplatin with vorinostat displayed synergistic cytotoxicity as well as an enhanced ATF3 expression compared to each agent alone in the cisplatin-resistant cell lines.

Conclusion

ATF3 has previously been identified as a key regulator of cisplatin cytotoxicity, however, the combination of cisplatin with other ATF3 inducers, such as vorinostat and topotecan, could represent a novel therapeutic approach against NSCLC.

Investigating the Immunogenicity of a Maraba virus Infected Cell Vaccine (MG1-ICV)

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Background

Therapeutic strategies aimed at harnessing a patient's immune system to target tumour cells have become a powerful ally in the fight against cancer. However, the success of current approaches is dependent upon prior knowledge of tumour associated antigens (TAAs). While these strategies have limited scalability and utility, we have found that Infected Cell Vaccines (ICVs) can be used to generate a robust tumour specific immune response with the potential for use across various tumour types without known TAAs. Particularly, we have shown that vaccination with irradiated tumour cells infected with an oncolytic rhabdovirus (MG1) expressing IL12 can promote tumour specific immune responses and improve median survival in several tumour models. Still, despite its efficacy, little is known about the key mechanisms contributing to the immunogenicity of the ICV and efforts to translate this approach into a clinical setting will require further investigations. It has been well documented that immunogenicity of cellular vaccines is dependent upon the release of immunogenic signals (HMGB1, extracellular ATP, Calreticulin), to stimulate uptake, processing, and presentation by Antigen Presentation Cells (APCs).

Objective

To determine whether immunogenicity of the MG1-ICV can be further optimized to improve APC activating ability.

Methods

Hallmarks of immunogenic cell death were evaluated in murine melanoma cells (B16F10) or murine breast cancer cells (4T1) following irradiation and infection with MG1, treatment with inducers of ICD or appropriate vehicle. The release of HMGB1 (ELISA), ATP (flow cytometry) and externalization of calreticulin (microscopy) were quantified at various time points. Either JAWSII (murine immature dendritic cell line) or primary Bone Marrow Derived Cells (BMDCs) were co-cultured with B16s treated with MG1-ICV, ICD inducers or controls as above and analyzed for changes in markers of activation including MHCI, MHCII, CD86, CD11b, and CD11c by flow cytometry.

Results

Irradiation and infection with MG1 resulted in a substantial decrease in quinacrine signal compared to untreated cells suggestive of increased ATP release. However, the change in ATP was noticeably less than the positive control. Both BMDCs and JAWSII cells demonstrated increases for all three activation markers compared to both negative and positive controls following exposure to MG1-ICV.

Conclusions

This work represents the foundation of characterizing the ICD and APC response of ICVs, which is crucial to understanding how they work and potential strategies for improving their immune stimulatory properties.

IL-12 expression is required in a Maraba infected cellular vaccine for the treatment of an orthotopic pancreatic cancer

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Background: Pancreatic ductal adenocarcinoma cancer (PDAC) is one of the highest fatality cancers with fewer than 10% of patients surviving past 5 years. Although immune modulating therapies have had a dramatic impact in other cancer types they have provided little benefit in PDAC because of an immunosuppressive environment. Infected cell vaccines (ICV) may prove to be an improved immunotherapy for treating PDAC as this whole cell vaccine allows tumour associated antigens to be presented in the context of a viral infection.

Objective: To explore whether an autologous ICV can stimulate a robust, durable anti-tumour immune response and improve survival in a murine model of pancreatic cancer.

Methods: PDAC was established by orthotopically implanting 1e5 Pan02 cells in the tail of the pancreas. Mice were randomized on day 15 into either an untreated group (No Sx) or a group that underwent surgical resection of the primary tumour (Sx). Resected tissue was analyzed by immunohistochemistry and flow cytometry. On day 18 mice from all groups began receiving intraperitoneal injections of either vehicle or ICV prepared by irradiating (60 Gy) Pan02 cells infected with a Maraba MG1 virus expressing the cytokine Il-12 (MG1-IL12, MOI: 10). A total of 6 doses were given twice weekly over a 3 week period.

Results: Histological examination revealed that the orthotopic tumours displayed 5 hallmarks of human PDAC. Further investigation of the resected tissue by flow cytometry revealed the presence of CD45+ cells within the tumour. Although both CD4+ (7.5% of CD45+) and CD8+ (6.8% of CD45+) cells were present suggestive of an immune response directed against the tumour tissue, CD4+Foxp3+ T regulatory cells were also present which is consistent with a poor prognosis in models of PDAC indicative of a potential suppressive tumour microenvironment. Consistent with the presence of an immune suppressive environment we did not find any long term survivors in mice receiving no treatment (n=0/11), Sx (n=0/7) or an MG1-ICV alone (n=0/7). In contrast, 54% of mice (n=6/11) receiving the MG1-IL12 ICV survived long term (>100days). Next the combination of an ICV treatment with surgical resection of the primary tumour was explored, and found to further increase survival of mice over surgery alone.

Conclusions: An ICV expressing the cytokine IL-12 demonstrates therapeutic efficacy in treating murine models of PDAC. Further investigation of integrating this vaccine with other therapeutic approaches including surgical resection may further improve survival in the murine model.

Potentiating Oncolytic Virotherapy in Sarcoma Using Vanadium-Based Compounds

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Background: Sarcomas are a heterogeneous tumour family originating from mesenchymal tissues. Although relatively rare, they are responsible for roughly 15% of pediatric cancers and 13% of cancer-associated mortality in youths. Despite advances in detection, survival rates of osteosarcoma patients have remained essentially unchanged in the last three decades thereby highlighting the need for novel therapeutic approaches.

Objective: This project explores the combination of tyrosine phosphatase inhibitor vanadate with oncolytic rhabdovirus VSV?51 as a novel treatment against sarcomas.

Methods: Cytotoxicity, viral spreading and replication were tested using a panel of murine (S180, 76-9, K7M2), human (HT1080, 143b, U2OS) and canine (D17) sarcoma cell lines. To assess its clinical significance, the combination therapy was tested *in vivo* using syngeneic sarcoma models. Survival, tumour progression as well as viral infection were monitored following intratumoural or intraperitoneal treatments of vanadium compounds and VSV?51.

Results: Vanadate increases cytotoxicity and viral spreading *in vitro* during viral infection. In our murine sarcoma models, the combination therapy led to increased infection within tumours as well as improved survival rates and tumour control.

Conclusions: Oncolytic virotherapy is a promising therapeutic avenue. However, recent clinical evidence reveals that oncolytic viruses may be insufficient to elicit a positive antitumour defense in some patients when administered as single agents. Overall, this project provides insights on the clinical relevance of combining virotherapy with vanadium compounds for the treatment of sarcomas.

Identification and Evaluation of Novel Antigens for Use with Oncolytic Vaccines

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

Methods: This methodology consists 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. We tested intracellular TNFa production as a T cell marker and observed a higher percentage of TNFa+CD8+GFP+ doublets.

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 rhabodmyosarcoma tumour model 76-9 will be incorporated into an oncolytic vaccine therapy.

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

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Background An impairment in early placental development is at the root of placental dysfunctions such as preeclampsia, which causes symptoms at later stages of pregnancy. However, no early biomarker or treatment currently exist. Maternal immunity tightly regulates trophoblast functions such as invasion and syncytialization, during placentation. Crucial to the establishment of pregnancy is the formation of a balance, at the maternal-fetal interface, between Th1-type and Th2-type immunity. Fibrinogen-like protein 2 (FGL2) has known roles in the regulation of this balance and in the formation of fibrin clots, making it a likely player in the pathology of preeclampsia.

Objective We aim to characterize FGL2 expression in previously described molecular subtypes of preeclampsia and to uncover the molecular mechanisms by which differential levels could contribute to each subtype.

Methods We stably overexpressed *FGL2* in invasive human trophoblast cell line HTR-8/SVneo and in syncytium-forming trophoblast cell line BeWo to first characterize the effect of abberrant FGL2 levels on trophoblast function. We measured chorionic villus FGL2 expression in a cohort of preeclamptic and healthy pregnancies. We examined gene expression correlation with FGL2 in the various subtypes of preeclampsia, as well as correlation between FGL2 level and subtype-specific histopathological lesions of the placenta.

Results *FGL2* overexpression did not alter proliferation, invasion or migration in HTR-8/SVneo cells. Preliminarily, *FGL2* overexpression resulted in impaired syncytialization of BeWo cells, demonstrated by reduced increase in expression of syncytium-specific genes after forskolin treatment. We found FGL2 to be most highly expressed in the cytotrophoblast and syncytiotrophoblast of human chorionic villi, suggesting an impairment in syncytialization could have serious consequences on placental function. In our previously identified molecular subtypes of preeclampsia, we found *FGL2* to be differentially expressed across subtypes, particularly high in an immune-driven subtype and particularily low in a canonical subtype. We found that across all subtypes, genes whose expression best correlates with that of *FGL2* are predominantly involved in inflammatory response, suggesting *FGL2* is involved in immune function regulation within the mature placenta. Further analysis is ongoing to evaluate correlation between FGL2 expression and prevalence of subtype-specific histopathological lesions.

Conclusions Aberrant FGL2 level in the placenta could be associated with the development of histopathological lesions that lead to specific subtypes of preeclampsia.

Single-cell RNA sequencing reveals transcriptional dynamics of the epithelial-mesenchymal transition in ovarian cancer

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Background

The epithelial-mesenchymal transition (EMT) is a phenotypic switch, where epithelial cells lose defining characteristics, such as stable cell-cell junctions, and transition towards a mesenchymal state, capable of migration and tissue invasion. It is critical for many biological processes, including embryo development and tissue homeostasis. The EMT is also thought to promote the progression of a variety of cancers, including ovarian cancer, where it is associated with metastasis, chemoresistance, and reduced overall survival. Ovarian cancer is currently the most lethal gynecological malignancy, and developing a comprehensive understanding of the EMT in this context would provide valuable insight into the progression of the disease. Much of the previous work has focused on the signalling cascades involved, and, despite the ubiquity of this process, relatively little is known about the transcriptional determinants coordinating it.

Objective

The objective of this study is to map transcriptional dynamics throughout the EMT in ovarian cancer, and to determine which components are critical for acquiring traits associated with cancer progression.

Methods and Results

To map the transcriptional events coordinating the EMT, we generated single-cell RNA sequencing libraries from the OVCA420 high-grade serous ovarian carcinoma cells that were treated with transforming growth factor beta-1 for 0, 1, 3, or 7 days. After sequencing and filtering, our dataset comprised expression profiles of 10,093 single cells. Principal component analysis revealed a time-dependent structure in the data, supporting that we successfully captured cells along various stages of the EMT. In order to infer the transcriptional dynamics throughout the process, we leveraged the cells' asynchronous response, using pseudotime analysis to map a transcriptional trajectory through the data and order cells based on their position along this trajectory. We then identified differentially expressed genes throughout the trajectory. Clustering these genes based on their pseudotemporal dynamics identified several waves of transcriptional events, including a transient repression of cell cycle genes that is restored towards the end of the trajectory. This repression is followed by the activation of EMT-associated genes, and interestingly, many components of cancer-related pathways are activated following this.

Conclusions

Here, we have generated a comprehensive map of transcriptional changes that occur throughout the EMT in ovarian cancer, resolving waves of gene expression that were previously uncharacterized. This will provide insight into the progression of the disease and may identify novel therapeutic targets. These findings may also be generalizable to other epithelial cancers.

Optimization of the Upstream Production of Vaccinia Virus for Clinical Trials

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Background:
The Biotherapeutics Manufacturing Centre (BMC) is a core facility at the Ottawa Hospital Research Institute that evaluates and manufactures viral and cell-based therapeutics for human cancer clinical phase I/II trials. One of the oncolytic viruses produced large-scale at the BMC is Vaccinia Virus (VV). We currently use HeLa cells for the upstream production of this virus. BSC-40 cells, derived from the African Green Monkey, have been specifically engineered to be particularly susceptible to VV.

Objective:
Here, our project objectives are two-fold – i) to further characterize and optimize upstream production conditions of VV in HeLa cells, and ii) to assess VV yield produced in BSC-40 cells compared to the current standard of HeLa cells.

Methods:
Using a high-throughput method of quantifying VV using fluorescence, we have investigated the effects of different multiplicity of infections and harvest time points on VV yield in HeLa and BSC-40 cells. We plan to confirm fluorescence results using traditional viral plaque assays, and measure genomes by qPCR to assess differences in functional vs. non-functional virus particles between the two cell lines.

Results:
Our preliminary results suggest that BSC-40 cells produce a log higher yield of virus compared to the current standard of practice using HeLa cells.

Conclusion:
Future work will investigate the effects of initial seeding cell density, and cell density at the time of infection on VV yield in both HeLa and BSC-40 cells. We also plan to assess whether VV yield produced in these cells lines at a small-scale is comparable during the scale-up to different vessels (ie. rollerbottles, and hyperstacks). Optimization of the upstream production of VV may ultimately save time and resources in GMP production.

Activating Transcription Factor 3 as a Novel Regulator of Chemotherapy Response in Breast Cancer

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Background:
Anthracyclines, such as doxorubicin, are used as first line chemotherapeutics, usually in combination therapies, for the treatment of advanced breast cancer. While these drugs have been successful therapeutic options, their use is limited due to serious drug related toxicities and acquired tumor resistance.

Objectives:
The goal of this study is to uncovwe the molecular mechanisms that mediate doxorubicin's cytotoxic effect which may lead to the identification of novel more efficacious combination therapies and/or allowing for reduced doses of doxorubicin to be administered while maintaining efficacy.

Results:
In our study we demonstrated that ATF3 expression was upregulated by doxorubicin treatment in a representative panel of human breast cancer cell lines MCF7, T47D, and MDA-MB-231. The upregulation of ATF3 was regulated by multiple cellular mechanisms including the activation of JNK and ATM signaling pathways. Loss of ATF3 expression resulted in reduced sensitivity to doxorubicin treatment in mouse embryonic fibroblasts (MEF)s and the MCF and T47D cell lines. Through a 1200 FDA approved compound library screen, we identified ATF3 inducing agents that enhanced doxorubicin cytotoxicity. The combination of the HDAC inhibitor, vorinostat, or the nucleoside analogue, trifluridine could synergistically enhance doxorubicin cytotoxicity in the MCF7 and T47D cell lines. We have also demonstrated that doxorubicin treatment can induce ATF3 expression in ex-vivo human breast and ovarian tumor samples.

Conclusion:
Taken together our results demonstrate a role for ATF3 in mediating doxorubicin cytotoxicity and provide rationale for the combination of ATF3 inducing agents with doxorubicin as a novel therapeutic approach.

The anti-tumour potential of Natural Killer Cells in prostate cancer

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Background: Prostate cancer remains one of the most lethal cancers, resulting in 11 deaths per day on average in Canada. In order to develop, the prostate tumour evades the immune system by undetermined mechanisms. Several immune cell types are involved in cancer immunosurveillance, including Natural Killer (NK) cells, a subset of cytotoxic innate lymphocytes with potent anti-tumour activity. Clinical studies have correlated NK cell infiltration and activity in prostate tumours with better prognosis. Therefore we hypothesize that *i)* there are inhibitory interactions in the prostate tumour microenvironment that suppress the ability of NK cells to kill cancer cells, and *ii)* that releasing NK cells from such inhibition will result in an effective immune response against the prostate tumour cells with therapeutic benefit.

Objective: We aim to identify the nature of the interactions that inhibit NK cells and allow for prostate tumour development, focusing on inhibitory checkpoint receptors. Ultimately, this knowledge will enable us to identify and develop targeted immunotherapies that harness the impressive ability of the immune system to fight cancer.

Methods: Prostate tumours were generated by injection of a mouse prostate cancer cell line, TRAMP-C2, subcutaneously or intra-prostatically into C57BL/6 mice, and monitored by palpation (intra-prostatic) or caliper measurements (subcutaneous). Select mice were also depleted of NK cells by regular injection of a NKR-P1C antibody. Cultured TRAMP-C2 were stained and analyzed by flow cytometry.

Results: In a preliminary experiment with a small cohort of mice, antibody-mediated depletion of NK cells resulted in more aggressive tumour growth compared to non-depleted controls. These data are in line with flow cytometry analysis of the TRAMP-C2 cell line, revealing expression of ligands for NK-activating receptors, making them a good target for NK cell recognition and killing. Furthermore, TRAMP-C2 cells expressed ligands for the inhibitory checkpoint receptors PD-1 and TIGIT, which may be the source of NK-inhibitory interactions. Lastly, TRAMP-C2 cells are highly susceptible to infection and lysis by the oncolytic virus VSVd51, a potential immunotherapy in this model. Interestingly, TRAMP-C2 up-regulated expression of the PD-1 ligand PD-L1 upon VSVd51 infection.

Conclusions: Our preliminary data suggests that NK cells play a role in the control of prostate cancer in this model. The rationale for two potential inhibitory interactions, and a potential combinatorial immunotherapy strategy, has been identified for further investigation *in vivo.*

Palbociclib treatment activates focal adhesion kinase (FAK) and use of palbociclib in combination with FAK inhibition enhances anti-proliferative activity in breast cancer cells

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Background:
Cell survival and proliferation is a tightly regulated process involving a number of interacting signaling pathways and proteins including the cyclin dependent kinases (CDKs). Palbociclib, a CDK4/6 inhibitor, has recently been shown to be effective in the treatment of estrogen receptor (ER) positive breast cancer, however many patients develop an acquired resistance through as of yet incompletely characterized mechanisms.

Objective:
As one possible mechanism for this resistance has been suggested to be compensation by CDK2, and focal adhesion kinase (FAK) has been previously shown to activate CDK2 in hepatocytes, we were interested in determining whether FAK-mediated upregulation of CDK2 occurs in palbociclib treated breast cancer cells, thereby limiting its efficacy.

Results:
ER positive MCF7, T47D and MDA-MB-134VI breast cancer cell lines were treated with increasing doses of palbociclib. We observed dose-dependent increases in phospho-FAK Y397, a marker of the active kinase following palbociclib treatment. We thus used a selective FAK tyrosine kinase inhibitor in combination with palbociclib, and observed an enhanced ability to inhibit cell viability as compared to use of either drug alone. This was concomitant with observed increases in the CDK4/6 inhibitor p27, and with decreases in phospho-RB which is indicative of reduced CDK2 activity, following treatment with FAK inhibitor.

Conclusions:
Our results highlight FAK activation as an unexpected result of palbociclib treatment which could potentially contribute to resistance due to activation of CDK2 and inhibition of p27.

Characterizing alterations in cytokine-mediated IFN? production in human Natural Killer cells following surgical stress

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Background: Surgical resection of solid tumours is an essential component of curative cancer treatment. However, surgical stress can lead to profound immunosuppression which is associated with increased metastases and disease recurrence in patients. Natural Killer (NK) cells, in particular, suffer a significant decrease in function following surgery. Normally, NK cells secrete IFNγ upon stimulation by cytokines such as IL-2, IL-12, IL-15, and IL-18. The Auer Lab has demonstrated that surgical stress completely block cytokine stimulated IFNγ production from patient NK cells for at least 5 days in the majority of patients. However, the mechanisms mediating this profound suppression remain elusive.

Objective: We hypothesize that the deficiency in IFNγ production following surgery is a result of an impairment in cytokine-mediated signalling events upstream of IFNγ gene transcription.

Methods: NK cells were isolated from the blood of cancer surgery patients prior to surgery and on post-operative day 1 (POD1) and subsequently cultured with cytokines known to stimulate IFNγ production including IL-2, IL-12, IL-15, and IL-18. Supernatants and cells were collected 24 hr later to measure IFNγ production (ELISA) and phosphorylation status of downstream signalling proteins including STAT4, STAT5, NF-kB and MAPK using flow cytometry. In parallel, we extracted RNA from isolated NK cells before and after surgery to assess changes in gene transcription by RNAseq.

Results: In agreement with our previous results, the IFNγ production of NK cells was significantly impaired on POD1 when compared to preoperative levels (3705pg/ml/1e6 cells vs 1679pg/ml/1e6 cells, n=3) when stimulated with a combination of IL-2, IL-12, and IL-15. Despite the significant reduction in IFNγ levels, the phosphorylation of downstream signaling pathway molecules and transcription factors was not decreased following surgery.

Conclusions: There are no significant changes in the activation of signaling pathway leading to the initiation of transcription following surgery. This suggests that the intracellular dysfunction may lie in transcription of the IFNG gene or in translational mechanisms, and further investigation is necessary.

Evaluation of cancer-testis antigen as immunotherapeutic targets for sarcoma

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Introduction: T-cell based cancer immunotherapies are a promising alternative to traditional cancer treatments due to their ability to direct the immune system target and eliminate cancer cells, leaving healthy cells unharmed. Our lab aims to develop a T-cell based immunotherapy for sarcoma. To do so, it is essential to identify a targetable immunogenic sarcoma antigen. Cancer-testis antigens (CTA) are a group of proteins that may serve as tumor-specific antigens for CD8+ T-cell recognition. However, antigen expression is not the only factor that warrants the success of an immunotherapy. Antigen recognition by CD8+T cells is dependent on the expression of a HLA-peptide complex on the surface of the cancer cell. Additionally, the outcome of an immunotherapy depends on the ability of antigen specific CD8+ T-cells to infiltrate sarcoma tumors. The goal of this study is to screen for CTA expression, HLA expression and tumor T-cell infiltration in human sarcoma samples.

Methods: Expression of CTA, HLA, and T-cell infiltration is identified by immunohistochemistry (IHC). We evaluated the expression NY-ESO-1, MAGE-A3, SSX and survivin in human sarcoma samples to identify which antigen is most commonly expressed. Additionally, we quantified the level of expression of CTAs to 1) determine if CTAs are heterogeneously expressed in different sarcoma subtypes and 2) to identify a CTA that is most highly expressed in sarcoma.

Results: Quantification of IHC staining for CTAs revealed positive expression in 78.5% of samples. Further evaluation of CTA expression in each sarcoma subtype revealed a heterogeneous expression pattern of CTAs. MAGE-A3, SSX and survivin are expressed at intermediate to very high levels. Contrastingly, NY-ESO-1 is expressed at low levels in the majority of sarcoma samples (60%). Additionally, evaluation of HLA staining confirmed HLA expression in all sarcoma samples. CD3 staining revealed tumor T-cell infiltration in 76.5% of sarcoma samples.

Conclusion: High expression of MAGE-A3, SSX and survivin in sarcoma samples indicates that these CTAs could be used as immunotherapeutic targets. HLA expression and tumor T-cell infiltration in sarcoma samples suggest that CD8+ T-cells generated in response to an immunotherapy will effectively infiltrate tumor and recognize HLA-antigen complexes on tumor cells. These results will contribute to the development of an immunotherapy for sarcoma.

An Emerging Role for PREX1 in Glioblastoma Microtubes and Invasion

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Background: Glioblastoma multiforme is the most lethal and most common primary brain tumor in adults. A defining feature of glioblastoma is its ability to grow rapidly, infiltrate tissue, and disseminate across the brain. Despite surgical and therapeutic treatment, 9 out of 10 patients experience tumor recurrence due to the infiltration of cancerous cells around the primary tumor site. This emphasizes the need to better understand the mechanisms orchestrating expansion and dissemination of the tumor population. Of these mechanisms, microtubes have an emerging functional role in tumor growth. These actin-based, membranous extensions can persist over long distances and support intercellular communication and the exchange of proteins. Recently, our lab established a role for PREX1, a Rac guanine nucleotide exchange factor, in glioblastoma invasion. We reported a PREX1 knockdown in primary glioblastoma cells that reduced cellular motility and invasion.

Objective: We hypothesize that a CRISPR/Cas9-based knockout of PREX1 in our mouse model of glioblastoma will reduce tumor invasion and spread. Furthermore, we hypothesize that the knockout of PREX1 will disrupt the formation and growth of microtubes in vitro.

Methods: We are using CRISPR/Cas9 to knockout PREX1 in primary glioblastoma cells obtained from patients at The Ottawa Hospital. After we assess the consequence of a PREX1 knockout on motility and invasion in vitro, we will perform intracerebral injections of PREX1-knockout primary cells into mice to assess tumor growth and invasion. Concurrently, we are assessing the role of PREX1 and Rac1 in the formation and growth of microtubes in vitro. This is being investigated using siRNA-mediated knockdowns and our knockout model.

Results: Using CRISPR/Cas9, we have achieved a high efficiency of PREX1 knockout in our primary glioblastoma cells and are using clonal selection to isolate cell populations with full deletion of PREX1. By performing siRNA knockdowns, we have demonstrated that a reduction in PREX1 and Rac1 protein levels impairs the growth of glioblastoma microtubes. Using GFP and mCherry, we have also identified a functional role for microtubes in glioblastoma whereby these structures allow the exchange of proteins between connected cells.

Conclusion: Overall, our results will demonstrate the importance of PREX1 to tumor invasion and will provide an avenue for future studies to therapeutically target the PREX1 pathway in glioblastoma. Thus far, we have shown that microtubes facilitate protein exchange between glioblastoma cells, and that PREX1 and Rac1 are required for their proper formation.

Optimizing the Manufacturing Process of an Autologous Infected Tumour Cell Vaccine for Clinical Use.

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Background: A clear clinical benefit, including long term durable cures, can be achieved following the vaccination of patients with their own cancer cells engineered to express immune modulating cytokines or adjuvants (autologous tumour cell vaccines). The success of the current platforms has been strongly correlated with the strength of the stimulated anti-tumour immune response suggesting strategies with enhanced immunogenicity could provide improved outcomes. Our lab has demonstrated that vaccination with autologous cells infected with a replicating oncolytic rhabdovirus expressing IL-12 is effective in stimulating a potent anti-tumour immune response, delaying tumour outgrowth and improving survival in several murine cancer models.

Objective: To develop an optimized process for manufacturing autologous infected cell vaccines from human cancer patient tumour samples.

Methods: Ascitic fluid (n=25) and primary tumour samples (n=18) were collected from patients diagnosed with lung, kidney, ovarian, liver, colon and breast cancers following informed consent. The cellular component of ascitic fluid was isolated following centrifugation and removal of red blood cells. Primary tumour samples were either sliced into 2 mm sections and cored prior to infection or dissociated using a commercially available kit or various mixtures of collagenase and DNAseI prior to mechanical dissociation. Tumour samples were infected with Maraba MG1 expressing GFP or IL12 and infectivity assessed by microscopy, flow cytometry and titration of supernatants or homegenates by plaque assay.

Results: Cells were collected from the ascitic fluid of 84% of patients (n=21/25). Although viability was high for all isolations (>85%) the average cellular yield was highly variable (mean: $1.36x10^6$/mL; range: $1.7x10^3$/mL – $9.3x10^6$/mL). A positive infection as observed by the presence of GFP was evident in 10 of 21 samples. Viability (61%) and yield ($1.5x10^7$/g of tissue) of dissociated tumour samples was unimpacted by the various dissociation protocols used. Infection of dissociated tumour samples and cores was observed in only 2 of 18 samples. The relatively poor infectability of tumour samples was not impacted by dissociation method or tumour type.

Conclusion: We have identified methods for generating viable single cell preparations from ascitic fluid and surgically resected primary tissue in yields that would permit the manufacture of the vaccine. However, poor infectability of primary tumour samples remains a significant barrier and the underlying contributing factors are currently being investigated.

PD-L1 expression is increased following repeated dosing with an IL-12 expressing autologous infected cell vaccine in a model of peritoneal carcinomatosis.

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Background

We have recently reported that an IL-12-expressing oncolytic virus (OV)-infected cell vaccine (ICV) is effective in treating murine models of peritoneal carcinomatosis (PC), the leading cause of terminal complications in gastrointestinal malignancies. Efficacy of the vaccine in a model of established peritoneal disease requires $CD8^+$ T-cells, suggesting an adaptive response is critically involved in controlling tumour growth. However, although treatment with MG1-IL12-ICV delays tumor growth and prolongs survival in models of established peritoneal disease (B16 and MC38) durable cures is not achieved. This suggests that immunosuppressive mechanisms counteract the immune response, eventually permitting tumour escape and outgrowth.

Objective: Investigate mechanisms of immunosuppression developing in the tumor microenvironment in response to treatment with different MG1-ICVs.

Methods: 1e5 B16F10 cells were injected i.p. to establish peritoneal disease. Animals were randomized into different treatment arms and received 5 doses over an 18 day period: vehicle alone, irradiated cells, MG1-ICV or MG1-IL12-ICV (or OVTstim-ICV) beginning on day 5. Immune infiltrates in the tumors, peritoneal cavity, and spleens were profiled on day 15 and 20 post tumor inoculation by flow cytometry and immunohistochemistry.

Results

Treatment with MG1-IL12-ICV resulted in a significant decrease in tumour growth and an increased infiltration of $CD4^+$ and $CD8^+$ T-cells and dendritic cells ($CD11c^+$) compared to other groups. In addition, MG1-IL12-ICV resulted in decreases in MDSCs ($CD11b^+$ $Gr\text{-}1^{hi}$) and minimal changes in T-regs ($CD4^+$ $Foxp3^+$) at both time points. However, despite the presence of a favourable immune infiltrate, the tumour volume continued to increase and long-term survival was not significantly improved. Further investigation revealed significant increases in PD-L1 expression on $CD11b^+$ $F4/80^+$ Macrophages in the peritoneal cavity and tumour. Further characterization of the tumor microenvironment environment revealed increased PD-1 expression on T-cell subsets without significant changes in in CTLA-4 or Lag-3 expression. These effects were more significant at latter timepoints, suggesting MG1-IL12-ICV induced immune activation is countered by increased PD-L1 expression suggesting that combination approaches which target PD-1/PD-L1 axis may improve efficacy.

Conclusion

Our data demonstrates that novel IL-12 producing ICV therapy can induce a favorable anti-tumor microenvironment in the peritoneal cavity, and that the PD1/PDL1 axis may be a barrier that needs to be overcome to attain durable cures. Further investigation into the tumor microenvironment is underway, as well as experiments investigating the therapeutic efficacy of combination therapy with checkpoint inhibitors and MG1-IL12-ICV.

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.

Oncolytic vesicular stomatitis virus expressing a reovirus fast protein as tumor treatment by enhancing anti-tumor immunity.

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Reovirus FAST proteins are the smallest known viral fusogens and efficiently induce cell-cell fusion and syncytium formation in multiple cell types. Syncytium formation enhances cell-cell virus transmission and may also induce immunogenic cell death, a form of apoptosis that stimulates immune recognition of tumour cells. These properties suggest that FAST proteins might serve to enhance oncolytic virotherapy.

Oncolytic viruses (OVs) constitute a form of cancer immunotherapeutic exhibiting promising results in a broad range of tumor type and Vesicular Stomatitis Virus (VSVd51) is an oncolytic viral platform that has shown a good therapeutic window in various studies and models.

The oncolytic activity of recombinant VSV encoding the p14 FAST protein (VSV-p14) was compared to a similar construct encoding GFP (VSV-GFP) in *in vitro* and *in vivo*. Compared to parental VSV, VSV-p14 exhibited increased oncolytic activity against MCF-7 and 4T1 breast cancer spheroids in culture, and reduced primary 4T1 breast tumour growth *in vivo*. VSV-p14 prolonged survival in both primary and metastatic 4T1 breast cancer models, and in a CT26 metastatic colon cancer model. As with VSV-GFP, VSV-p14 preferentially replicated *in vivo* in tumors and was cleared rapidly from other sites. Furthermore, VSV-p14 increased the numbers of activated splenic CD4, CD8, NK, and NKT cells, and increased the number of activated CD4 and CD8 cells in tumors.

FAST proteins may therefore provide a multi-pronged approach to improving oncolytic virotherapy via syncytium formation and enhanced immune stimulation.

Surgical Stress Suppresses Natural Killer Cell IFN? Release in Colorectal Cancer Patients

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Background:

Surgical stress results in profound immune suppression. Natural Killer (NK) cells play a central role in clearance of metastases and NK cell dysfunction, as measured by NK cell cytotoxicity (NKC), following surgery has been linked to cancer metastases in animal models. However, NK cell activity (NKA), as measured by secretion of interferon-γ (IFNγ), is a global measure of NK cell function since it measures activity from both the $CD56^{bright}$ and $CD56^{dim}$ subsets. NKA has been correlated with cancer prognosis in numerous clinical studies of varying cancer types. The effects of surgery on NKA have not been previously reported.

Methods:

A total 22 healthy participants and 38 colorectal cancer surgery patients were enrolled in an observational study (May 2016 to June 2017). Peripheral blood was collected at baseline and on postoperative day (POD) 1, 3, 5, 28, and 56. NKC, measured by ^{51}Chromium assay, NKA, measured by production of IFNγ following cytokine stimulation, and immunophenotyping by flow cytometry, was compared. Statistical analysis was performed using Mann-Whitney non-parametric testing.

Results:

NKC: NK cell cytotoxicity was reduced on POD1 to 65% of preoperative levels ($p=0.0046$, $n=13$). *NKA (Figure 1):* The median preoperative IFNγ levels for CRC patients (267 pg/mL) was significantly lower than that of healthy controls (855 pg/mL, $p<0.001$). However, surgical stress was associated with a complete loss of IFNγ secretion on POD1, with a median of 0 pg/mL ($p<0.001$, $n=37$). The impairment persisted until POD28 in 72% ($n=18$) of patients. Immune cell profiling did not reveal any differences in either the total NK cells ($CD56^{+}$ $CD3^{-}$) or the percentage of $CD56^{bright}$ and $CD56^{dim}$ subsets.

Conclusion:

Immediately following surgery there is significant decrease in NK cytotoxicity which is accompanied by a near complete loss of NK cell IFNγ production in all patients which persists for up to 1 month, and is not related to NK cell numbers. NKA is a more sensitive measure of postoperative NK cell dysfunction, as compared to NKC. Future work will study the effects of postoperative suppression of NKA on surgical outcomes and cancer recurrence.

Bifluorescent analysis of alpha-synuclein aggregation in vivo

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BACKGROUND

Parkinson's disease is a neurodegenerative synucleinopathy with no cure. The prodromal phase has a long time course during which symptoms such as constipation and difficulty swallowing suggest a role of the enteric nervous system in early disease propagation. The Braak hypothesis suggests that alpha-synuclein propagates from neuron to neuron in a prion-like fashion.

OBJECTIVES

We will be addressing the critical initiation mechanisms of Parkinson's disease *in vivo* using our own model. We want to determine the site of initiation, route of propagation, and whether the *in vivo* form displays a prion-like spread of alpha-synuclein aggregates.

METHODS

For the first time, we will be conducting our experiments using our bifluorescent synuclein (BiSyn) transgenic mice, whereby two alpha-synuclein monomers associate to promote the assembly of a functional fluorescent protein. We will be injecting alpha-synuclein fibrils into the gut wall of our BiSyn mice and extracting tissue systematically throughout the gastrointestinal tract and along the length of the vagus nerve for analysis.

RESULTS

If our findings coincide with the gut hypothesis of Parkinson`s disease, our model will hold strong validity in the future assessment of Parkinson`s mechanisms and therapeutics.

CONCLUSIONS

In future experimentation, we will develop a time course for the spread of alpha-synuclein, with an aggregate-promoting stimulus delivered by gavage into the stomach. If our findings coincide with the gut hypothesis of Parkinson`s disease, our model will hold strong validity in the future assessment of Parkinson`s initiation mechanisms and novel therapeutics.

Age-associated ovarian fibrosis promotes a pro-tumor niche through the recruitment of immunosuppressive cell populations

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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. 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 unclear, the number of lifetime ovulations and age are the main non-hereditary risk factors. Ovulation results in cycles of extensive tissue remodeling and age-associated ovarian fibrosis.

Objective: We hypothesized that regulatory T cells (Tregs) would be recruited to quench autoimmunity that could result from chronic fibrotic inflammation, but also create a locally immunosuppressive pro-tumor niche within the aged ovary.

Methods: Formalin-fixed paraffin-embedded (FFPE) murine and human ovaries were analyzed by immunohistochemistry for the presence of immune populations and fibrosis. RNA was isolated from FFPE human ovarian cortex using automated microdissection and subsequently run on Nanostring immune cell signaling panel. Nanostring analysis was performed using nCounter software.

Results: Immunohistochemical analysis of aged murine ovaries (>20months) revealed extensive fibrosis that correlated with the appearance of FOXP3+ Tregs and CD206+ M2-polarized macrophages with negligible amounts of these populations in younger ovaries (<20 months). Interestingly, using the 4-vinylcyclohexene diepoxide model of ovarian aging that does not produce stromal fibrosis, no Tregs or M2 macrophages were present showing that fibrosis and not simply menopausal status is required for the recruitment of these cells. Next, RNA was isolated from the ovarian cortex of human ovaries (age 36-69) for Nanostring analysis. Unsupervised hierarchical clustering revealed two distinct clusters that correlated with age and the amount of cortical fibrosis by Masson's trichrome staining. Differential gene expression analysis revealed 333 downregulated genes and 62 upregulated genes in fibrotic compared to non-fibrotic ovaries. Next, nCounter analysis predicted the presence of type 1 regulatory T cells (CD4+CD49b+LAG3+) and M2-polarized macrophages (CD206+) in fibrotic ovaries, which was validated by double immunohistochemical staining.

Conclusions: Our results show that both murine and human ovaries acquire an immunosuppressive phenotype with aging that correlates with fibrosis, offering a novel explanation for the age and ovulation-associated risk of ovarian cancer. Current studies aim to prevent and treat fibrosis with the goal of maintaining functional immune surveillance within the aging ovary, opening up a novel avenue for ovarian cancer prevention

Targeting Oncogenes in Cervical Cancer

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GGPP Synthase as a Therapeutic Target: Enhancing Afatinib Activity in Squamous Cell Carcinoma

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Background and Objective:
Epidermal growth factor receptor (EGFR) is highly expressed in head and neck squamous cell carcinomas (HNSCC) and non-small cell lung cancer (NSCLC). Tarceva inhibits ligand induced EGFR signaling but shows limited activity as a single agent in EGFR wild-type expressing cancers. Novel strategies will likely require its combination with an agent(s) that will more efficiently target the EGFR. Recently, we have demonstrated that combining statins, an inhibitor of the mevalonate pathway, with Tarceva induced synergistic cytotoxicity through the induction of activating transcription factor 3 (ATF3). In our Phase I clinical trial combining Rosuvastatin with Tarceva, statin-induced myopathies limited their use. Therefore, alternative strategies are warranted. Targeting geranylgeranyl diphosphate (GGPP) synthesis, a downstream mevalonate metabolite, may circumvent statin- associated toxicities, but retain the efficacy in combination with EGFR inhibitors.

Results:
In this study, we evaluated the effect of the addition of a GGPP synthase inhibitor, digernyl bisphosphonate (DGBP), with the EGFR inhibitor, Afatinib. ATF3 expression was upregulated in HNSCC *in vitro* and in a cohort of *ex-vivo* tumor tissues following the treatment of both DGBP and Afatinib. Furthermore, the combination of DGBP and Afatinib induced synergistic cytotoxicity in HNSCC cell lines that was dependent on the expression of ATF3. Co-administration of GGPP inhibits the induction of ATF3 and cytotoxicity associated with DGBP treatment. We have also shown that the upregulation of ATF3 was regulated by the Integrated Stress Response pathway.

Conclusion:
Taken together, these results reveal the role ATF3 in mediating the cytotoxic effects of DGBP and Afatinib and suggest the potential of clinical utility of combining GGPP synthase inhibitors with Afatinib as a novel and more refined therapeutic approach.

Envelope exchange in oncolytic measles virus for neutralization escape

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Background
The use of oncolytic viruses (OV) to precisely target and eliminate tumors is a rapidly 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), a member of the *Paramyxoviridae* family, the patient's pre-existing neutralizing antibodies will impair delivery of the OV before it can attack the tumor as most individuals have been immunized against this agent. The neutralization response towards MeV targets predominantly the fusion (F) and hemagglutinin (H) envelope glycoproteins displayed at the particle surface.

Objective
In this project, we aim to effectively shield an attenuated oncolytic measles virus against the host's immune response by substituting its two envelope proteins with the glycoproteins of other viruses.

Methods
The panel of viruses assembled consists of closely related *Paramyxoviridae* such as Newcastle Disease Virus (NDV) in addition to distantly associated viruses, for instance, the Vesicular Stomatitis Virus (VSV) of the *Rhabodoviridae* familly. The viral candidates selected for MeV pseudotyping have no pre-existing neutralizing antibodies against them due to a low incidence of contact. Furthermore, the pseudotyping compatibility of these candidates with MeV to form viable infectious particles has been assessed.

Results
The envelope exchange cloning strategy has been confirmed in this MeV genomic backbone. Plasmids containing full-length recombinant viral genomes containing chimeric VSV G glycoprotein, enhanced green fluorescent protein, and NDV transgenes have been generated. Newly synthesized MeV pseudotyped with NDV F and H viruses are currently being produced.

Conclusions
A neutralization evasion strategy may improve the delivery process of the virus to the tumor location in patients immunized for measles or if they have been previously treated with the oncolytic agent. 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.

Characterization of a second generation oncolytic Vaccinia

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Viral Sensitizers Potentiate Infection of Cancer Cells via NF-kappaB

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Background: Genetically engineered oncolytic viruses (OVs) have been proven to be effective anti-cancer agents and many are advancing in clinical trials. However, the heterogeneity of tumours and the attenuation of these viruses can limit their efficacy. Our group has previously shown that a novel class of small molecules called Viral Sensitizers (VSes) are used in combination with OVs, they potentiate the infection of cancer cells by oncolytic VSV (VSV?51) up to 1000-fold. Of particular interest is a group of small molecules, including VSe1 and its analogues, which have been shown to inhibit various antiviral genes including IFNβ. Additional investigation revealed that these small molecules interact with glutathione-s-transferase- π (GSTP1-1), an enzyme induced by oxidative stress.

Objective: In this study, we aim to investigate the effects of VSe1 and analogues on the innate cellular antiviral response and to identify their mechanism of action.

Methods: The expression of various antiviral cytokines and the activation and nuclear translocation of transcription factors involved in the antiviral response were analyzed using immunoblotting, ELISA, and real-time PCR techniques. Glutathione-S transferase (GST) inhibition assays and GSTP1-1 knockdown experiments were used to assess the VSes' effects on GST. In addition, flow cytometry, HPLC, and a fluorescent microplate reader were used to assess glutathione levels and oxidative stress via various redox sensitive probes.

Results: Our results show that our class of viral sensitizers inhibit virus-induced nuclear translocation of NF-κB and expression of antiviral cytokines IFNβ, TNFα, and IL-6. In addition, the sensitizers induce oxidative stress, inhibit GST activity and deplete cellular glutathione. Furthermore, we demonstrate that inducing oxidative stress with H_2O_2 enhances VSV?51 activity and inhibits NF-κB nuclear translocation and transcriptional activity in a similar manner to our viral sensitizers.

Conclusions: Our results so far demonstrate that VSe1 and analogues inhibit nuclear translocation and transcriptional activity of NF-κB in response to virus infection, which in turn, dampens the expression of antiviral and pro-inflammatory cytokines IFNβ, TNFα, and IL-6. Our data suggests that inhibition of NF-κB may be a result of redox-mediated post-translational modifications. Future work will aim to determine whether these viral sensitizers are acting through direct binding of NF-κB or through indirect modification of redox-sensitive residues of NF-κB.

Inhibition of the Ste20-Like Kinase Restores Myogenesis Downstream of TGF-b

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Background

TGF-β levels are significantly increased in patients with muscular dystrophy as well as dystrophic animal models. Elevated levels of TGF-β in muscular dystrophy worsens the disease state by preventing satellite cells from efficiently repairing damaged muscle tissue. Inhibition of TGF-β signalling improves muscle function and decreases muscle damage. Therefore, blocking the effects of TGF-β would be an effective therapeutic target to mitigate disease progression in muscular dystrophy.

The Ste20-Like Kinase (SLK) is a ubiquitously expressed protein with predominant expression throughout embryonic and adult skeletal muscle. We have previously shown that muscle specific deletion of SLK delayed muscle regeneration and caused a mild myopathy in older mice, demonstrating that, although important, SLK is dispensable for muscle formation and regeneration. More recently, we have shown that SLK knockdown prevented TGFβ induced EMT in mammary epithelial cells. We speculated that SLK may similarly block TGF-β signalling in myoblasts.

Objective

SLK knockdown prevented TGFβ induced EMT. Given the repressive role of TGFβ in myogenesis as well as the higher levels of TGFβ in muscular dystrophy, we predicted that SLK inhibition/knockout would rescue myogenesis downstream of TGFβ signalling. We would therefore observed an increase in myogenesis and muscle function in SLK deficient dystrophic satellite cells.

Method/Results

The knockdown of SLK in C2C12 and primary myoblasts resulted in a partial rescue of myogenesis. Interestingly, we found this effect was independent of Smad2/3 activity as well as Smad4 target gene activation. Further investigation revealed that SLK knockdown altered RhoA activity, which may lead to increased expression of myogenic proteins. SLK deletion in the mdx model led to increased expression of myogenin and eMHC, indicating enhanced myogenesis in these muscles. Additionally, there was a decrease in CD45+ cells indicating lower immune infiltration. However, total collagen deposition remained unchanged.

Conclusions

Deletion of the Ste20-Like Kinase in the mdx model increased muscle regeneration and decreased immune infiltration. Additionally, SLK deletion restored myogenesis following treatment with TGFβ These results provide evidence that targeting SLK in dystrophic muscle will enhance muscle function. Further work is required to understand the molecular basiss behind these findings.

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 high impact scientific journals over the past years including integrated Genomic Characterization of Carcinomas. The most recent publication was in the January 2017 issue Nature for the characterization of oesophageal carcinoma.

TOH-Ontario Tumour Bank team, Dr. Harman Sekhon (PI); Dr. Angel Arnaout and Dr. Sebastien Gilbert (Co-PI's); TOH-OTB staff, Nikita Rayne.

Identifying mechanisms of resistance to oncolytic virotherapy in acute leukemia through a genome-wide CRISPR screen

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Background
Approximately half of all adults diagnosed with acute leukemia (AL) relapse after standard chemotherapy, and therefore, alternative treatment options are needed for this aggressive malignancy. We have previously shown that vaccination with irradiated autologous tumour cells infected with an oncolytic virus (OV) can elicit a durable, tumour specific, T cell-mediated response in a mouse model of AL. In the context of this AL infected cell vaccine (ICV) model, infection of autologous cells *ex vivo* with an OV is essential for stimulating a lasting immune response. While the murine AL line L1210 can be robustly infected with Maraba MG1, there remains a large fraction of cancers that are resistant to OV infection.

Objective
Therefore, we sought to utilize a genome wide CRISPR-Cas9 screen to identify genetic factors that mediate OV resistance in this model of AL.

Methods
L1210 cells stably expressing Cas9 were transduced with the mouse GeCKOv2 library, which contains 130,209 gRNAs against 20,611 genes within the mouse genome. Following selection, cells were treated with Maraba MG1 at an MOI of 10. Genomic DNA from resistant populations was sequenced to identify genes enriched in resistant cells relative to mock treated cells.

Results
Candidates identified in this screen will be validated in a panel of AL cell lines to determine their relevance in AL.

Conclusions
The findings from this study have the potential to elucidate novel mechanisms of resistance to OV therapy in AL and other cancers, as well as identify biomarkers, which may be useful in determining the feasibility of creating and administering an ICV using patient derived tumour cells.

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: Increasing global incidence of obesity has highlighted the role of fat cells in cancer progression. In breast cancer, fat cells are abundant and active in the tumour microenvironment (TME). Ovarian tumours demonstrate 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. Our lab is interested in harnessing oncolytic viruses (OVs), a virus-based anti-cancer therapy, for the treatment of cancer. It is an attractive therapeutic of choice for its proven efficacy and established clinical safety profile.

Objective: 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 influence OV responses.

Methods: An *in vitro* model of high-grade ovarian serous adenocarcinoma (OVCAR8) was cultured in breast adipocyte conditioned media (BACM), to mimic a 'fatty' TME, and infected with a fluorescently-tagged OV. Changes in the transcriptome of the cell, in the presence of BACM and with/without OVs, was determined by microarray analysis. Flow cytometry was harnessed to evaluate changes to cancer cell metabolism and OV entry. Furthermore, *in vivo* studies of tumour OV infectivity in a breast cancer 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 pan-virus resistance to infection in BACM-cultured cancer cells in comparison to its growth media cultured counterparts. Microarray data suggests priming of uninfected, BACM-cultured cells with an anti-viral phenotype. Preliminary studies evaluating virus entry indicate viral resistance at early stages of the viral life cycle. A closer look at changes in cancer cell metabolism reveal that cells that were cultured with BACM have increased intracellular lipid accumulation. Interestingly, a recovery of OV titers can be achieved when lipids in BACM are removed with a calcium silicate hydrate. I*n vivo* studies show that tumours localized in the fat pad display lower OV infectivity in comparison to subcutaneously seeded tumours.

Conclusions: *In vivo* results recapitulate *in vitro* results suggesting that adipocyte secreted factors confer virotherapy resistance. Future work will aim to elucidate the molecular events that constitute this phenotype. Ultimately, the long-term goal of this project is to bioengineer a tailored OV that is more efficacious in a ‘fatty’ TME.

Tailoring oncolytic viruses for the treatment of pancreatic cancer

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The role of sCD127 in mediating T-cell homeostasis in vivo

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Interleukin 7 is an essential cytokine that plays a major role in the development and homeostatic maintenance of T cells. The presence of soluble forms of various cytokine receptors has been proposed to be involved in the endogenous regulation of cytokine activity. For instance, IL-7 can signal by binding to its membrane receptor, but can also interact with the soluble form of IL-7Rα (sCD127). Due to the natural ability of sCD127 to bind to IL-7, there is an interest in its potential application as an immunotherapeutic agent in diseases where IL-7 has been found to be relevant. However, little is known in regard to the role of sCD127 in regulating IL-7 functions. In our study, we hypothesize that sCD127 will enhance T-cell proliferation by increasing the bioavailability of IL-7. Using an *in vivo* T-cell depletion model, T-cell reconstitution in mice receiving exogenous sCD127 will be investigated, as well as the dynamic change in IL-7 blood levels during the rebound phase. To assess the effect of administering sCD127 in vivo, we injected non-lymphopenic mice with recombinant sCD127 protein. The results revealed that there is no significant difference in the overall distribution of T-cell in peripheral blood and secondary lymphoid organs between treated and untreated mice. Our preliminary data demonstrates a substantial loss in T-cell population one week after receiving anti-CD4 and anti-CD8 depleting antibodies. The pattern of T-cell reconstitution was different between the T-cell types; an almost complete T-cell reconstitution was observed in CD4+ T cells 6 weeks following depletion, while CD8+ T cells were partially reconstituted as compared to their basal level before depletion. The plasma level of IL-7 in mice was undetectable during the reconstitution process as measured by cytometric bead array. Our future goal is to dissect the role of exogenous sCD127 on T-cell reconstitution and proliferation *in vivo* following depletion, which in return will provide critical information for understanding its potential therapeutic use and impact on IL-7 function.

Peri-Ovulatory Supplementation of L-Ornithine to Increase Reproductive Success in Old Mice

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Background:
As maternal age increases, conception becomes difficult and comes with increased risk of birth defects and spontaneous abortion. Due to the nature of oogenesis, mammalian females have a set number of oocytes at birth. Quantity and quality of this oocyte reserve decrease with maternal 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 improve oocyte quality and increase reproductive success in older mice, identifying putrescine as a possible treatment for older women. However, since ODC activity and putrescine levels are low or undetectable outside of the peri-ovulatory ovaries, we wish to explore an alternative to systemic putrescine administration. We reasoned that ODC substrate L-ornithine, a non-protein amino acid widely available as a health supplement, might be a better choice since it can only be converted to putrescine in tissues expressing ODC. Furthermore, while both compounds are relatively non-toxic, L-ornithine has oral rat LD_{50} of 10,000mg/kg (DrugBank), compared to 2,000mg/kg for putrescine.

Objective:
This study was designed to determine if peri-ovulatory L-ornithine supplementation is capable of increasing putrescine levels in the ovaries resulting in similar reproductive benefits to putrescine supplementation.

Methods:
Old C57BL/6 mice were given L-ornithine via oral gavage and sub-cutaneous injection 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. In addition, old C57BL/6 mice were mated with young BDF1 male mice in the presence or absence of L-ornithine in water to determine the effect on reproductive outcome.

Results:
L-ornithine delivered via oral gavage or sub-cutaneous injection can increase putrescine levels in the ovaries only when ODC is active (e.g. during ovulation). Moreover, L-ornithine can dose-dependently increase ovarian putrescine concentrations. Compared to putrescine supplementation, L-ornithine specifically increases ovarian putrescine concentrations. Chronological breeding with L-ornithine appears to increase the number of pups and proportion of pregnant mice versus control.

Conclusions:
Peri-ovulatory L-ornithine supplementation specifically and dose dependently increases ovarian putrescine levels. L-ornithine also appears to have a beneficial effect on mating in old animals. Continuing work will determine levels of L-ornithine before and after both ovulation and L-ornithine supplementation. The effects of L-ornithine on reproductive outcome are continuing to be evaluated.

Characterizing Thymic Stromal Lymphopoietin Expression in Human Adipocytes

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BACKGROUND/OBJECTIVES: Thymic stromal lymphopoietin (TSLP) is a cytokine that plays a role in regulating inflammatory responses. TSLP has also recently been implicated in platelet activation. Administration of recombinant human (rh) thyroid-stimulating hormone (TSH) to thyroid cancer survivors raises pro-inflammatory cytokines and activates platelets. We aimed to measure platelet microparticle levels after rhTSH stimulation in vivo, and characterize TSLP expression in TSH-stimulated human adipocytes.

SUBJECTS/METHODS: Blood samples from thyroid cancer survivors before (day 1) and after rhTSH administration (day 5) were obtained for total and platelet microparticle analysis. TSLP mRNA expression, protein expression, and protein secretion were measured in TSH-stimulated differentiated adipocytes differentiated from stromal preadipocytes that were isolated from adipose tissue of surgical patients.

RESULTS: We observed a 5-fold increase of platelet microparticles in thyroid cancer patients after rhTSH administration. TSLP is upregulated by TSH partially through a protein kinase A (PKA)-dependent pathway, as inhibiting PKA with H89 inhibited 66% of this response. TSH, as low as 5 mU/mL, stimulates TSLP protein secretion in differentiated adipocytes, with higher TSH (50 mU/mL) resulting in a 5-fold increase.

CONCLUSIONS: TSLP is a novel adipokine that is upregulated by TSH. Future studies are required to elucidate signaling pathways regulating adipocyte-derived TSLP and its role in TSH-dependent platelet activation.

A microtubule-organizing center (MTOC)-based Ca2+ signaling system required for spindle assembly in Xenopus oocytes

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Background: High calcium has been considered as a factor of microtubule depolymerization for more than 40 years; our lab, however, recently shows that fast calcium chelator BAPTA (1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid), but not slow chelator EGTA (ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid), causes immediate spindle collapse in *Xenopus* oocyte. By engineering and employing a novel microtubule-binding fluorescent calcium-indicator EMTB-GCaMP3, we discovered that, for the first time, highly concentrated calcium foci enriched at the spindle pole and formed a tube-like structure at the spindle pivot. However, the calcium signaling pathway and underlying mechanism involved in spindle function remains unclear.

Rationale and objective: Originated from the proof of principle experiment that fast calcium chelator cause spindle collapse and the observation that calcium foci are at the spindle MTOCs, we hypothesize spindle assembly are regulated by nanodomain calcium signaling. My overall objective is to establish the presence of spindle-based Ca^{2+} transients and to identify the functional components including the calcium source, sensor and effector. Specifically, we hypothesize that vesicular sub-compartment ER serves as calcium storage and release calcium through inositol 1,4,5-triphosphate receptors (IP3Rs). Calcium is then bound by calmodulin (CaM) within tens of nanometers of IP3Rs and Ca^{2+}/CaMs activate downstream effectors at the MTOC to promote spindle assembly.

Significance: This work would reveal a novel spindle- or MTOC- regulating mechanism based on nanodomain calcium signaling. It would also elucidate a new role of calcium signaling reconciling the long-standing puzzles regarding the role of calcium in microtubule polymerization and spindle assembly.

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 (SCs) are seminiferous tubule (SFT) somatic cells that provide spatial and nutritional support to developing male germ cells. Also, they phagocytose residual bodies and apoptotic germ cells for intracellular degradation. To date, information on properties of SCs is limited, thus demanding a proper protocol for their primary culture. However, this is challenging as SCs are only 5% of total SFT cells in adult rodents, making it difficult to obtain their pure population. Since SCs from 20-day old rodents constitute 30% of SFT cells, their primary culture is available and used instead in ex vivo studies. However, the assumption that SCs from prepubertal and adult rodents are the same is likely incorrect, since testosterone/FSH/LH levels at these two ages are different.

Objectives: 1. Obtain a pure population of SCs from adult mice. 2. Compare their properties with SCs from 20-day-old mice.

Methods: Optimum concentrations of SCs + germ cells of collagenase/trypsin/ hyaluronidase digested SFTs were empirically determined for culture dish plating. Germ cells were gently removed from SCs selectively adherent to the substratum. SCs were identified by their specific markers and ability to form tight junctions and phagocytose apoptotic germ cells. Cell division was determined by BrdU staining and counting. Lipidomic and proteomic analyses were performed by LC-ESI-MS/MS and SWATH-MS, respectively.

Results: In culture, SCs constituted >90% of adherent cells, as revealed by their marker-Wilms tumor protein and ability to form tight junctions and secrete signature proteins (e.g., prosaposin, clusterin). On average, we obtained 1.7 million SCs/adult mouse. SCs from adult mice phagocytosed more carboxyfluorescein-labeled apoptotic germ cells than SCs from 20-day-old animals. These results agreed with the higher levels of triacylglycerols, cholesteryl esters, and seminolipid in adult SCs. However, adult SCs no longer divided despite treatment with rFSH. This was in contrast to SCs from prepubertal mice, which remained dividing for one month. These results corroborated the MS data, revealing higher levels of antimullerian hormone and other proliferation promoting proteins in SCs from prepubertal mice. Notably, SC primary cultures from both ages could be revived after cryopreservation, thus facilitating their use in research studies.

Conclusion/Significance: Our primary culture method allows studies of properties/roles of adult SCs. This is particularly relevant when male infertility/subfertility stems from SC dysfunctions in adulthood such as that occurring in *Arsa*$^{-/-}$ mice.

Altered type I interferon signalling facilitates the eradication of HIV-infected monocyte-derived macrophages by the oncolytic rhabdovirus, MG1

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Introduction: Impairment of the type 1 interferon (IFN1) response is common to multiple diseases, including HIV infection and cancer, and may be exploited for therapeutic benefit. For example, novel oncolytic viruses, such as the recombinant Maraba virus, MG1, have been designed to selectively kill tumor cells with impaired IFN1 responses. Similar strategies may prove useful for the eradication of HIV-infected cells *in vivo*. However, IFN1 signalling in the context of persistent infection, such as that seen in myeloid cells, remains poorly understood.

Hypothesis: IFN1 responses are impaired within HIV-infected macrophages, and serve as a target for MG1.

Methods: Monocyte-derived macrophages (MDM) were infected with the reporter virus, HIV NL4-3 BAL-IRES-HSA, and HIV-infected (HSA^+) cells were measured by flow cytometry. To assess IFN1 signalling, MDM were stimulated with IFN-alpha or 5'ppp dsRNA, and the induction of two IFN-stimulated genes (ISG), PKR and ISG15, was measured by flow cytometry. Following MG1 infection, HIV p24 antigen and integrated HIV DNA were measured by ELISA and qPCR, respectively. Finally, cell viability was measured by AnnexinV staining by flow cytometry.

Results: IFN-alpha- and 5'ppp dsRNA-induced ISG expression was lower in HSA^+ MDM, in comparison to uninfected cells. MG1 infection resulted in a reduction in p24 release, integrated HIV DNA, and number of HSA^+ MDM. As well, MG1 infection caused the induction of apoptosis, as measured by AnnexinV staining, in HSA^+ MDM but not HIV-uninfected MDM. Finally, UV-inactivated MG1 had no impact, confirming the need for actively replicating MG1.

Conclusion: IFN1 responses were found to be altered in HIV-infected MDM. This was associated with a decrease in HIV production following MG1 infection, as well as a significant induction of apoptosis in HSA^+ MDM. These results may therefore suggest the eradication of HIV-infected MDM *in vitro* by a known oncolytic virus. This is of particular relevance to current HIV cure strategies, which require persistent HIV reservoirs to be eliminated in a highly specific manner, without harm to uninfected bystander cells.

Retrospective analyses of pancreas biopsies from diabetes prone rats reveal a hepatic clue to the onset of type 1 diabetes

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Background: Type 1 Diabetes (T1D) is an autoimmune disease in which the insulin-producing β cells are destroyed by the patient's immune system. Numerous immune and non-immune susceptibility genes are involved and the environment is an important player. Only a small proportion of individuals with diabetes risk genes develop T1D. It is unclear what biological processes are essential for disease development and when these processes are first impaired.

Objective and methods: To investigate this problem, pancreas biopsies from diabetes-prone rats were taken at 30 days of age and the animals were followed until development of the disease to determine which rats developed diabetes and which remained disease-free. Bioinformatic analysis of microarray data showed several genes involved in ER stress, apoptosis, glucoregulation, and metabolism.

Results: RT-qPCR validation of selected pathway associated genes revealed downregulation of the ER stress pathways, increased pro-inflammatory genes and reduced insulin gene expression in the pancreas. Interestingly, this imbalance was more striking in the liver of 30-day rats, and over time became more accentuated in the pancreas. This impaired metabolic stress response occurred very early in the prediabetes period, long before morphological changes, immune infiltration of islets or signs of disease.

Conclusions: These results suggest that in the prediabetic period, before significant immune involvement, a metabolic imbalance in the pancreas is being triggered initially by the liver.

A successful protocol of artificial insemination in mice

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BACKGROUND: Artificial insemination (AI) is useful for pup production from cryopreserved sperm and sperm from mice with mounting problems. It can also be used for determining the effects of sperm treatments on *in vivo* fertilization/pregnancy. AI methods involve transcervical sperm injection into females. In most described protocols, % pregnancies and eggs fertilized are either unavailable or <50%. Success of AI depends on several factors. Capacitated sperm have to be delivered into the uterus around the ovulation time. This in turn requires female boarding in a 10 h/14 h or 12 h/12 h dark/light cycle and reliable methods to track the estrous cycle. Synchronizing the estrous cycle in a female population also expedites the procedure. The right apparatus to inject sperm and methods to gauge AI success are needed.

OBJECTIVE: Provide an AI protocol for high fertilization/pregnancy rates.

METHODS: Females were induced to synchronize their estrous cycle by progesterone injection or housing in cages with male bedding. Estrous cycle stages were tracked by cytological changes in the vaginal lavage. Females in the proestrus/estrus transition were transcervically injected with sperm 1 h before the ovulation time. Sperm prepared by Percoll-gradient centrifugation and capacitated were delivered through a blunt-ended,1.8-cm 23 G needle attached to a syringe. *In vivo* fertilization was assessed by formation of zygotes or 2-cell embryos, retrieved from females 14 and 42 h post-injection. Embryos were further cultured to become blastocysts. Pregnancy was assessed by belly bulging or pup delivery 19-20 days postAI.

RESULTS: Each estrous cycle stage was successfully detected: proestrus containing nucleated epithelial cells (NE), estrus-cornified (C) cells, metestrus-NE + C + leukocytes, and diestrus-leukocytes. Progesterone induced ~40% of 87 mice to synchronize their estrus cycle, whereas male bedding induced synchrony only within cages (n=32). Fifty microliters of 5 million capacitated sperm (~90% hyperactivatedly motile) were easily delivered through the mouse cervix, and sperm entry to the oviduct was evident 12-18 h postAI. Among 29 female mice inseminated, fertilization = 81±13% (n=441 zygotes/2-cell embryos + unfertilized eggs) and 89% of the two-cell embryos (n=293) became blastocysts upon culturing. Alternatively, among 26 females artificially inseminated, 24 mice (92%) became pregnant, delivering pups with a normal litter size (9.6).

CONCLUSION: Our AI protocol gave higher fertilization and pregnancy rates than others.

Acute Effects of Hemodialysis on Circulating Microparticle Levels

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Background: Individuals with end stage kidney disease (ESKD) are at increased risk of cardiovascular complications. Previous studies have shown that levels of circulating microparticles are increased in ESKD and that an increase in circulating endothelial microparticles is a predictor of cardiovascular morbidity and mortality (Amabile, 2012).

Objective: The purpose of this study was to examine the effect of a single hemodialysis session on levels of circulating microparticles in patients with ESKD.

Methods: We studied 23 patients (age 58±3 years, 12 M/11F) undergoing a single hemodialysis session. Levels of circulating total, endothelial, leukocyte, and platelet microparticles were assessed by flow cytometry immediately prior to, and at the completion of, hemodialysis (3 times weekly, 4 hour sessions).

Results: Participants had been treated by hemodialysis for 50±8 (mean±SEM) months prior to enrollment. The mean ultrafiltration volume on the day of study was 2.06 ±0.17 L. The level of total microparticles was significantly reduced by ~55% following hemodialysis ($5.15x10^7$ ±1.1 $x10^7$ [Pre] vs $2.32x10^7$ ±$4.27x10^6$ [Post], P<0.05). Similarly, the level of platelet microparticles was significantly reduced by ~75% following hemodialysis ($4.33x10^7$ ±1.06 $x10^7$ [Pre] vs $1.06x10^7$ ±$3.8x10^6$ [Post], P<0.05). In contrast, the level of leukocyte microparticles was not altered by hemodialysis ($2.65x10^6$ ±3.19 $x10^5$ [Pre] vs $2.15x10^6$ ±$3.2x10^5$ [Post], P=n.s). The level of endothelial microparticles also remained the same before and after hemodialysis ($2.09x10^6$ ±4.85 $x10^5$ [Pre] vs $1.39x10^6$ ±$2.94x10^5$ [Post], P=n.s). There was no correlation between the degree of ultrafiltration and the reduction in platelet, leukocyte, or endothelial microparticles.

Conclusion: Hemodialysis is associated with reductions in circulating total and platelet microparticles with no impact on circulating endothelial or leukocyte microparticles. These results suggest that dialytic clearance selectively influences the levels of circulating microparticle subpopulations in ESKD patients undergoing hemodialysis. Consideration should therefore be given to the timing of sampling for circulating microparticles in any study involving hemodialysis patients.

High Content Imaging Core

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The ArrayScan is a high content imaging instrument that offers a wide set of functions for the high and medium throughput study of cell biology in a single modular platform. From the development of high-content assay, through basic cell biology research to systems biology and drug discovery and toxicology, this system has been engineered to deliver robust data with minimal effort and with the fastest "image-to-answer" currently available.

The high content imaging core is composed of two separate ArrayScan VTI instruments, both equipped with an Orbitor RS, capable of processing of up to 100 plates (fully automated). Each system has their own specific modules (live cell chamber, confocal unit, apotome, etc), enabling a wide variety of applications. With a 7-channel LED light engine and the HCS Studio software, the ArrayScan VTI allows the creation of personalized analyses suited for any experimental design.

Our core facility offers full training, 24/7 access, experimental support as well as technical support.

Profiling the transport of serine, a likely component of one-carbon metabolism in oocytes

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Background:
Products of one-carbon metabolism play a variety of important roles in cellular systems, including neutralizing of reactive oxygen species (ROS), synthesis of purines and thymidylate for DNA synthesis, and providing methyl groups for methyltransferases, including for epigenetic methylation. Folate is actively taken up during oocyte growth, and it is known that the folate cycle is a key component of one-carbon metabolism, for which serine is the main methyl donor. While the transport of many amino acids have been studied in both oocytes and preimplantation embryos, the transport of serine has largely been overlooked despite its potential role in many important oocyte processes.

Objective:
We aim to profile the transport of serine in oocytes and identify the responsible transporter(s).

Methods:
We are using radiolabeled [3H]serine and competitive inhibition with the substrates of known transporters of serine to establish candidates for the major transporter of serine in mouse oocytes. The focus was on transport systems already established to exist in oocytes, and a competition assay for candidate systems was carried out using an excess of the system substrates. Serine uptake was also measured with and without sodium (Na+), since amino acid transport has classically been categorized based on sodium dependent or independent activity.

Results:
We measured uptake of serine at 12 time points throughout meiotic maturation and found that the serine uptake rate is highest in the 4 hours following germinal vesicle breakdown (resumption of meiosis), ranging between 0.04 and 0.07 fmol/oocyte/min (1uM [3H]serine in the medium), after which it is inactivated. At 4 hours, where the transport rate was consistently high among measurements, we carried out our experiments and found that substrates that would inhibit labeled serine uptake by systems B0,+ and
b0,+, system A, or GLYT1 did not affect uptake of [3H]serine. On the other hand, alanine and cysteine in media nearly eliminated [3H]serine uptake into oocytes. Excess leucine also inhibited serine transport. In addition, labeled serine transport was eliminated in Na+-free media.

Conclusions:
Serine transport is transiently activated during meiotic maturation of mouse oocytes. ASCT2 (SLC1A5) appears to be the most probable transporter of serine in oocytes during meiotic maturation, as ASCT2 requires Na+ to function and is a known transporter of small neutral amino acids including serine, alanine, cysteine, and unlike ASCT1 (SLC1A4), also carries leucine. Expression of this candidate transporter will be confirmed using RT-PCR.

A Comprehensive Systematic Review that Compares the Diagnostic Accuracy Performance of Blood Markers, Synovial fluid and Tissue Testing in Peri-prosthetic Joint Infections (PJI)

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BACKGROUND: PERIPROSTHETIC JOINT INFECTION (PJI) IS A SERIOUS COMPLICATION OF TOTAL JOINT REPLACEMENT SURGERY ENTAILING HIGH COST AND POOR QUALITY OF LIFE IN PATIENTS WITH JOINTS DISEASE. DIAGNOSING PJI EFFICIENTLY REMAINS THE MAIN CHALLENGE FOR CLINICIANS TREATING PJI.

OBJECTIVE: TO CONDUCT THE FIRST SYSTEMATIC REVIEW THAT COMPARES DIAGNOSTIC ACCURACY PERFORMANCE OF ALL EXISTING BLOOD, SYNOVIAL AND TISSUE TESTS IN DIAGNOSING PJI.

Methods: We search MEDLINE, Embase, Cochrane Library and grey literature, and screened the retrieved records in an online systematic review software program (Distiller Systematic Review (DSR) Software©) at level one (title and abstract), and level two (full text). We assessed the quality of the included studies via the QUADAS-2 tool. Both screening and quality assessment were carried out independently by two reviewers, and disagreements were resolved through consensus or third party adjudication. This review is registered with PROSPERO (CRD42015023768).

RESULTS: WE SCREENED 10525 BIBLIOGRAPHIC RECORDS AFTER REMOVING DUPLICATES AT LEVEL ONE. OF THESE 9395 WERE EXCLUDED AND 1130 PASSED TO LEVEL 2 SCREENING. 974 RECORDS WERE EXCLUDED AS THEY DID NOT MEET THE ELIGIBILITY CRITERIA AND 156 PASSED LEVEL 2 OF WHICH 149 WERE INCLUDED. OF THESE STUDIES, 55 PROVIDED DATA ON SERUM TESTS, 70 ON SYNOVIAL TESTS, 75 ON TISSUE TESTS, AND 3 ON CLINICAL EXAMS.
We obtained data on diagnostic performance of 83 index tests (20 blood , 45 synovial , 14 tissue , and 4 clinical exam), and conducted meta-analyses for 16 (5 serum tests, 6 synovial tests, and 5 tissue tests). The comparison of tests across groups demonstrated that synovial alpha-defensin is the test with almost perfect performance (but need to be confirmed in the future research), followed by four other synovial tests (synovial CRP, LER strips, synovial PMN%, and synovial WBC). Compared with the four synovial tests, tissue culture, aspiration culture, histological analysis based on >= 5 PMNs/HPF and >=10 PMNs/HPF might have slightly better mean summary specificity and equivalent summary ROC curves at the cost of lower mean summary sensitivity. Serum tests generally have worse HSROC curves than the synovial and tissue tests mentioned above, but serum CRP and ESR might have slightly better mean summary sensitivity than aspiration culture and histological analysis at the cost of lower mean summary specificity.

Conclusion: Clinicians should emphasize the utilization of these synovial tests compared to serum CRP, ESR recommended by current guidelines as a starting point for diagnosing PJI.

Eye Care Utilization and its Determinants: Baseline Findings from the Canadian Longitudinal Study on Aging (CLSA)

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Background: Visual impairment is one of the most feared health conditions and is a substantial economic burden costing approximately $15.8 billion per year in Canada. Routine eye examinations are important for the timely detection of refractive error and the early identification and management of eye diseases like cataract, glaucoma, age-related macular degeneration, and diabetic retinopathy.

Objective: To provide the prevalence and potential determinants of eye care utilization over the last 12 months among Canadians between the ages of 45 and 85 years old.

Methods: This analysis used baseline data from the 30,097 participants in the Comprehensive Cohort of the Canadian Longitudinal Study on Aging (CLSA). Participants were community-dwelling adults between 45 and 85 years old living within 25-50 km of one of 11 data collection sites across 7 Canadian provinces. Eye care utilization was defined as the self-report of a visit to an optometrist or ophthalmologist in past 12 months.

Results: Fifty-seven percent of adults visited an eye care provider in the last year although there was great heterogeneity between provinces. The highest eye care utilization was found in Ontario at 62%, while the lowest was in Newfoundland and Labrador at 50%. Of concern, 1 in 4 people with diabetes over age 60 had not seen an eye care provider in the last year. Current smokers were less likely to use eye care compared to never smokers (odds ratio (OR)=0.76, 95% CI 0.66, 0.86). Men compared to women (OR=0.67, 95% CI 0.62, 0.71), people with less than a Bachelor's degree compared to more than a Bachelor's degree (OR=0.86, 95% CI 0.79, 0.94), and people making less income (linear trend $P<0.05$) were also less likely to use eye care.

Conclusions: Disparities exist in eye care utilization in Canada. Efforts should be made to reduce these disparities to reduce avoidable vision loss.

Pectoralis major tears diagnostic accuracy of MRI and ultrasound

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Objective:
To determine the accuracy of magnetic resonance imaging (MRI) and ultrasound for characterization of perctoralis major muscle (PM) injury and identify surgical candidates, having as reference the surgical and clinical outcome. In addition to assessing the newly suggested classification system of pectoralis injury on MRI based on the new defined anatomy of the distal PM tendon.

Method:
A retrospective search of the radiology database (from October 2004 to November 2016) reveal 40 patients of pectoralis major tears with total number of 32 MRI and 15 Ultrasound examinations. The study was separated into two parts.
In part one, retrospective review of the primary MRI and US reports were performed and compared with the surgery and clinical outcome.
In part two, the MRIs were independently reviewed by two MSK radiologists (2.5 and 10 years of experience) blindly to the reports, clinical outcome and the surgical results. The injuries were classified according to the new classification system. The sensitivity, specificity and accuracy of the MR review for the detection and localization of the tears compared with surgery and clinical outcome were calculated . Inter and intra-observer agreements of the site of the tear were assessed.

Results:
40 patients (ages 18-59, mean 35, 73 males) were left after application of the exclusion criteria. In part one the ultrasound examinations showed 80% sensitivity, 50% specificity and 71% accuracy while MRI showed 100% sensitivity, 57 % specificity and 87% accuracy for the diagnosis of pectoris muscles tear that need surgery.

The second MRI review demonstrated respectively sensitivity, specificity and accuracy of 100%, 57% and 87% for reader 1 and 94%, 71% and 87% for reader 2. Inter-observer kappa for the presence of a tear was 0.74. The agreement of the site of tear between reader 1 and the surgery was 0.74 for MTJ tears and 0.71 for tendon tears. The intra-observer kappa for reader 1 for the surgical cases was 1. Based on the new classification system of PM tear, there was good inter observer agreement to detect insertional tendon tears but there was poor agreement to decide if the other tears were at the tendon substance or at the myotendinous junction.

Conclusion:
The diagnostic ultrasounds and MRIs were both helpful to identify surgical candidates. Based on the new classification system of PM tear, MRI is reliable to identify insertional tendons tear but unreliable to separation between intra-substance tendinous vs myotendinous tears.

Effectiveness of Outpatient versus Operating Room Hysteroscopy for the Diagnosis and Treatment of Uterine Conditions: A Systematic Review

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Background: Common international clinical practice guidelines recommend gynecology units provide uterine assessment and treatment in an outpatient setting. However, current diagnosis and treatment of intrauterine pathology requires multiple heath care visits and continues to occur in the operating room (OR) under general anesthesia which presents significantly more complications and risks to the patient.

Objective: To systematically review the effectiveness of outpatient hysteroscopic procedures and hysteroscopic procedures performed in the operating room (OR) to diagnose and/or treat intrauterine pathology.

Methods: We searched computerized databases including Medline, EMBASE, and the Cochrane Library for clinical trials and observational studies that investigated the effects of outpatient hysteroscopic procedures in the diagnosis and treatment of intrauterine pathology. Screening and data extraction were done independently by two reviewers. Our primary outcome of interest is diagnostic accuracy, while our secondary outcomes included pain, patient and/or practitioner satisfaction, treatment success, adverse events, and cost.

Results: 19 full-text studies met our inclusion criteria including a total of 4,074 women. Seven of the 19 studies were randomized controlled-trials, while 12 were observational studies. No study compared the diagnostic accuracy of outpatient hysteroscopy to any gynecological procedure performed in the OR. 80% (8/10) of studies reported a higher satisfaction in patients receiving an outpatient hysteroscopy, while 100% (9/9) of studies reported fewer adverse events that occurred in the outpatient setting. Of the studies reporting pain, 57% (4/7) reported lower or no difference in pain scores in the outpatient group, while 60% (3/5) of papers reported more success in the surgical setting. Lastly, all seven studies reporting on cost concluded that the cost for outpatient hysteroscopic procedures is substantially lower than hysteroscopic procedures performed in the OR.

Conclusions: Outpatient hysteroscopy is a safe and less costly alternative to treating intrauterine pathology compared to hysteroscopic procedures performed in the OR.

Clinical outcomes of immunoglobulin use in solid organ transplant recipients: protocol for a systematic review and meta-analysis

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Background: Post-solid organ transplant complications such as infection are common. Immunoglobulin prophylaxis has been used to prevent infection. However, its efficacy is not clear.

Objective: We sought to investigate the effects of immunoglobulin in patients with solid organ transplant.

Methods: We searched MEDLINE, Embase and Cochrane Central Register of Controlled Trials up until September 2015 for retrospective and prospective studies reporting clinical outcomes of immunoglobulin use during peritransplant period in solid organ transplant recipients. Outcome of interest were infection, hospitalization, ICU admission, mortality, graft rejection, allograft survival and adverse events. Where appropriate, data were pooled for meta-analysis. The Cochrane Risk of Bias assessment tool was used to evaluate quality of included prospective studies and the Downs and Black risk of bias assessment tool for the retrospective studies. Pooled odds ratio estimates and 95% confidence intervals (CI) were calculated using random-effects model.

Results: Thirty-three studies were included (2863 patients): 11 were prospective RCTs, 4 were prospective cohorts, and 18 were retrospective studies. The majority was kidney (18), followed by heart (6), lung (6) and liver (3). Data were grouped and analyzed based on study design and organ transplanted. There was no statistical difference in infection, acute rejection and graft loss in kidney transplant recipients when looking at prospective studies (OR 0.72 CI 0.45-1.17, 1.02 CI 0.61-2.02 and 0.47 CI 0.27-1.26 respectively). There was no statistical difference when looking at infection and mortality when looking at kidney transplant recipients in retrospective studies (OR 0.86 CI 0.49-1.49 and OR 0.53 CI 0.17-1.68 respectively). There was a statistically significant reduction on acute rejection and graft loss in the same population (OR 0.53 CI 0.30-0.93 and OR 0.17 CI 0.06-0.45 respectively). There was a statistically significant reduction in mortality of heart transplant recipients when looking at retrospective studies (OR 0.27 CI 0.13-0.59). It was not the case for recipients of lung transplants when looking at retrospective data (OR 1.10 CI 0.82-1.46). We were not able to analyze other outcomes due to low study number, inconsistent reporting, and significant heterogeneity. Quality of RCTs was very low to low. Similarly, quality of retrospective studies was low.

Conclusions: In summary, there is not enough evidence to conclude the effect of immunoglobulin prophylaxis either universally or targeted and treatment to those with hypogammaglobinemia in solid organ transplant recipients.

Comparing Defensive Functioning Rated from Adult Attachment Interviews (AAIs) in Adults with Binge Eating Disorder (BED) in a Randomized Controlled Trial Testing Group Psychotherapy

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BACKGROUND: This study examined change in level of defensive functioning following Group Psychodynamic Interpersonal Psychotherapy (GPIP). We used the Defense Mechanisms Rating Scale (DMRS) to assess defensive functioning in Adult Attachment Interviews (AAIs) administered pre-treatment and post-treatment in the context of a randomized controlled trial for treatment of adults with binge eating disorder (BED). This is the first study to use the DMRS with the AAI and observe change in defensive functioning in BED following group psychotherapy relative to a control condition. We hypothesized that (1) the DMRS can be reliably rated from AAI transcripts, and (2) that level of defensive functioning will improve to a greater extent at post-treatment in those who received group psychotherapy compared to those in a wait-list control condition.

OBJECTIVES: We assessed inter-rater reliability of the DMRS when used with the AAI, as well as differences in defensive functioning at post-treatment between control and treatment conditions.

METHODS: Treatment-seeking individuals diagnosed with BED were randomly assigned to one of two conditions. Those in the treatment condition received 16 weeks of GPIP, whereas those in the control condition were wait-listed. AAIs were administered at pre-treatment and at 6 months post-treatment and post-waitlist control. Seven coders were trained to use the DMRS to rate defensive functioning from AAI transcripts. Coders, blind to study condition and time-point, coded pairs of pre-treatment and 6-month post-treatment AAIs using the DMRS (GPIP: n = 18; Control n = 18). Inter-rater reliability was measured by intra class correlation coefficients (ICCs). Difference in post-treatment overall defensive functioning (ODF) score between treatment and control groups was assessed by ANCOVA in which pre-treatment ODF score was the covariate.

RESULTS: Inter-rater reliability as measured by ICCs were adequate for ODF score and all subscales of the DMRS, except for Major Image Distortion and Action Defenses. Those who received GPIP showed significantly higher ODF scores compared to the control group at the post-treatment time-point.

CONCLUSIONS: Adequate ICCs for inter-rater reliability on ODF score as well as most subscales of the DMRS support the continued use of DMRS to code AAI transcripts for level of defensive functioning. A significant difference in post-treatment defensive functioning following GPIP indicated the improved use of adaptive defense mechanisms with the GPIP treatment modality for those with BED.

Ilioinguinal/iliohypogastric nerve blocks as a treatment for pelvic pain in a gynecologic population

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Introduction: Ilioinguinal/iliohypogastric nerve blocks (IINBs) are a simple, minimally invasive option for managing pain associated with ilioinguinal/iliohypogastric neuropathy (IIN). This study assessed the safety and efficacy of IINBs in women with IIN associated pelvic pain.

Methods: A retrospective chart review of patients who received IINBs at The Ottawa Hospital between January 1st, 2012 and July 13th, 2017 by a single physician was performed. Patient demographics, history, examination findings, and block data were extracted. Chi-square and Mann-Whitney U tests explored differences in patient characteristics between women with and without effective response to IINBs.

Results: Amongst 106 meeting inclusion criteria, 381 IINBs were performed. Most women had a history of chronic pain (*n*=97,90%), endometriosis (*n*=69,65%), and/or lower abdominal surgery (*n*=95,90%). On examination, 56 women (53%) had tenderness within the IIN distribution, 59(56%) had point tenderness, 20(19%) had a positive Carnett's sign, 21(20%) had allodynia, and/or 7(7%) had hyperalgesia.

Data regarding effectiveness was available for 301/381 blocks. Of these, the majority (*n*=227,75%) greatly improved pain, 11%(*n*=33) somewhat improved pain, 10%(*n*=31) had no effect, and 3%(*n*=10) worsened pain. Sixty women (57%) had at least one block which greatly improved pain. Five women (5%) reported complications which could be directly related to blocks. No significant differences in patient characteristics were observed between women with and without effective response to IINBs.

Conclusion: IINBs may provide pain relief for women with complex pelvic pain and features of central sensitization with a low rate of complications. Future prospective studies on IINB safety and efficacy are warranted.

The Impact of Prenatal Methadone or Suboxone Exposure on Infant and Child Development: A Review

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Background: There is a poor understanding of the long term impact of opioid replacement therapy (ORT) on child development. Pregnant women with opioid addiction may receive ORT with either methadone or suboxone to decrease unregulated drug exposure of the fetus. The definitive *long term* effects of this exposure remain largely unknown.

Objective: This scoping review summarizes the current state of knowledge in the literature. *Methods:* This review initially identified one hundred and seventy-one articles for analysis. Following abstract screening and full text review for exclusion criteria, fifty-three articles were identified for inclusion. Study quality was assessed using the STROBE Statement Checklist.

Results: There is evidence to suggest a negative impact of methadone exposure on the development of the infant's visual system and sleep patterns within the infant's first year. Findings are conflicted with respect to the long term impact of methadone use on infant growth and development. Infants born to mothers with ORT were generally smaller at birth, but this appears to resolve over time. Studies suggest that any differences in the exposed child's development may be attributable to decreased maternal attachment. This phenomenon is postulated to be due to compromised social situations rather than ORT itself. Maternal attachment is an appealing target for early intervention. Despite increasing clinical use, there is little data regarding the long term impact of suboxone use in pregnancy and long term child development, suggesting an area for future research. There was considerable heterogeneity in the identified studies, limiting the ability to generate conclusions.

Conclusion: The conflicting literature on the long-term offspring effects of ORT in pregnancy is not surprising due to the complexity of substance abuse. The relationship is highly confounded by social exposures separate from methadone or suboxone use. It is important to consider these complexities and continue to implement early intervention programs and treatment for both mother and child to ensure best possible outcomes.

Brain-derived neurotropic factor Val66Met polymorphism and post-traumatic stress disorder among survivors of a major flood in China

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Background: Brain-derived neurotropic factor (BDNF) has been implicated in various psychiatric disorders, such as post-traumatic stress disorder (PTSD), bipolar disorder, schizophrenia and anxiety. BDNF Val66Met polymorphism, which affects synaptic localization and plays a crucial role in activity-dependent secretion of BDNF from the neurons, is a functional single nucleotide polymorphism of the BDNF gene. The role of BDNF Val66Met polymorphism in PTSD has been widely explored. However, studies in populations with different types of trauma exposure have yielded inconsistent results when the association between BDNF Val66Met polymorphism and PTSD was examined, suggesting the type of trauma exposure may affect this association. Up to now, there has been no study attempting to examine the association between BDNF Val66Met polymorphism and PTSD among flood survivors.
Objective: This study aimed to examine the association between BDNF Val66Met polymorphism and PTSD among flood survivors.

Methods: Individuals who experienced the 1998 Dongting Lake flood, a major flood in Chinese history, in Southeast Huarong, China were enrolled in this study. Qualified health personnel carried out face-to-face interviews with participants. PTSD was identified using PTSD Checklist-Civilian version (PCL-C). Blood samples were collected from the participants to extract DNA for genotyping.

Results: A total of 175 participants were enrolled in this study. The prevalence of PTSD among flood survivors at 17-year follow-up was 16.0%. Individuals with PTSD were more likely to be female, experienced at least three flood-related stressors, experienced at least three post-flood stressors, and carried the Met than those without PTSD. Compared with Val/Val homozygotes, Met carriers had higher scores of PCL-C (mean ± standard error: 27.19±9.48 versus 23.60±7.23, $P<0.05$). Multivariable logistic regression analysis indicated that Met carriers (aOR=4.76, 95%CI=1.02-22.15, $P<0.05$) were more likely to develop PTSD than Val/Val homozygotes.

Conclusions: Met carriers for BDNF rs6265 are at increased risk of developing PTSD and also exhibit more severe PTSD symptoms than Val/Val homozygotes among flood survivors in China.

How pervasive are unit of analysis errors in cluster randomised trials and what can be done about it: A review of diabetes quality improvement RCTs

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Background: Cluster randomised trials (CRTs) can lead to spurious conclusions if clustering is not taken into account during analysis (i.e., unit of analysis error). Meta-analysis of CRTs with unit of analysis errors may lead to incorrect review conclusions. Standard methods have been developed to correct unit of analysis errors, however these methods ideally require cluster sizes and intraclass correlation coefficients (ICCs) to be reported.

Objectives: To determine the proportion of CRTs that have unit of analysis errors (and whether they provide data to correct for errors) in a large systematic review of diabetes quality improvement strategies.

Methods: Two researchers independently assessed the analysis and reporting of 13 outcomes (4 continuous and 9 binary) across 73 CRTs to determine whether: 1) analyses appropriately adjusted for clustering, 2) adjusted estimates could be extracted (or calculated), 3) outcome-specific ICCs were reported.

Results: The proportion of appropriately analyzed studies varied across outcomes ranging from 25% for Harms to 81% for LDL. For HbA1c, only 37 of 54 studies (69%) adjusted for clustering. For continuous outcomes, only 3-4% of studies reported standard errors adjusted for clustering. Although many studies reported other measures of variance (e.g., 95% CI, p-value) to calculate adjusted-standard errors indirectly, approximately half of the studies still had unit of analysis errors. ICCs were reported rarely across all outcomes (0-27% of studies). Correction of unit analysis errors for most studies will therefore require external estimates of ICCs.

Conclusions: Cluster RCTs pose important methodological challenges for systematic reviews. Reviewers need to be aware of potential unit of analysis errors and adjust estimates accordingly. While many CRTs in the diabetes QI review adjusted for clustering, study reports did not give enough information to extract or adjust estimates required for meta-analysis.

The association of incident ST and non-ST elevation myocardial infarction in chronic kidney disease

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Background: Cardiac disease is the most common cause of morbidity and mortality among patients with chronic kidney disease (CKD). CKD is defined by a decrease in kidney function (measured by the estimated glomerular filtration rate) and/or the presence of proteinuria (measured by the albumin to creatinine ratio). Low levels of eGFR are associated with a propensity for vascular calcification whereas higher proteinuria with thrombosis.

Objective: The objective of this study wa to determine the association between the type of incident acute coronary syndrome (ACS), ST-elevation myocardial infarction (STEMI, a thrombosis-based event) or non-STEMI (a calcification-based event) by eGFR and ACR category.

Methods: Using administrative, population-level data from Ontario, Canada, we examined seniors (>66 years of age) with a measure of outpatient eGFR and ACR for incident ACS from April 2002 to March 2015. Multivariate Fine & Gray sub-distribution hazards, adjusted for demographics, comorbidities, health resource utilization and medications accounting for the competing risk of death were used.

Results: In a total of 248,438 patients, STEMI, NSTEMI and mortality occurred in 1,435 (0.58%), 4,431 (1.78%) and 30,015 (12.08%) patients, respectively. The crude rate of STEMI and NSTEMI was higher across increasing ACR categories and lower eGFR categories (ACR>30 mg/mmol and eGFR<30 mL/min/1.73 m^2: HR 5.60, CI 4.18-7.52, HR 7.67 CI 6.49-9.07 for STEMI and NSTEMI, respectively). In adjusted models, the increase in risk for STEMI was higher across increasing ACR relative to declining eGFR (HR = 4.53, CI 3.30-6.21). Conversely NSTEMI risk was higher with low eGFR categories relative to higher ACR (HR = 4.42, CI 3.67-5.32).

Conclusions: Patients with CKD are at a high risk for both ST- and non-ST- elevation MI. Specifically, a high ACR, in contrast to a low eGFR, confers a higher risk of STEMI possibly by an increased thrombosis risk.

Reporting Bias in Imaging Research: Do studies with higher diagnostic accuracy estimates get published sooner?

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Background: Selective reporting and publication of primary studies can introduce substantial bias into systematic reviews and meta-analyses. Although the prevalence and impact of reporting bias has been well documented for therapeutic intervention studies, there is less evidence of this phenomenon for diagnostic accuracy research in imaging journals. Prior work has documented delayed publication of studies with inferior test accuracy estimates. This is problematic as it may lead to inflated accuracy estimates in systematic reviews and meta-analyses used in every day clinical decision making.

Objectives: The objective of this study was to evaluate whether higher reported accuracy estimates are associated with shorter time to publication among published imaging diagnostic accuracy studies.

Methods: We included primary diagnostic accuracy studies from meta-analyses in Medline-indexed systematic reviews that were published in 2015. We extracted data on accuracy estimates, participant recruitment period, and publication dates. Using correlation and Cox hazard regression analyses, we assessed for associations between time to publication and Youden's index (calculated as sensitivity + specificity - 1) as our primary outcome.

Results: Our sample included 55 meta-analyses and 781 primary studies. The median time from completion to publication was 20 months (IQR 14 - 29). Youden's index was negatively correlated with time from completion to publication (rho = −0.106; *p =0.009*). These publication time lags remained significant in multivariable Cox regression analyses after adjusting for year of publication, journal impact factor, number of authors, continent of first of author, type of data collection, study duration and sample size. The hazard ratio of publication was 1.09 (95 % confidence interval [CI] 1.03 to 1.15; $p = 0.002$) per unit increase in logit-transformed estimates of Youden's index.

Conclusion: Higher accuracy estimates are weakly associated with a shorter time to publication of diagnostic accuracy studies. Therefore, the impact of reporting bias on systematic review findings may be less important in diagnostic accuracy research than it is in evaluation of therapeutic interventions. Further study into factors associated with publication (e.g. association of accuracy estimates with publication of conference abstracts, registered protocols or ethics-approved diagnostic accuracy studies) may add to our understanding of reporting bias in imaging diagnostic accuracy research.

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.

Assessing variability in surgeon empathy using a validated assessment measure at a tertiary care centre

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Introduction:
Physician empathy has been associated with better outcomes, fewer malpractice claims, and greater patient safety and remains an essential attribute of the patient-physician relationship which is a key determinant of patient experience. Identifying modifiable and non-modifiable factors to improve patient experience as it relates to the patient-physician relation are not known. The Consultation and Relational Empathy (CARE) measure was developed by Mercer and colleagues at Glasgow and Edinburgh Universities based on empathy in the context of a therapeutic relationship within a consultation. It has been used widely in general practice, but not yet in orthopaedic surgery.

Objective:
Our objective was to assess whether CARE scores are stable for a given surgeon and to what extent they vary between surgeons.

Methods:
The validated CARE survey asks ten questions which holistically assess caregiver empathy. The answer options range on a scale of 1 to 5, with 1 being poor and 5 being excellent, with a total score of 50. With the intention of sampling 19 orthopaedic surgeons over the course of several clinics, surveys were distributed to patients upon arrival at the clinic (by the clerk) and they were asked to return them to the front desk after their visit with the surgeon. To assess how attributable the survey is to the surgeon, SPSS version 22.0 was used to measure intraphysician correlation coefficient (Intraclass correlation coefficient).

Results:
Seven hundred and thirty one CARE surveys were completed for nineteen surgeons. Reliable data (ICC > 80%) was obtained for 11 surgeons, and 8 surgeons were lacking questionnaires. The highest number of questionnaires from a surgeon with an ICC lower than 80% was 28, suggesting that more than 30 questionnaire per surgeon are needed to obtain a reliable estimate of surgeon empathy. Of the 11 surgeons with reliable estimates, the overall average CARE score for all surgeons was 45.65 out of possible maximum score 50 (standard deviation: 2.46). Average individual surgeon scores ranged for 42.21 to 50. Three surgeons were statistically significantly above overall average, three were under, and five were not significantly different from average.

Conclusion:
The results show that the CARE measure scores appear stable for each surgeon. CARE scores variability between orthopaedic surgeons appears very similar to physician normative scores, with similar averages, ranges, and distributions. Future studies are needed to determine the impact of CARE scores on patient outcomes in orthopaedic surgery, and ability of interventions to modify CARE scores.

Design for Intelligent Stance-Control System with Local Sensor Gait Phase Recognition for Real-Time Orthosis-Control

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Background: Emerging microprocessor-controlled knee-ankle-foot orthoses (M-SCKAFOs) use multiple sensor systems to guide knee control during gait, and can provide enhanced functions across daily walking activities. M-SCKAFO systems could use machine learning based gait phase detection and/or rule-based gait phase detection for natural walking. Most on the market today use rule-based control algorithms and complex sensor systems located at all orthotic-segments, producing increasingly expensive and difficult to personalize devices.

Objective: To evaluate an optimized machine learning based gait phase recognition system along with rule-based artificial intelligence algorithm to develop a real-time stance-control system that uses a local sensor system (thigh and knee) to be used in orthosis-control.

Methods: A de-identified data set provided gait dynamics for thigh angular velocity, acceleration, and knee angle for various ground level conditions and speeds. Each stride was segmented into the four gait phases desired for classification (loading response, push-off, swing, and terminal swing). 21 features were extracted from this data using a 0.1s sliding window to train and test a logistic model decision tree. The classification function was then integrated with a rule-based algorithm to determine appropriate instances for stance-control switch settings.

Results: Logistic model decision tree gait phase recognition integrated with a rule-based algorithm for real-time stance-control provided suitable switching instances along each stride. Low-resistance valve switch (for knee flexion during swing) and high-resistance valve switch (for knee-support during stance) was determined at appropriate times during all strides. This design for an intelligent stance-control system that uses local sensors is robust enough to handle uneven ground condition and different walking speeds.

Conclusion: A novel local sensor stance-control system for application in M-SCKAFOs is presented. Local sensor signals at the thigh and knee integrated with machine intelligence algorithms are viable for effective real-time stance-control across uneven ground conditions and different speeds. This is an ideal system for M-SCKAFOs that will lead to inexpensive modular unit that can fit under trousers, is easily customizable for end-users, and comes with sophisticated knee-control functionality able to be used throughout daily walking activities.

The Efficacy of Turmeric in the Treatment of Digestive Diseases: A Systematic Review

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Background: Digestive disorders contribute considerably to the global burden of disease and result in significant costs to healthcare systems, as well as marked reductions in the quality of life of affected individuals. Alternative medicines such as turmeric continue to gain popularity as treatment options for digestive diseases, although rigorous assessments of its effectiveness in improving disease outcomes are limited.

Objective: To systematically review the evidence for the efficacy of turmeric and its derivatives or compounds in the treatment of digestive diseases, including irritable bowel syndrome (IBS), dyspepsia, gastroesophageal reflux disease (GERD), peptic ulcers, and inflammatory bowel disease (IBD).

Methodology: A literature search was conducted using Medline, EMBASE, AMED, the Dissertations & Theses Database, and the Cochrane Central Register of Control Trials from inception to May 2017. The searches yielded 710 studies (following duplicate removal). Of these, 675 were excluded after abstract screening, and 1 additional study was included via a manual search. Thus, 36 full articles were screened and after 22 further exclusions, 14 were included in the final review. The primary outcomes assessed were remission and relapse rates and relief of GI symptoms, whilst secondary outcomes included changes in quality of life (QOL), and the occurrence of adverse events.

Results: Due to high heterogeneity between studies in terms of treatment outcome measurements and the administration of curcumin, a meta-analysis was not feasible. Four studies reported treatment success in terms of the proportion of patients experiencing symptom improvement. The percentage of patients experiencing symptom improvement in the turmeric arm was higher than the proportion experiencing improvement in the placebo arm in 2 of the 4 included studies. The percentages of patients experiencing symptom improvement ranged from 25.8-71.1% in the turmeric arm, and 27.1-65.2% in the control arm. Among studies evaluating the proportion of patients experiencing clinical improvement (n=4) clinical improvement was observed in 18.9-56.5% of patients in the turmeric group, and 0.0-36.4% of patients in the placebo group. Turmeric groups were also found to experience greater improvement in QOL in comparison to placebo or conventional treatment arms.

Conclusion: Turmeric may be effective for treating and managing digestive disorders, however, due to the limitations imposed by the variability in the quality of studies, small sample sizes, the short duration of interventions, the lack of long-term data availability and the small number of relevant studies, the evidence for this remains insufficient.

Analysis of plasma lipid biomarkers in acute myocardial infarction after percutaneous coronary intervention by direct infusion mass spectrometry

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Background: Acute myocardial infarction (MI) remains a major cause of morbidity and mortality worldwide. Immediate and prompt revascularisation with percutaneous coronary intervention (PCI) or thrombolysis can reduce acute myocardial ischemic injury, limit MI size, decrease in-hospital mortality, and improve the long-term outlook in survivors of the acute phase. However, reperfusion can itself induce cardiomyocyte death, known as myocardial reperfusion injury. Although reperfusion (I/R) injury is one of the main reasons for myocardial cell death and heart failure, the exact pathophysiological mechanism underlying myocardial ischemia reperfusion (I/R) injury is not clear. The underlying pathological mechanisms are triggered when reperfusion injury occurs. The exact pathophysiology mechanism is not well understood. Lipid has a very important role in regulating signal transduction, and can regulate many pathological processes. So far, there has been no study on the dynamic changes of lipid during the process of MIRI, which may reveal how lipid plays a part in regulating MIRI.

Objective: The purpose of this study is to analyze the plasma lipid biomarkers in acute myocardial infarction after percutaneous coronary intervention by direct infusion mass spectrometry, the metabolic pathway of those lipid biomarkers, and to find out the signal pathways regulated by lipid biomarkers and the mechanism of lipid role.

Methods: We conducted a prospective, single-center, randomized trial on the lipid composition and content changes of the blood sample in patients before and after percutaneous coronary intervention treatment by direct infusion mass spectrometry. The dynamically changes as the ruperfusion times of 15 types of specific lipid markers (myristic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, stearic acid, oleic acid, linoleic acid, γ-linolenic acid , Eicosatrienoic acid and eicosatrienoic acid, eicosatetraenoic acid, eicosatetraenoic acid, eicosatetraenoic acid, and eicosapentaenoic acid) at different intervals (1 hour, 4 hours, 8 hours, 24 hours, 48 hours, 7 days before PCI and after PCI) were identified by direct infusion mass spectrometry and analyzed the changes of lipid composition and content.
Results: The endogenous ligands of PPAR? played an pivotal role on anti-inflammation by activating PPAR?signal pathways.

Conclusion: The dynamic changes of lipid markers have a close relationship with PPAR?which has a key role on anti-inflammation. The underlying mechanism of MIRI may involve in inflammatory response. Those lipid markers may be not only the promising therapeutic targets, but also the potential lipid drugs as the agonist of PPAR treating myocardial I/R injury.

Modelling and Characterization of Lower Extremity Powered Exoskeleton Ankle Mechanics for Very Slow Walking

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Lower extremity powered exoskeletons (LEPEs) allow people with spinal cord injury to stand, walk, and perform activities of daily living. However, current LEPEs walk slowly and cannot be used for extended periods because users tire due to crutch requirements for balancing and weight transfer. The ankle contributes to speed and stability during able-bodied walking, but most LEPEs lack biomimetic ankle design. This research investigated biomimetic ankle design requirements using a full 3D body model of a real LEPE (ARKE, Bionik Labs) attached to a human model, driven by 3D motion capture data of 29 able-bodied individuals walking at realistic LEPE slow walking speeds. Ankle range of motion, quasi-stiffness calculated from linear and quadratic regressions, work, peak moment, and peak power were compared between human and human+ARKE models, across four gait phases and four slow walking speeds. Ankle quasi-stiffness was significantly different across all gait phases and between human and human+ARKE models, and increased significantly with speed for controlled dorsiflexion and active plantarflexion phases. The human+ARKE model's ankle absorbed more total energy and produced negative net work, even for the fastest speed, compared to the human only model that produce positive net work for the fastest speed. R^2 values for quadratic regressions were significantly greater than linear regressions while RMSE values were significantly lower for quadratic versus linear regressions ($p<0.05$). These results suggest that passive variable stiffness ankles incorporating quadratic elastic spring elements could achieve biomimetic ankle function for lower extremity powered exoskeletons.

Trans-tibial Amputee Gait and Satisfaction with Elevated Vacuum Suspension System

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Background:
Unity suspension system consists of a mechanical vacuum pump and a hypobaric sealing membrane around an Iceross Seal-In V liner to remove the need for an external sleeve. Research is lacking on the Unity system's effect on trans-tibial amputee gait performance and comfort.

Objective:
The main purpose of this study was to evaluate effects of the Unity elevated vacuum system on level walking performance while the vacuum was active (ON) and inactive (OFF).

Methods:
The Ottawa Hospital Research Ethics Board approved the protocol. Twelve people with unilateral transtibial amputation were fitted with the Ossur Unity elevated vacuum suspension system. 3D motion analysis was completed using a virtual Park scenario within the CAREN Extended System (Motek Medical, Amsterdam, NL). Participants completed two walking trials while the vacuum was active (ON) and two walking trials while the vacuum was inactive (OFF). The order for ON and OFF conditions was randomized and blinded for each participant.

Results:
All participants reported no movement inside the socket and improved proprioception when the vacuum was ON compared to vacuum OFF and their previous suspension system. Statistically significant differences ($p<0.05$) were found between vacuum conditions for most temporal spatial, kinematic, and kinetic gait parameters; however, effect sizes were small ($r<0.3$). The largest difference between vacuum conditions was observed for step length. Symmetry index indicated a more symmetrical step length with vacuum ON (SI=7.42 (SD=5.41)).

Conclusions:
Active vacuum effects on gait parameters were small during level walking and were not clinically significant. While elevated vacuum suspension can provide other benefits, including proprioception and comfort, level walking gait effects are small if a well-fitting liner-based socket is provided. Future investigations across other surfaces encountered in daily living, such as slopes, are also needed to better determine the effects of active vacuum on gait parameters.

Understanding High Frequency Use of the Emergency Department (ED) for Chronic Pain Patients: Results from a Qualitative Study exploring Patient Experiences and Cost Effective Health Care Solutions

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Background: During the 2012-2013 fiscal year, we found that chronic pain (CP) was the primary reason for presentation among 36.4% of patients who had more than 12 ED visits over a 12-month period at The Ottawa Hospital. The ED is generally not the most appropriate setting for CP treatment and management; it is associated with greater risk of harm, does not provide opportunity to find and implement longer term solutions to improve patients' pain and function, and it puts pressure on limited ED resources, contributing to longer wait-time and overcrowding.

Objective: The purpose of this study was to identify factors leading to ED presentation and to learn about the experiences of CP patients with multiple visits to the ED in order to explore solutions that will reduce ED presentation and improve health outcomes**.**

Methods: A qualitative design was used. Participants were CP patients who had visited the Ottawa Hospital ED at least12 times or more in the 2013-2014 fiscal year. Seven women and five men between the ages of 26 - 68 years (M = 46.17; SD = 13.17), agreed to participate in one semi-structured interview.

Analysis: Applied Thematic Analysis was employed to analyze and code the transcribed interviews. This process involved having multiple members of the research team identify codes for each interview and a reiterative process of refining the themes and subthemes.

Results: Five themes emerged: factors contributing to ED visits; patient experiences of chronic pain in the ED; factors influencing patient experience; patient experience with family physician or specialist; and possible alternatives to ED visits. Factors contributing to the ED visits included: fear (e.g., pain escalation and impact on quality of life); needing relief; an inability to successfully manage their pain; physician limitations; helplessness; lack of support from their primary care physician; and pain intensity. The patients' experiences at the ED consisted of positive, negative, and mixed experiences. Positive experiences included experiencing pain relief and believing their needs were being met, while also feeling acknowledged and supported by the physicians and staff. Conversely, negative experiences involved: the patients believing their needs were not met; long wait times; feeling judged by physicians and staff by inquiry into drug seeking behaviors and an overall disregard for their pain history.

Conclusion*:* Patients with chronic pain who are frequent users of the ED presented complex pain experiences, yet were unanimous in wanting to find better ways to manage their chronic pain.

A systematic review of interventions for smoking tobacco cessation in lower socio-economic status populations

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Background:
There is a large amount of evidence confirming that smoking tobacco prevalence is much greater among people who use drugs (PWUD) and people experiencing homelessness (PEH) than in the general population. The health inequities between these populations are widened due to the increased morbidity and mortality related to all smoking related diseases in the lower socio-economic status populations. Spontaneous quitting is negligible in this population. There are to date no well-established interventions to promote tobacco smoking cessation in this vulnerable population.
Objective:
To conduct a systematic review to assess the efficacy of smoking tobacco reduction and cessation interventions in populations experiencing homelessness or poverty.
Methods:
We conducted data base search in November 2016 and updated it in April 2017, to identify Randomized Controlled Trials (RCT) with the primary outcome of smoking tobacco cessation in lower socioeconomic populations. The search was conducted in Embase and Medline and included RCT published between 1946 and 2017.
Results:
The search yielded 1340 articles, 32 of which met inclusion and exclusion criteria (Inclusion: 1. RCT, 2. Low income/homeless/lower socio-economic status 3.Tobacco dependence intervention, end point is quitting or reducing tobacco smoking 4. Tobacco dependant adults. Exclusion: 1.No abstract or article available 2. Secondary analysis or study protocol 3. Not active smokers 4. In treatment centres (rehab, hospitals)). Two independent reviwers selected articles after screening the titles and abstracts; and then they extracted the data. A third reviewer resolved any discrepancies. We also examined the back references of the included articles for any new studies. Due to significant heterogeneity, meta-analysis could not be performed. Preliminary results showed that a variety of interventions were studied in this population, some of which included nicotine replacement therapy, counseling sessions, and behavioural interventions. Several of these interventions demonstrated significantly increased biochemically-verified smoking abstinence rates. Some studies, such an internet-based smoking cessation advice intervention, were specifically tailored for low-socioeconomic-status populations and demonstrated efficacy while being economically viable.
Conclusions:
While the smoking cessation interventions were heterogenous, there were interventions that demonstrated significant improvement in tobacco smoking abstinence rates among PWUD and PEH. These interventions have the potential to reduce the health inequity between this group and the general population. Future projects should include community-based participatory research to determine which interventions are the most acceptable and feasible to specific communities and should assess the long-term sustainability of interventions.

Long-term care placement and mortality after dementia diagnosis: A population-based study

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Background: The prevalence of dementia in Canada is projected to more than double between 2008 and 2038, due in large part to Canada's aging population. Dementia is a major risk factor for the need for formal home care services, placement in a long-term care (LTC) facility (or nursing home), and mortality. To date, studies on dementia outcomes have been limited to non-population based cohorts, restricting their generalizability, and have not explored all three outcomes together.

Objective: To describe the outcomes for individuals newly diagnosed with dementia, and the predictive factors associated with mortality within 5 years of diagnosis.

Methods: A population-based retrospective cohort study was conducted to examine the rates of home care, LTC placement and death for all community-dwelling Ontarians aged 65 and over diagnosed with dementia between July 1, 2007, and June 30, 2010. Using routinely-collected administrative data, outcomes were described at 1, 3 and 5 years after dementia diagnosis. Logistic regression was used to identify predictive factors associated with mortality 5 years post-diagnosis.

Results: 83,919 individuals were identified as being diagnosed with dementia during the study period. Home care services were accessed soon after diagnosis and by a large proportion of individuals, while LTC placement took longer and was less frequent (although still common). By the end of the 5-year follow-up period, 76% of the cohort had received home care, 39% had been placed in LTC, and 53% had died. Men were significantly more likely to die than women at all time points examined, while women were more likely than men to receive home care and be placed in LTC. The most common trajectory after dementia diagnosis was home care followed by LTC placement and death (25.3% of cohort). Age was by far the most significant predictor of death, while other key predictors included male sex, congestive heart failure, renal failure and chronic obstructive pulmonary disease (COPD).

Conclusions: This population-based study provides a detailed analysis of the outcome trajectories following dementia diagnosis and predictive factors of mortality that can be used by patients, their families and physicians to better anticipate disease course. The results are also valuable for policymakers in health systems planning in response to the increasing prevalence of dementia.

Incidence of post-operative urinary retention (POUR) in males undergoing total hip and knee arthroplasty at The Ottawa Hospital (TOH)

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Background
Postoperative urinary retention (POUR) is defined as the inability to void in the presence of a full bladder. This condition is common after total hip and knee arthroplasty (THA and TKA) procedures prevalent in males over fifty years of age. The current standard treatment of POUR is bladder catheterization, which is linked to complications and patient dissatisfaction. Alternative methods of treating and preventing POUR are necessary to mitigate complications and decrease hospital costs. In order to implement such methods, understanding the incidence in the male population is essential and has not been explored at The Ottawa Hospital (TOH). In the literature, there are inconsistencies of reported incidences of POUR among different age cohorts across the country. Therefore, isolating the age group at highest risk is necessary to determine which age cohort will be most appropriate for intervention.

Objective
The objective of this study was to determine the age group at highest risk for POUR at TOH, in order to further delineate the group in which an intervention would be most effective and appropriate.

Methods
The incidence of POUR at TOH was determined between July 1st 2016 and June 30st 2017 through a retrospective chart review design in males over 50, based on representative incidence (5% SE). The integrated progress notes were thoroughly examined and keywords pertaining to the presence of POUR were used to assist the search. To determine which age group was at higher risk, we ran Pearson chi-square between age 50-60 and 60+ cohorts to better focus future intervention with p-value of 0.05 as significance.

Results
Incidence of POUR for THA patients between the ages of 50-60 and 60 and over was 26.3% and 33.6%, respectively (p= 0.397). Incidence of POUR for TKA patients for same age cohorts was 8.8% and 26.8% respectively (p= 0.026). The overall incidence for TKA and THA procedures combined between the ages of 50-60 and 60 and over was 18.1% and 30.1%, respectively (p= 0.041).

Conclusions
Overall, males over the age of 60 are statistically at a significantly higher risk of developing POUR than males between the ages of 50 and 60. This indicates that this age group will be most efficient for us to trial an intervention on. This may have the capacity to decrease incidence of POUR and patient catheterization.

Does trial registration reduce research bias? A comparison of registered and unregistered trials in diabetes quality improvement interventions

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Background: In 2005, the International Committee of Medical Journal Editors (ICMJE) implemented a policy on trial registration to prevent selective publication and selective reporting of outcomes. It is currently unclear whether trials continue to be conducted without registration, and whether unregistered trials are at greater risk of selective publication than registered trials. In this study, we compare the characteristics of registered and unregistered trials of diabetes quality improvement (QI) interventions.

Objectives: 1) To estimate what proportion of trials on diabetes quality improvement (QI) interventions are unregistered; 2) To compare study characteristics of unregistered trials to registered trials; 3) To examine whether there is greater likelihood of selective publication among unregistered trials.

Methods: In a systematic review of diabetes QI interventions, we identified 119 trials published between 2010 and 2014 reporting on HbA1c as an outcome. We assessed the proportion of trials that were unregistered, and compared characteristics of unregistered and registered trials including ethics approval, sample size, number of study arms, cluster- versus patient-level randomisation, study duration, number of outcomes reported. We used meta-analysis to assess whether trial registration status is related to reported effect size and funnel plots to examine selective publication.

Results: Of the 119 trials examined, 44 (37%) trials were not registered in a clinical trials registry. Compared to registered trials, unregistered trials had a shorter median duration of interventions (6 vs 12 months) and shorter median follow-up (9 vs. 12 months). Cluster randomization was less common in unregistered compared to registered trials (13% vs 28%). The effect size of interventions on HbA1c was significantly larger in unregistered trials (-0.49, 95%CI -0.65, -0.34) compared to registered trials (-0.19, 95%CI -0.25, -0.13). Finally, funnel plots demonstrated important asymmetry suggesting greater likelihood of selective publication among unregistered trials.

Conclusions: Despite the ICMJE policy, a large proportion of diabetes quality improvement trials published after 2010 remain unregistered. Unregistered trials report significantly larger effects on HbA1c. The distribution of effect sizes suggests that unregistered trials are at greater risk of selective publication. These findings may have implications on how unregistered trials are to be handled in a systematic literature review of diabetes QI interventions.

Mindfulness is a Significant Predictor of Mental Health-Related Quality of Life in Breast Cancer Survivors with Neuropathic Pain

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BACKGROUND:
Chronic neuropathic pain (CNP) is defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system and affects up to 50% of breast cancer survivors. There is no cure for CNP and pharmacotherapeutic treatments remain limited; however, mindfulness has been linked to mental health-related quality of life (HRQoL), thus Mindfulness-Based Stress Reduction (MBSR) may be a promising intervention given its demonstrated benefits. The current project involved the analysis of baseline data from a larger randomized controlled trial investigating the effect of MBSR among breast cancer survivors with CNP.

OBJECTIVE:
To examine the relationship between mental HRQoL and mindfulness 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: Five Facets Mindfulness Questionnaire (FFMQ), Perceived Stress Scale (PSS), Brief Pain Inventory (BPI), Pain Catastrophizing Scale (PCS), Patient Health Questionnaire-9 (PHQ-9), and Short Form Health Survey-12v2 (SF-12v2). Pearson correlations were completed to examine the relationships between variables, and hierarchical regression analyses were performed to examine whether mindfulness predicted mental HRQoL after controlling for pain intensity, interference, and catastrophizing.

RESULTS:
Participants reported neuropathic pain for an average of 2.9 years (*SD* = 1.8). Analyses revealed that mindfulness correlated negatively with depressive symptoms (r = -.49, p < .001), pain interference (r = -.23, p < .05), stress (r = -.62, p < .001), pain catastrophizing (r = -.39, p < .001), and positively with mental HRQoL (r = .58, p < .001). After controlling for age, pain intensity, and interference, mindfulness predicted 28% (p < .001) of variance in mental HRQoL.

CONCLUSIONS:
Consistent with existing research, we found mindfulness to be associated with mental health-related quality of life. These results contribute to evidence supporting the role of mindfulness in improving mental health in breast cancer survivors with CNP. Future work will examine whether changes in mindfulness, through training, predicts improvement in mental HRQoL among breast cancer survivors living with CNP.

Does EUS Performed by a Trainee Affect Procedure Quality? A Large Single-Center Study

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Background/Objective
While many procedures are performed within gastroenterology, limited data exists on the quality of those completed by trainees. Endoscopic ultrasound (EUS) is a core diagnostic modality used in the evaluation of gastrointestinal diseases, but only a few studies have examined trainee procedural quality. As such, we wanted to identify the impact on EUS procedures performed by trainees with staff (TS), using diagnostic yields (DY) and adverse events (AE) as quality indicators.

Methods
A retrospective chart review of patients who underwent EUS at The Ottawa Hospital (TOH) between September 2009 and May 2015 was performed. Data was collected on patient demographics, procedure details, and DYs. AEs were identified by reviewing emergency department visits and hospitalizations within 30 days of the procedure.

Results
1651 EUS cases were analyzed. The median patient age was 64 (IQR 53-73) years with 50% being male. The overall EUS DY was 80% and the risk of an AE was 3.5% (58 patients). Of all cases, 27% were performed by TS. We found TS procedures to not be associated with a significant decrease in DY (82%, p = 0.30) or an increased AE risk (4.9%, p = 0.064) in comparison to staff alone (SA). However, trainee DY did improve every 4 months (79%, 81%, 85%), while AE risk was highest in the first and last 4 months of training (6.8%, 2.1%, 5.9%). This trend was not seen for SA (DY = 81%, 80%, 78%; AE risk = 2.9%, 2.6%, 3.5%).

Conclusion
TS procedures demonstrate a U-shaped trend for AE risk. This warrants further evaluation to determine how excess risk to patients can be avoided during training.

Mindfulness-based Stress Reduction Changes the Brains of Breast Cancer Patient Survivors with Chronic Neuropathic Pain: an fMRI Study

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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. Although there is currently no cure for CNP, mindfulness has been shown to be related to decreased levels of pain and psychological distress. Previous neuroimaging studies that looked at participation in Mindfulness-based stress reduction (MBSR) found changes in the brain involved in attention, memory and emotional processing.

Objective: As part of a larger randomized control trial looking at the effects of mindfulness training among breast cancer survivors with CNP, this study evaluates the impact that MBSR has on the brain activity during emotional processing of breast cancer survivors with CNP.

Methods: Eighteen women (Mean age = 52.5, *SD* = 11.1) with CNP following breast cancer treatment were randomized (9 treatment, 9 control) and completed an Emotional Stroop task (EST) while undergoing functional magnetic resonance imaging (fMRI) pre and post-MBSR.

Analyses: Each participant's EST fMRI data was post-processed and analyzed individually followed by group comparisons, using Statistical Parametric Mapping 8. **Results:** There was significantly less activity within the treatment group post-MBSR in the primary and secondary somatosensory cortices (bilateral). Furthermore, compared to the controls post-MBSR, treatment participants had significantly less activity in the caudate tail (bilateral) and in the right mid-anterior insula.

Conclusions: This not only demonstrates that MBSR can significantly affect areas of the brain involved in attentional, emotional and interference processes, but the changes in activation suggests that MBSR can improve body awareness, emotional regulation, and inhibitory control.

Patients Perspectives and Pilot Outcomes: Remote Cardiac Monitoring of At-Risk Syncope Patients (REMOSYNC Study)

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Background
Approximately 2-3% of patients with syncope suffer from arrhythmia or death (due to unknown cause) after emergency department (ED) disposition. Advances in technology now allow remote cardiac monitoring of patients that are at-risk for arrhythmias without the need for hospitalization. However, remote cardiac monitoring strategy is underutilize and not well integrated into Canada's health care system for patients discharged from the ED with syncope. Furthermore, patient experiences and opinions of external cardiac monitoring are not well documented.

Objective
The objectives of this study were to determine the occurrence of arrhythmias detected by the remote cardiac monitoring, and to assess patient-centered outcomes such as safety and comfort

Methods
We conducted a pilot phase of a double-blind, randomized controlled trial at The Ottawa Hospital, Civic and General Campuses. We enrolled adults (≥18 years of age) who presented to the ED within 24 hours of syncope, who were at-risk for arrhythmia/death (Canadian Syncope Arrhythmia Risk Score ≥ 3) and were discharged home. We excluded those that were previously enrolled, suffered prolonged LOC (>5 min), had changes in mental status, had obvious witnessed seizure, presented with significant trauma, were intoxicated (alcohol or illicit drug), or had a language barrier. Patients were monitored for a period of 15 days using the Mobile Cardiac Telemetry or a live external loop recorder, Cardiophone. A survey questionnaire was provided at the follow-up appointment to collect the patient-centered outcomes. Data was analyzed using descriptive statistics.

Results
Of the enrolled patients, 23.1% suffered arrhythmias with 15.4% requiring procedural interventions. 80% of the patients stated they felt 'very comfortable' or 'comfortable' wearing the device. 90% of the patients indicated they felt 'very safe' or 'safe' while being monitored.

Conclusions
Important arrhythmias were detected during the monitoring period. The majority of the patients had a positive experience using the external cardiac monitoring devices. Implementation of remote cardiac monitoring has the potential to increase the detection of occult arrhythmias and improve patient morbidity, mortality and quality of life while optimizing resources. With funding from the Cardiac Arrhythmia Network of Canada (CANet), we are in the process of initiating the study at multiple centers across Canada.

Diagnostic Utility of Creatine Kinase in Patients Presenting to the Emergency Department with Chest Pain

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Background

An estimated six million Emergency Department (ED) visits in the USA are associated with chest pain annually. One of the most challenging diagnoses to rule out in these patients is a Non-ST Elevation Myocardial Infarction (NSTEMI). Troponin (TNI) and creatine kinase (CK) have been traditionally measured for the workup of an NSTEMI in the ED. CK measurement is not recommended for the diagnosis of ACS according to the most recent American Heart Association/American College of Cardiology guidelines. The diagnostic utility of measuring CK in patients who present with chest pain in the ED is still not well understood.

Objective

The aim of this study is to assess the additional value of CK in the work up of an NSTEMI in patients presenting with chest pain in the ED.

Methods

This was a prospective observational cohort study conducted at the Civic and General Campuses of The Ottawa Hospital from March 2014 to March 2016. We enrolled adults (≥18 years) for whom TNI and CK were ordered for chest pain or non-chest pain symptoms concerning for NSTEMI within 24 hours. We excluded all patients with code STEMI. Medical histories, demographics, labs and medication history was collected in addition to all CK values during the ED visit from documentation available in print and electronic format. Mean values of CK were calculated and a sensitivity and specificity analysis was completed for both TNI and CK.

Results

We enrolled 1,791 patients and included 1,735 in the final analysis. 85 patients (5.3%) suffered an NSTEMI, the mean of the highest CK values was 226.16 umol/L with a standard deviation of 251.14 umol/L. Although on average the highest CK values falls within an elevated range, a large variation was observed. The specificity and sensitivity of CK was 96.4% and 31.8% respectively. The specificity and sensitivity of troponin was 70.4% and 96.4% respectively. TNI measurements were observed to provide the added benefit of ruling out NSTEMI's in addition to ruling it in over CK.

Conclusions

CK measurements for the work up of an NSTEMI were found to be of no additional value. Serial TNI is enough for the workup of NSTEMI patients in the ED. This would reduce overall health care and emergency department costs, improve resource allocation and utilization in both the healthcare system and the emergency department, and streamline the management of patients who come into the ED with chest pain.

Avoiding overtreatment: population-based trends in radical prostatectomy practice in eastern Ontario

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Background:
An estimated 15% of men in Canada are diagnosed with prostate cancer in their lifetime. Overtreatment of prostate cancer is a public health concern because it exposes men to risks that may not be necessary, considering that not all men with prostate cancer have a high risk of cancer-specific mortality. Canadian guidelines recommend against population-based screening for prostate cancer due to the risk of overdiagnosis and treatment. Historically, 50-75% of patients with low-risk cancer received radical treatment such as surgery or radiotherapy. We have previously shown that active surveillance is the most common initial treatment approach for patients with low-risk disease at The Ottawa Hospital. Contrary to low-risk cancers, studies demonstrate that men with intermediate and high-risk disease are at significant risk of metastasis and cancer-specific death, and treatment is advocated.

Objective: This population-based study from eastern Ontario examines the characteristics of patients with prostate cancer receiving surgery. We hypothesized that the proportion of patients with intermediate and high-risk cancer at surgery increased over time.

Methods:
This study queried the Prostate Cancer Community of Practices' prospectively maintained database for all patients receiving prostatectomy in eastern Ontario from 2009 to 2015. Year-by-year trends in clinical (biopsy findings, clinical stage, PSA level, National Comprehensive Cancer Network risk group (NCCN)) and pathological characteristics (Gleason score, pathologic stage) were examined. Adjusted analyses were performed to determine if the proportion of patients with clinically significant cancer (Gleason ≥7 or pT3) increased over time.

Results:
1897 patients received prostatectomy in eastern Ontario during the study period (mean 271/year). The mean pre-operative PSA was 8.5 ng/ml (SD 8.9) and age was 62.3 years (SD 6.3). The proportion of patients with NCCN intermediate or high-risk disease increased from 46.7% in 2009 to 90.2% in 2015. The proportion of men with clinically significant cancer (Gleason ≥7 or pT3) on prostatectomy increased from 59.7% in 2009 to 93.1% in 2015 indicating only 6.9% of patients had disease that may be considered at low-risk of cancer-specific mortality. Adjusted analyses indicate the proportion of patients with clinically significant cancer increased by 4%/ year during the study period independent of the location of treatment (academic vs community hospital).

Conclusions:
There has been a dramatic change in the tumor characteristics of patients receiving prostatectomy in eastern Ontario. In recent years almost all patients have clinically significant cancer. These data suggest that overtreatment of prostate cancer has decreased.

Long-Term Outcomes in Syncope Patients Who Present to the Emergency Department: A Systematic Review

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Background: Approximately 50% of patients presenting to the Emergency Department (ED) for syncope will have no cause identified. The short-term outcomes in ED syncope patients have been thoroughly studied; however long-term outcomes are not as well known to ED physicians making investigation and follow-up planning difficult.

Objective: The objectives of this study were to: 1) Conduct a systematic review on long-term outcomes (≥one year) among ED patients with syncope and; 2) Investigate incidence of all-cause mortality, device insertion (pacemaker or ICD), recurrence of syncope requiring hospitalization, and new significant arrhythmia.

Methods: We conducted a systematic review of all major databases from inception to June 2017. The following databases were accessed during the electronic component of the systematic review: Cochrane Central Register of Controlled Trials (CENTRAL) via OVID, Medline and Medline in Process (via OVID), PubMed, Embase (via OVID), and the Cumulative Index to Nursing and Allied Health Literature (CINAHL). Articles were first screened by title, then abstract, and finally full text. We included articles of adult patients (>16 years old) presenting to the ED with syncope with follow-up of at least one year. We excluded articles that included pre-syncope and syncope mimics (seizures, stroke etc), <16 years old, patients that did not first present to the ED and follow-up less than one-year.

Results: 2,094 articles were screened and 16 were included in the meta-analysis. Pooled analysis showed: 6% deaths at 1-year; 14% syncope recurrence requiring hospitalization; and two studies reporting new arrhythmia (0.8 – 11.5%).

Conclusions: An important proportion of ED patients with syncope suffer outcomes at 1-year. There is a 6% mortality rate and 14% of patients will need to be hospitalized for further investigation. There was a large degree of heterogeneity between the studies; in particular many important outcomes were not investigated. Further prospective research is needed with more thorough methodology to investigate long-term morbidity and mortality among ED syncope patients. At this time there is no validated long-term syncope risk score available for physicians to appropriately follow-up and investigate syncope patients to aid in prevention of long-term morbidity and mortality.

The Audit & Feedback Meta-Laboratory: Improving healthcare outcomes by optimizing performance with Audit & Feedback

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Background

Audit and Feedback (AF) is a summary of clinical performance provided to a healthcare professional to change their behaviour. A 2012 Cochrane review of 140 trials of AF showed a 4% absolute improvement of the behavior in question with an interquartile range of 1-16%. Knowing that AF is effective, future studies need to evaluate comparative effectiveness of different methods of delivering AF, rather than continuing to compare intervention vs. control. This however, will require large sample sizes that are unlikely to be realized in one off research projects. Research teams integrated into healthcare systems already undertaking programs of research can address the need for large sample sizes while conducting research directly relevant to healthcare system priorities. These so-called implementation laboratories provide an opportunity for implementation researchers to work with health system partners to embed sequential randomized trials testing different ways of delivering AF in head-to-head comparisons at scale. To ensure efficiency of these implementation laboratories, we propose developing a meta-laboratory; a cross-laboratory steering group that allows for shared learning across studies and implementation laboratories and provides opportunities for planned replication of studies.

Objective

To develop an international meta-laboratory that coordinates individual implementation laboratories aiming to improve global healthcare outcomes by optimizing clinical performance with AF.

Methods

The meta-laboratory will work with individual implementation laboratories to provide input on their AF interventions and implementation efforts. It will collect data from each implementation laboratory with the intention of sharing results to inform the development of other AF interventions and plan replication studies. This will be facilitated with the creation of the AF meta-laboratory website where evidence, information, and expertise can be accessed and shared, as well as hosting annual international conferences to discuss the state of the science.

Results

The meta-laboratory steering group has been established including colleagues from the United States, United Kingdom, Netherlands, and Canada and the meta-laboratory website has been developed. Large scale implementation laboratories from the United Kingdom and Canada are currently conducting trials of AF and are reporting to the meta-laboratory to ensure that data is shared as appropriate.

Conclusions

The AF meta-laboratory will encourage the optimization of AF as a behavior change intervention and thus will contribute to global improvement of healthcare outcomes and delivery.

Results from a pilot study of smartphone-assisted problem-solving therapy for men who at-risk for self-harm.

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Background: Patients who present to Emergency Departments (ED) after an intentional self-harm receive variable levels care in Ontario. Many are not assessed by a psychiatrist and psychological services available in the community are typically not covered by OHIP. These patients are more likely to die by suicide after an index episode of self-harm and this risk is elevated in men, who represent two-thirds of all completed suicides (2). One way of addressing this is to offer psychotherapy facilitated by the use of a smartphone application, which has the potential to extend the reach of traditional face-to-face therapy with this high-risk group by providing patients with immediate and time-delayed communication with their therapist.

Objective: To assess the acceptability and feasibility of smartphone-assisted problem-solving therapy in men who intentionally self-harm.

Method: We are piloting the use of ACHESS, a smartphone application that was initially developed for individuals with substance misuse, which has been adapted for use in men who present to the ED at The Ottawa Hospital with intentional self-harm. Participants receive six sessions of problem-solving therapy over a period of five weeks supplemented by the use of the ACHESS mobile application.

Results: Participants report a high degree of interaction with the smartphone application as part of the problem-solving therapy treatment program. We will describe the role of smartphone technology in the treatment of self-harm.

Conclusion: Preliminary results show that smartphone technology has the potential to extend the reach of face-to-face therapy in the treatment of self-harm.

Trigeminal Neuralgia in Systemic Sclerosis

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Background and Objective:
Trigeminal neuralgia (TN) is characterized by pain and spasms affecting one or more divisions of the fifth cranial nerve. Of note, TN is one of the peripheral nervous system manifestations of systemic sclerosis (SSc), reported to be present in approximately 4% of patients. Proposed pathophysiologic mechanisms in this context have included nerve entrapment and compression from mandibular bone resorption, a phenomenon seen in SSc due to pressure ischemia from overlying tight sclerotic skin compromising blood supply to the bone. Previous studies of TN identified an association with overlap syndromes notably in patients with inflammatory myositis (IM), arthritis and interstitial lung disease (ILD). It has also been suggested that facial numbness can precede or follow other manifestations of SSc. However, since there is a paucity of evidence concerning TN in SSc, we undertook a nested case-control study to identify associations between SSc and TN in a multi-centered SSc cohort.

Methods:
Data were retrieved from the Canadian Scleroderma Research Group (CSRG) registry, an open cohort of 1652 SSc subjects enrolled since 2004. Subjects with a physician-reported diagnosis of TN were identified at the baseline study visit (prevalent cases) and during follow-up (incident cases). Four SSc subjects without TN and matched to each case on study visit were identified as controls for either prevalent or incident cases. Sociodemographic, clinical and serological characteristics of cases and controls were compared. P values < 0.05 were considered statistically significant.

Results:
43 (43/1652; 2.6%) prevalent and 36 incident (36/6193 total person-years follow-up; incidence rate 5.8 per 1000 person-years) TN cases were identified and matched to 144 and 172 controls, respectively. There were no significant differences in mean age, gender distribution and mean disease duration

Severe Traumatic Brain Injury Management and Outcome at The Ottawa Hospital: A 2-Year Cohort

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Background Severe traumatic brain injury (sTBI) is associated with significant morbidity and mortality. Aggressive management of the initial injury, as well as prevention of secondary injuries and complications, are known to improve patient survival. Despite little level 1 evidence to guide the management of these patients, the Brain Trauma Foundation has produced management guidelines on the best available evidence. As part of an internal practice audit, we present a complete 2-year cohort of patients with sTBI managed at TOH.

Objective This cohort provides an overview of the epidemiology, clinical presentation, management considerations and clinical outcomes of patients with sTBI at a single academic tertiary care hospital.

Methods This is a retrospective cohort of consecutive patients with sTBI admitted to TOH between 01 January 2014 and 31 December 2015. Patients were identified using the TOH Trauma Registry and the Ottawa Hospital Data Warehouse (OHDW). We screened patients from the Trauma Registry with an AIS >2, admission, length of stay >48 hours or death within 48 hours, as well as all patients from the OHDW with the diagnostic code for head injury who had an ICU admission, or death within 48 hours. From these samples we included patients that: (a) were ≥ 16 years of age, (b) had sustained blunt head injury, and (c) had a post-resuscitation Glasgow Coma Score (GCS) <9, or deteriorated to GCS <9 within 24h of presentation. For eligible patients, we collected and merged data from the TOH trauma database, the OHDW, and from primary chart review. Data points of interest included baseline characteristics, clinical presentation, hospitalization characteristics and interventions, and clinical outcomes.

Results We identified 795 patient records and classified 188 patients as meeting criteria for inclusion. Median (Q1-Q3) age was 62 years (32 – 79) and 179 (95%) required ICU admission with a median ICU length of stay 7 days (3 – 13). Median hospital length of stay was 15 days (5 - 29). 35 (19%) patients required immediate operative intervention at the time of admission and 7 (4%) underwent craniectomy. Additionally, 34 (18%) required a tracheostomy, 28 (15%) required a gastrostomy tube and in-hospital all-cause mortality was 40%.

Conclusion This comprehensive cohort identified using health administrative data, provides an overview of patients with sTBI managed at TOH during a two-year period. Future work will include a multi-disciplinary review of the treatment these patients received to identify opportunities to optimize quality of care.

The Role of Methotrexate in Polymyalgia Rheumatica

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Background:
The mainstay treatment for polymyalgia rheumatica (PMR), an inflammatory disorder, is glucocorticoids. However, discontinuation of therapy can take several years. Long term glucocorticoid use is associated with side effects including: neuropsychiatric and metabolic disturbances, hypertension and osteoporosis. Methotrexate is an inhibitor of tetrahydrofolate dehydrogenase. In high doses, it is used to treat malignant conditions, while in low doses it is used as a disease-modifying anti-rheumatic drug. Despite conflicting research regarding the role of methotrexate in PMR, the European League Against Rheumatism and the American College of Rheumatology suggest the early introduction of methotrexate in PMR alongside glucocorticoids.

Objective:
The objective of this review is to elucidate the clinical benefits and harms of methotrexate for patients with PMR.

Methods:
We conducted a Cochrane review to address this question specifically searching for randomized control trials (RCT) within the databases CENTRAL, MEDLINE and EMBASE. Four RCTs were included totaling 194 participants.

Results:
The mean cumulative dose of glucocorticoids for the methotrexate group was 2.57g ± 1.61 versus 3.25g ± 1.40 in the placebo group ($p < 0.05$), representing a relative effect of -0.91 (95% CI -1.25, -0.57). The proportion of patients that experienced at least one relapse was 43.3% in the methotrexate group versus 63.6% in the placebo group ($p < 0.05$), representing a relative effect of 0.65 (95% CI 0.47, 0.88).

Conclusion:
Although the quality of the evidence was mostly low, the results suggest that methotrexate use may be warranted in individuals with PMR as demonstrated by the major and minor outcomes investigated.

Audit and feedback to address prescribing of high-risk medications in long-term care: theory-based process evaluation alongside a pragmatic, factorial, cluster-randomized trial

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Background: Audit and feedback involves measuring a healthcare professional's practice and comparing it to professional standards: the results are fed back to the individual to encourage change. Providing feedback to long-term care physicians may reduce prescribing of high-risk medications (including antipsychotics and benzodiazepines). However, how audit and feedback is designed and presented impacts its effectiveness. Partnering with Health Quality Ontario, we are conducting a pragmatic, 2×2 factorial, cluster-randomized trial to evaluate: A) positive vs. negative framing of performance (number of patients not prescribed vs. prescribed medications); and B) comparing performance to the top quartile vs. the provincial median.

Objective: We investigated mechanisms of action, and the contextual factors shaping how the intervention works in practice.

Methods: In this theory-based, mixed-methods process evaluation, all physicians who downloaded their feedback were invited to complete a structured online questionnaire assessing intention, self-efficacy, outcome expectations, descriptive norms, and goal prioritization. We compared post-intervention scores using independent samples t-tests. We conducted semi-structured interviews to explore underlying views relating to these constructs. Themes were mapped to the Consolidated Framework for Implementation Research (CFIR).

Results: In total, 89 physicians downloaded their feedback; 33 (37%) completed the questionnaire. Significant differences in descriptive norms were observed between groups receiving different comparators: those receiving the median were more likely than those receiving the top quartile to agree that their colleagues are appropriately adjusting their antipsychotic ($t(31)=3.248$, $p=0.003$) and benzodiazepine ($t(22.653)=2.749$, $p=0.012$) prescribing. Five interviews were conducted. Key themes reflected the CFIR domains "Characteristics of Individuals" and "Implementation Process". Appropriate antipsychotic prescribing was a pre-existing priority (not initiated by the feedback, but by Ministry and media attention). The feedback enhanced self-efficacy when individual prescribing rates were close to the comparator. Participants who received the median comparator and those who received the top quartile (a higher target) aimed to achieve similar prescribing rates to the comparator. However, problems with identification with the comparator, which was not case-mix adjusted, were apparent.

Conclusions: Comparing performance to the top quartile may enhance audit and feedback effectiveness. However, this may not be mediated by descriptive norms, which were more likely to be enhanced by the median comparator (which represents the physician population as opposed to the 'top performers'). Limited influence on other constructs and prioritization driven by other sources may undermine effectiveness.

A pilot trial design - HAVARTI: Step towards a cure for HIV infection?

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HIV remains a threat to human health despite effective treatment. While HIV viremia can now be effectively controlled with sustained anti-retroviral therapy (ART), there is no cure and few examples of remission of viremia without continuous ART. Rebound HIV viremia with ART interruption is felt to be due to reactivation of latent infection and lack of effective acquired immunity during ART or with experimental adjuvant treatments. Last year, an unexpected finding of sustained remission of plasma viremia level in a rhesus macaque nef-deficient SIVmac239 infection model was reported (Byrareddy et al., Science 2016). In that study, SIV-infected ART-treated animals received a series of monoclonal antibody (mAb) infusions targeting mucosal homing receptor α4β7 integrin. ART and then the mAb were discontinued. Infected animals had initial low level viral rebound (6/8) and all 8 went on to reach sustained, immunologically mediated suppression of plasma viremia without ongoing ART. Vedolizumab (humanized anti-α4β7 integrin mAb) is licensed in humans for treatment of inflammatory bowel disease, where it has been shown to be safe. We propose a dose-finding pilot trial in 12 healthy adults with ART-treated chronic HIV infection to assess vedolizumab safety and sustained anti-HIV effect after analytical treatment interruption (ATI). The proposed trial is of a short duration and simple design to assess short-term safety and tolerability of vedolizumab according to dose, to assess whether plasma viremia rebound and remission occur after ATI, and to evaluate acquired immunity and residual compartments of latency of HIV infection. Participants will be serially allocated to receive seven infusions of vedolizumab at 300mg, 150mg, or 75mg per infusion. ATI will occur after the third infusion to allow for assessment of the anti-HIV effects of anti-α4β7 integrin mAb treatment. The planned duration of study follow-up is 12 months. This trial design accommodates the collection and cryopreservation of biological specimens (sera, plasma, peripheral blood lymphocytes, rectal biopsy samples, CSF, and stool) that will permit ongoing mechanistic, immunological and virological studies to be done if virological remission of chronic HIV infection is achieved in humans as in an animal model. Regulatory and administrative permissions have been granted, and drug supply secured for trial execution to begin September 2017.

Risk factors for urologic injury in women undergoing hysterectomy for benign indication

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Background:
Urinary tract injuries are known complications during abdominopelvic procedures. Among these procedures, hysterectomies account for a majority of iatrogenic urologic injuries, likely due to the close proximity of the bladder and ureters to the female reproductive organs.

Objective:
To determine the risk factors associated with iatrogenic urologic injury in women undergoing hysterectomy for benign indication.

Methods:
A retrospective cohort study on women undergoing hysterectomy for benign indication from 2011-2015 was conducted using the National Surgical Quality Improvement Program database. Women without urologic injury were compared to women with injury to identify risk factors. Multivariate logistic regression models were constructed to control for patient demographic, clinical, and surgical factors.

Results:
125,571 women underwent hysterectomy for benign indication, and the overall urologic injury rate was low (0.63%). Patient demographic factors associated with urologic injury included age less than 40, lower BMI, and Asian or Indigenous ethnicity ($p<0.05$). Associated surgical factors included abdominal versus laparoscopic or vaginal approach and total versus subtotal hysterectomy ($p<0.05$).
Following adjustments for potential confounders, patients who underwent total hysterectomy had increased odds of urologic injury compared to subtotal (adjusted odds ratio [AOR] 1.58, 95% confidence interval [CI] 1.20-2.08). Patients with Class III obesity had decreased odds of injury compared with patients who were of a normal weight (AOR 0.63, 95%CI 0.45-0.87), overweight (AOR 0.61, 95%CI 0.45-0.85), and Class I obesity (AOR 0.67, 95%CI 0.49-0.93).
Interaction between variables was observed between surgical approach and indication for hysterectomy. Laparoscopic compared to abdominal approach to hysterectomy was associated with decreased odds of urologic injury for women with endometriosis (AOR 0.31, 95%CI 0.17-0.56), pelvic pain (AOR 0.19, 95%CI 0.08-0.48), menstrual disorders (AOR 0.14, 95%CI 0.04-0.44), and fibroids (AOR 0.49, 95%CI 0.33-0.71). Laparoscopic compared to vaginal approach was associated with decreased odds of urologic injury for women with endometriosis (AOR 0.38, 95%CI 0.17-0.86), menstrual disorders (AOR 0.03, 95%CI 0.003-0.34), and pain (AOR 0.13, 95%CI 0.02-0.72).
Iatrogenic urologic injury was associated with increased measures of healthcare utilization including OR time ($p<0.0001$), length of hospital stay ($p<0.0001$), and return to OR ($p=0.04$).

Conclusion:
While the risk of urologic injury during hysterectomy for benign indication is low, the risk is modified by certain patient factors - such as BMI and type of gynecologic pelvic disease - and certain surgical factors - such as total or subtotal hysterectomy and surgical approach.

Post-treatment liver stiffness measurements predict the development of liver-related complications in patients with HCV cirrhosis who achieve SVR post-DAA therapy.

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Background: Chronic hepatitis C virus (HCV) infection may lead to cirrhosis (1) and liver-related complications (LRC) such as hepatocellular carcinoma (HCC), ascites, hepatic encephalopathy (HE) and esophageal varices (2). Transient elastography (TE) is a non-invasive measurement of liver fibrosis in HCV (3, 4, 5), and may predict LRC (6). HCV therapy with sustained virologic response (SVR) appears to decrease liver stiffness (LS) (7) however, whether this is also associated with fewer LRC is unclear.

Objective: To evaluate whether a reduction in LS post-HCV treatment with SVR is associated with a lower incidence of LRC in cirrhotic patients within 24 months of therapy.

Methods: We included all cirrhotic patients (LS >12.5 kPa) treated with direct acting antivirals (DAAs) between May 1, 2013 and June 1, 2016 with SVR and pre- and post-treatment TE. We excluded patients with new/worsening LRC before post-treatment TE. Those with baseline LRC were included, and evaluated for worsening LRC, as defined by progression of post-treatment grading of the LRC compared to baseline. The absence of new/worsening LRC was recorded as 'non-event'. ROC curves and Kaplan-Meir analysis were used. Person-time was calculated from the post-treatment TE date to the last clinic visit up to 24 months post-treatment.

Results: Of 57 patients, we excluded 4 patients with new LRC prior to post-treatment TE. 40/53 (75.5%) patients had reduction in LS, with a mean decrease of 10.7 kPa (SD 10.4). There were no differences in baseline characteristics of patients with/without decreased LS. The incidence rate of new/worsening events for patients with increased LS was 0.47/100 person-weeks, vs. 0.19/100 person-weeks for patients with decreased LS (RR 2.5, p=0.40, 95% CI: 0.26-24.0). All events occurred in individuals with LS >20.75 kPa; no events occurred in individuals with LS score <20.75 kPa (4/20 vs. 0/33, p=0.02). This LS cutoff had the best AUC (0.786) with a sensitivity of 100% and specificity of 67%. Post-treatment, 20/53 (37.7%) patients still had a LS above 20.75 kPa.

Conclusions: In our cohort of patients with early cirrhosis (Child-Pugh class A), successful antiviral therapy led to a reduction in LS in most patients. Prior studies have identified a LS cutoff of 20 kPa as associated with clinically significant portal hypertension (8), and this was confirmed in our post-treatment cohort. Many (37.7%) patients remained above this cut-off and require LRC monitoring post-SVR. The predictive value of long-term, serial LS measurements requires evaluation.

Clinical and radiologic predictors of response to anti-TNF alpha therapy in patients with perianal Crohn's disease.

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Background

Perianal fistulas are a frequent manifestation of Crohn's Disease (CD) and often result in substantial morbidity. Pelvic magnetic resonance imaging (MRI) is commonly performed prior to initiating therapy to evaluate fistula anatomy, and the presence of complications. These factors are critical for determining if therapy can be safely initiated. Although anti Tumor Necrosis Factor alpha (anti-TNF) therapy has emerged as the most effective treatment for perianal CD (PCD), a substantial proportion of patients will not achieve clinical remission.

Objective

The aim of this study is to determine clinical and radiologic factors associated with clinical remission in patients with PCD.

Methods

A retrospective, observational study was performed between 2005-2016. Patients with PCD who underwent a pelvic MRI were identified by a search of our institutional electronic picture archiving and communication system (WEB PACS). Study inclusion criteria included patients over the age of 18 with PCD who underwent a pelvic MRI within 12 months of starting anti-TNF therapy. Clinical remission, defined as a lack of fistula drainage without clinical evidence of abscess, was assessed at 3 months after initiating therapy. A single, experienced radiologist using a standardized template reinterpreted each MRI study. Clinical and radiologic factors were selected a priori and were compared among patients with and without clinical remission. Chi square and Fisher exact tests were used to compare variables where appropriate.

Results

Seventy-seven patients met ourinclusion criteria. Twenty-five (32.5%) patients achieved clinical remission at 3 months and 52 (67.5%) did not. Age, gender, and smoking status, and were similar between both groups of patients. Age of diagnosis, Montreal Classification of disease characteristics, and duration of disease were also similar between both groups. Patients who did not achieve remission required more examinations under anesthesia (OR 2.4; p=0.076), and receive a higher number of setons (p=0.063) prior to initiation of anti-TNF therapy. Patients who did not achieve remission were more likely to have multiple primary fistula tracts (OR 4.8; p=0.035), multiple liquid containing tracts (OR 2.57; p=0.06), and a greater number of primary enhancing tracts (p=0.047) seen on MRI.

Conclusion

Multiple radiologic features are associated with a lack of clinical remission in patients with PCD and may help in patient counselling, and to determine which patients should be treated aggressively.

The efficacy of postoperative iron therapy in improving clinical and patient-centred outcomes following elective surgery: a systematic review and meta-analysis

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Background: Postoperative anemia is a common occurrence in surgical patients and leads to an increased risk for allogeneic blood transfusions. The efficacy of iron therapy in treating postoperative anemia has not been firmly established.

Objective: The purpose of this systematic review was to evaluate the efficacy of postoperative oral and intravenous iron therapy in improving clinical and patient-centred outcomes following elective surgery.

Methods: The databases Medline, EMBASE, CENTRAL, the Transfusion Evidence Library, and ClinicalTrials.gov were searched. Eligible studies were RCTs or prospective cohorts having a control group, where postoperative oral or intravenous iron was administered to elective surgery patients. Primary outcomes were hemoglobin levels and patient-centred outcomes of quality of life and functioning. Secondary outcomes were the safety of postoperative iron and blood transfusion requirement. Meta-analysis using a random effects model was performed.

Results: Seventeen relevant studies were identified, of which 7 investigated IV iron, 7 investigated oral iron, and 3 compared IV to oral iron. Postoperative oral and IV iron therapy were ineffective in improving quality of life and functioning (GRADE: moderate-low quality). Compared to control, IV iron increased mean hemoglobin levels by 3.40 g/L (95% CI: 1.18, 5.62) (GRADE: moderate quality), however this increase is likely not clinically meaningful. Overall, oral iron was ineffective in increasing hemoglobin concentrations compared to control (MD = 0.77, 95% CI: -1.48, 3.01) (GRADE: moderate quality). Postoperative iron therapy did not significantly reduce the risk of blood transfusion (RR = 0.75; 95% CI: 0.53, 1.07) (GRADE: low quality). IV iron was not associated with a significant increased risk of adverse events (RR = 4.50, 95% CI: 0.64, 31.56). There was insufficient information to determine the risk of adverse events for postoperative oral iron.

Conclusion: This systematic review found no evidence to support the routine use of postoperative iron therapy in all elective surgery patient populations; however, results are based largely on studies with non-iron deficient patients pre-operatively. Further research on the role of postoperative IV iron is warranted for certain high risk groups, including patients with iron deficiency or anemia prior to surgery.

Long-term health outcomes and health system care costs associated with surgical site infections: a population based, retrospective matched cohort study

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Background
Surgical site infection (SSI) are associated with mortality, increased utilization and cost of healthcare. The study **objectives** were to: 1) examine the association between SSIs and health outcomes, including all-cause mortality and hospital readmissions; and 2) estimate the attributable short-term and long-term costs of SSIs from the perspective of the health care system.

Methods
We conducted a population-based, retrospective matched cohort study of all adult patients at The Ottawa Hospital who underwent surgery and were monitored using the National Surgical Quality Improvement Program (NSQIP) between 2010 and 2016. We used a logistic regression technique to estimate propensity score, using all available patient-related and operation specific factors. Then, those with SSIs (n=794) were matched 1:3 to patients without SSIs by surgery specialty, wound class, and a propensity score. The index date was defined as a SSI diagnosis date for both exposed and unexposed groups. The primary outcomes were measures of long-term health outcomes, including all-cause mortality and hospital readmission. The secondary outcomes were the average total healthcare costs. All outcomes were estimated at 30 days, 90 days and 1 year following index date. We used Cox-proportional hazard models to examine the association between the SSIs and health outcomes.

Results
The final cohort included 794 patients with postoperative SSIs and 2,382 matched controls. Patients with and without SSIs were highly similar with respect to baseline characteristics. Our preliminary analyses reveal that postoperative SSIs were associated with a significant increase in mortality at 30, 90 days and 1 year (HR=2.42, 95% CI 1.34-4.38, HR=2.41, 95% CI 1.59-3.70, and HR=2.01, 95% CI 1.53-2.62, respectively), as well as with increase in hospital readmission at 30, 90 days and 1 year after discharge (HR=2.83, 95% CI 2.20-3.44, HR=2.23, 95% CI 1.85-2.68, and HR=1.96, 95% CI 1.71-2.25, respectively). Preliminary estimates of mean costs associated to SSIs at 30 days following index date amounted to $11,564 (95%CI 10,593-12,536), at 90 days were $17,802 (95%CI 16,113-27,696), and at 1 year amounted to $26,939 (95%CI 22,181-27,696).

Conclusions
SSIs contribute markedly to adverse health outcomes and healthcare costs. The results of this study can be used to determine the benefits to patients and the size of savings if effective preventive programs are implemented and maintained. Further analyses will be performed to adjust for remaining confounding factors, such as health care utilization in a previous year and body mass index.

Development of a Human Accompanying Wheelchair using Ultrasonic Tethering

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In recent years, a study has shown that 20% of all powered wheelchair related accidents are caused by distracted driving. These accidents include bumping into objects and/or people that can injure a person, damage the wheelchair, or tip and fall. In many social situations, people who use a powered wheelchair must divide their attention between navigating the chair and conversing with people. These conversations with the person accompanying could lead to increased mental stress while navigating and distraction from maneuvering the chair. This project aims to eliminate the need to manually control a powered wheelchair to aid its users in conversing with an accompanying person. A system is being developed that will assist in side-by-side following and wirelessly tether the wheelchair to a person. The device is designed to identify a person on the side of a wheelchair and detect motion. Transducers, such as, Infra-Red (IR) and cameras are highly dependent on environmental light conditions. Ultrasonic sensors that can detect an object are inexpensive and independent of environmental conditions. Person identification is achieved by using ultrasonic PING sensors that detect a signal from a transmitter module on the person. Motion is detected by triangulation using ultrasonic transducers. Both these methods of identifying and detecting an accompanying person's motion are at different frequencies, 20KHz and 40KHz respectively. Simulation of this system in Matlab & Simulink achieved the goal of maintaining a conversational distance of 80 centimeters between the wheelchair and person. A drive control algorithm that achieves tight turns (i.e., hard right and hard left) and follows the person on a curved path is required to make the system robust.

Feasibility of pre-operative patient blood management in liver resections patients at The Ottawa Hospital

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Introduction:
Patient blood management (PBM) is a multidisciplinary, evidence-directed and goal-oriented approach to minimizing patient exposure to blood transfusion and improving surgical outcomes [1]. In patients undergoing liver resections, pre-operative anemia has been associated with an increase in risk of blood transfusion, increased length of hospitalization, increased risk of major morbidity, and inferior overall survival [2-4]. Additionally, pre-operative anemia and perioperative blood transfusion may be associated with poorer oncologic outcomes in patients undergoing resection for primary or metastatic malignancies [4-8].

Objective:
The purpose of this retrospective chart review was to determine the feasibility of offering pre-operative blood management to patients undergoing liver resections for oncologic indications at The Ottawa Hospital (TOH).

Methods:
Retrospective review was conducted on electronic medical records of TOH patients who underwent liver resections for oncologic indications between 2010 and 2017

Results:
372 patients underwent liver resections for oncologic indications between January 2010 and May 2017 at TOH. The median pre-operative time period between obtaining the surgical consent and the date of the surgery was 24 days (range 3-127 days). Approximately one-third (32.6%) of the patients were anemic at time of surgery. At the time of consent for the surgery, 36.8% of patients had a recent (within 3 weeks) complete blood count (CBC), and 3.0% had a recent test of blood ferritin or vitamin B12. Only 2 patients had a CBC, blood ferritin test and vitamin B12 test at the time of consent (0.5%).

Conclusions:
Pre-operative anemia (32.6%) and red cell transfusion (22.3%) occurred at a relatively high rate in oncologic liver resections patients at TOH between 2010 and 2017. Most patients did not have a recent CBC available to the hepatobiliary surgeon at the time of consent, and very few patients had laboratory testing for serum ferritin or vitamin B12. Lack of timely anemia screening prevents the application of PBM interventions to improve pre-operative hemoglobin levels and eventual surgical outcomes. Pre-operative PBM therapies can be implemented based on the median time period patients wait for liver resection after consenting to surgery [9-11]. Collaboration between the TOH blood conservation program and the hepatobiliary surgery service could ensure adequate anemia screening at the time of consultation.

Risk factors for venous thromboembolism in women undergoing hysterectomy

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Background:
Venous thromboembolism (VTE) is a severe complication of surgical procedures that can be prevented with the use of prophylactic measures. As surgical approaches advance and hospital stays shorten, it is essential to update current guidelines on VTE prophylaxis for hysterectomies for benign conditions to reduce morbidity and mortality associated with VTE.

Objective:
The objective of this study was to determine the prevalence of venous thromboembolism (VTE) and to identify risk factors associated with VTE in women undergoing hysterectomy for benign indication.

Methods:
A retrospective cohort study of women undergoing hysterectomy for benign indication from 2011-2015 was conducted using the American College of Surgeons National Surgical Quality Improvement Program (NSQIP) Participant Use Data Files. Women diagnosed with a venous thromboembolic event (pulmonary embolism and deep vein thrombosis) within thirty days post-operatively were compared to women who did not have VTE. Multivariate logistic regression models were constructed to control for patient clinical factors (age, BMI, diabetes mellitus, smoking, cardiovascular disease, bleeding disorder, ASA classification) and perioperative factors (surgical approach, blood transfusion and operative time).

Results:
A total of 125,729 women underwent hysterectomy for benign indication, and the overall VTE incidence was low (0.33%). Patient characteristics associated with increased risk of VTE were elevated BMI, African American race, and ASA classification. Associated operative factors included abdominal route of surgery, intra- or post-transfusion, and prolonged operative time ($p<0.05$ for above comparisons).
Following adjustment for potential confounding variables, factors associated with increased odds of VTE were African American ethnicity (aOR1.27, 95% CI 0.98-1.64), elevated BMI (aOR1.62, 95% CI 1.10-2.38), severe systemic disease (aOR 3.00, 95% CI 1.05-1.76).
Abdominal hysterectomy was associated with greater odds of VTE compared with laparoscopic (aOR 2.10, 95% CI 1.67-2.62) or vaginal approaches (aOR 2.75, 95% CI 1.84-4.11).
Elevated odds of VTE were also observed with prolonged OR time (>150 vs <90 minutes, aOR 1.85, 95% CI 1.37-2.50), intraoperative transfusion (aOR 2.28, 95% CI 1.44-3.60), and postoperative transfusion (aOR 2.70, 95% CI 1.66-4.40).

Conclusion:
The risk of VTE is low in women undergoing hysterectomies for benign indications. Identified patient risk factors include BMI, race, and ASA classification, while operative risk factors include route of surgery, occurrence of blood transfusion, and operative time. These findings have important implications in the assessment of VTE risk and potential prevention for patients undergoing hysterectomy for benign conditions.

Cost analysis of Omega-3 supplementation in critically ill patients with sepsis

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Introduction: Nutritional supplement of omega-3 fatty acids have been proposed to improve clinical outcomes in critically ill patients. A recent systematic review showed that omega-3 supplementation of patients with sepsis was associated with a significantly lower ICU and hospital length of stay. However, the financial impact of omega-3 supplementation is unexplored. Objective: To evaluate the impact on ICU & hospital costs by conducting a cost analysis on omega-3 supplementation. Methods: We extracted individual length of stay in ICU and length of stay in hospital from the data reported by 12 prospective, randomized control trials involving 925 patients included in the systematic review from Lu et al. The Cochrane Collaboration tool was used to assess the risk of bias in these studies. The average daily ICU and hospital costs per patient were obtained from a cost study by Kahn et al. We estimated the ICU and hospital costs by multiplying the mean length of stay by the average daily cost per patient in ICU or Hospital. Adjustments for inflation were made according to the USD annual consumer price index. We calculated the difference between the direct variable cost of patients with omega-3 supplementation and patients without omega-3 supplementation. 95% confidence intervals were estimated using bootstrap re-sampling procedures with 1000 iterations.

Results: A total of 12 RCT involving 925 patients were included in this cost analysis. Septic patients with omega-3 supplementation had significantly lower ICU length of stay [mean difference (MD) -3.79 days [95% CI: -5.49, -2.09; $P < 0.0001$, $I2= 82\%$]. Septic patients supplemented with omega-3 had a lower average weighted cost in ICU and hospital at $15,274 and $17,088 respectively, compared to the average weighted cost in ICU and hospital of $18,172 and $19,778 in the control patients. Sensitivity analyses were conducted to investigate the impact of different study methods on the LOS. The results were still consistent with the overall findings. Conclusion: Septic patients who received omega-3 supplements had significantly shorter LOS in the ICU and hospital which results in lower direct variable costs than those who did not receive omega-3 supplements. The 12 RCT used in this analysis had a high risk of bias according to the Cochrane Collaboration tool. Large-scaled, high-quality, multi-centered RCTs on the effectiveness of this intervention is recommended to improve the quality of the existing evidence.

Usage of patient-centred outcomes in the management of anemia: a systematic review

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Background: Patient-centered outcomes (PCOs) evaluate the impact of disease on patient wellbeing. Patient-centred tools are critical for managing chronic disease. Anemia is a diagnosis that affects all ages, sexes, and ethnicities, and is a frequent consequence of acute and chronic conditions. Treatments for anemia including iron supplementation, erythropoiesis-stimulating agents (ESAs), and transfusion are associated with improvements in hematological parameters and survival, but the impact of anemia and its management on quality of life remains poorly understood.

Objective: To critically assess published studies that have measured PCOs to investigate the effects of anemia management on quality of life and patient wellbeing. To evaluate the usage of PCO tools and the quality of PCO reporting in studies of anemia management.

Methods: We searched PUBMED, EMBASE, PsychInfo, and CINAHL for relevant studies published until January 2017. Eligibility criteria included full-text observational studies, case series (N >10), and randomized-controlled trials (RCTs) published in English. We included studies with anemic patients undergoing any intervention for treatment of anemia that reported hemoglobin levels and PCOs before and after anemia intervention. Risk of bias was assessed in interventional and observational cohort studies. Study methodological and patient characteristics, PCOs, and additional outcomes was qualitatively synthesized. Subgroup analysis was performed by disease category and by type of intervention for anemia. The efficacy of PCO tools was assessed, and this analysis was performed separately for studies where the anemia intervention improved hemoglobin levels versus studies where hemoglobin levels did not increase following treatment. Finally, the prevalence of individual PCO tool use was reported, both overall and for each disease category.

Results: Of the 2230 articles found during database searching, 406 were read in full text and 93 met all inclusion criteria. Preliminary analysis found that 47.3% (44/93) of articles pertained to anemia in oncology populations and of all the PCO tools used, the FACT-Anemia questionnaire was used 22.9% of the time. Furthermore, 78.5% of the studies utilized ESAs as an intervention for anemia. Preliminary descriptive analysis of the reporting quality of PCOs demonstrates that while 58.1% of the studies included were RCTs, 27.8% of these studies utilized QOL as a primary endpoint. Final descriptive analysis and disease-specific subgroup analysis results are forthcoming.

Conclusions: These findings suggest that the majority of articles address PCOs in anemic oncology patients and that most anemia studies do not utilize PCOs as a primary endpoint. Final conclusions regarding PCOs in anemia await forthcoming analysis.

Do Young Women Regret Their Hysterectomy? A Survey of Women 35 years of Age and Under

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Background: Hysterectomy is one of the most commonly performed gynecological procedure. It is often an effective way of controlling patient symptoms. Traditional teaching dictates that young women will regret this irreversible procedure, but there have been no studies investigating the incidence of regret.

Objective: To determine if young women regret a hysterectomy to manage a benign gynecologic condition.

Methods: A cross-sectional study was performed at a tertiary level academic centre.
Patients aged 35 or under with hysterectomy performed by a single surgeon for benign indications between January 1, 2008 and December 31, 2015 completed a validated decision regret survey and patient health questionnaire.

Results: Of patients who met inclusion criteria, 24/27 contacted patients agreed to participate and completed the study (response rate 89%). No differences in group mean parity, BMI, or indication for surgery existed between contacted and non-contacted patients, except a younger age in the latter group (mean difference 2.8 years).
Median age at time of hysterectomy was 32 years (range 27-35). Median time from hysterectomy to participation was 4 years (range 1-8.5). The most common indications for hysterectomy were endometriosis (50% of cases), fibroids (20.8% of cases), and pelvic pain (15.4% of cases).
Of the 20/24 participants who disagreed or strongly disagreed to the statement "I regret the choice that was made," 8 were nulliparous and 9 still had medical management options available. 4/24 participants neither agreed nor disagreed to this statement. 87.5% of participants agreed or strongly agreed that "I would go for the same choice if I had to do it over again." Most participants (71%) felt the decision for hysterectomy was shared between the physician and participant; no participants noted that the decision was made mostly by the physician or by the physician only.

Conclusion: Gynecologists are concerned about future patient regret when considering a hysterectomy. This pilot study is the first to investigate the incidence of regret after hysterectomy. Our results suggest that patients who are young, nulliparous, or haven't failed all forms of medical management, do not regret their decision for hysterectomy.

Validating the use of a protein gap to identify gammaglobulinopathies

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Background:
Although the protein gap is thought to be predictive of hypergammaglobulinemia or hypogammaglobulinemia, this has never been validated in patients with suspected paraproteinemia or in patients with suspected immunodeficiencies.

Objective:
We set out to determine the relationship between a protein gap and hypergammaglobulinemia or hypogammaglobulinemia.

Methods:
This study is a retrospective cohort analysis of patients who received a serum protein electrophoresis at The Ottawa Hospital from March 2014 to July 2017. A total of 17 277 patients had received a SPE as well as data on serum protein and albumin and were included in the analysis of hypergammaglobulinemia. A total of 16 815 patients had a gamma globulin level and were included in the analysis of hypogammaglobulinemia. The primary outcome was identification and correlation of the protein gap, defined as [total protein] – [albumin] in serum, with results from SPE as a gold standard for hypergammaglobulinemia, and total gammaglobulin levels as a gold standard for hypogammaglobulinemia.

Results:
The area under the curve (AUC) for predicting a positive SPE result using protein gap data was 0.663 (95% CI, 0.649-0.678). The sensitivity and specificity for predicting a positive SPE using a gap of >41g/L were 38.0% and 84.6%, respectively. Multiple regression analysis found age, sex, WBC, protein gap and total protein as being significantly associated with the presence of a positive SPE result. The AUC for the prediction of a hypogammaglobulinemia using the protein gap was 0.705 (95% CI, 0.694-0.716). Sensitivity and specificity analyses using a gap of <30g/L to identify a hypogammaglobulinemia were 47.6% and 86.2%, respectively.

Conclusions:
A very high or very low protein gap may reflect a gammaglobulinemia, but a normal gap does not have the sensitivity to rule out its presence. If a paraproteinemia is suspected, a gold-standard test should be still be performed in addition to the protein gap.

Transitioning to Living Systematic Reviews: Lessons learned from a large scale review on diabetes quality improvement interventions

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Background: Diabetes incidence continues to rise globally, along with diabetes-related health expenditures, complications, and death. Correspondingly, evidence evaluating professional/organizational quality improvement (QI) strategies designed to optimise diabetes management is also rapidly growing, with almost 20 new randomized controlled trials published in English each year. Traditional systematic review methodology to synthesize this evidence is no longer sustainable or appropriate, as reviews are in danger of being out of date by the time they are published. Living systematic reviews (LSR), defined as systematic reviews which are continually updated, incorporating relevant new information as it becomes available, have been proposed as a solution to ensure rigorous, timely evaluation of diabetes QI evidence.

Objective: To describe our transition of a large scale systematic review into a LSR, and to provide solutions for challenges encountered.

Methods: An existing, large-scale systematic review of 278 studies that evaluated diabetes QI interventions targeting professionals and organizations was transitioned into a LSR in 2017. Operationalizing the transition of this review into a LSR required numerous methodological considerations, including when and how to update our search strategy, what databases to search and how often, what screening platforms to use, when to update analyses, and the role of machine learning. The publication model to be utilized also required deliberation, with challenges including the need to maximise visibility while minimizing author/editor workload, and the desire for new citations/DOI with each publication.

Results: Our presentation will review decisions that were made to ensure the successful transition of our systematic review into a LSR. We will reflect on the expert opinions we received that informed our final decisions, and will integrate this knowledge with our own experiences. Methods to facilitate and streamline the process will be discussed, with particular focus on capabilities of automation/machine learning. We will provide our final recommendations and thoughts, including suggestions on how other research teams might conceptualize the transition of their own systematic review into a LSR.

Conclusions: The transition of an existing large-scale systematic review into a LSR has numerous challenges that require critical a priori thought by the review team. By detailing our decisions and experiences, we hope to contribute to the discussion of the methodology for this novel, emerging field. Furthermore, we hope to provide researchers with the tools they require to make informed decisions for their own LSR.

Investigating the Efficiency of Using Bacteriophage as a Potential Therapeutic Adjunct to Antibiotics for Staphylococcus aureus Biofilm Infections

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Background: Biofilm formation by bacteria is a major clinical challenge contributing to antibiotic treatment failure. Alternative strategies have been explored to overcome biofilm tolerance to antibiotic therapy. One of these strategies is the use of lytic bacteriophages to eradicate bacteria residing in biofilms. Bacteriophages can target biofilm associated bacteria at localized sites of infection by penetrating and disrupting biofilm matrices.

Objective: The main aim of our research is to assess whether bacteriophage treatment can enhance the antibiotic efficacy against biofilm *Staphylococcus aureus*.

Methods: A lytic bacteriophage, SATA-8505, that is known to infect *S. aureus*, and a biofilm-positive *S. aureus* isolate (ATCC 35556) were used. The minimal inhibitory concentrations (MIC) and minimal biofilm eradication concentrations (MBEC) of cefazolin and vancomycin, commonly used to treat *S. aureus* infections, were assessed. The ability of bacteriophage to enhance the antibiotics efficacy against biofilm cells was evaluated by exposing an established 24 h-old biofilm cultures to one of four treatments: i) varying concentrations of antibiotic alone, ii) bacteriophage alone at a concentration of 10^6 PFU/mL, iii) a combination of the two treatments, either simultaneously, or sequentially. Following treatment, the biofilm cultures were scraped off, and then plated to assess viability. Statistical analysis with a mixed model was performed to determine if the combined treatment of bacteriophage and antibiotics against *S. aureus* biofilms resulted in synergistic interaction which is defined as an interaction that leads to greater reduction of bacteria when the treatments are combined than the sum of the individual effects.

Results: MIC results suggest that cefazolin and vancomycin were effective at eradicating planktonic bacteria (MIC= 0.5 and 4 µg/mL, respectively) but ineffective against biofilm (MBEC >1024 µg/mL). Viable bacterial counts revealed that there was minimal efficacy against *S. aureus* biofilm cultures treated with: bacteriophage or antibiotics alone or simultaneously. However, when biofilm is treated with bacteriophage followed by antibiotics, there was a significant reduction in bacterial count up to 3-Log CFU. Vancomycin and cefazolin exhibited synergistic interaction with bacteriophage, particularly at lower antibiotic concentrations.

Conclusions: This study is designed to provide a proof of principle that bacteriophages can be effective in treating *S. aureus* biofilm infections. The sequential combination of lytic bacteriophage followed by antibiotics had the most robust biofilm eradication than each individual agent used alone. This observation suggests that bacteriophage can effectively disrupt *S. aureus* biofilms thereby enhancing the penetration and the bactericidal activity of antibiotics against biofilm-producing bacterial strains.

Classification and Recognition of Aggressive Movements using Smartwatches

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Background

Aggressive behaviour can occur in clinical and elderly care settings with people suffering from dementia, mental disorders, or other conditions that affect memory. Since identifying the nature of the event can be difficult with people having memory and communication issues, other methods to identify and record aggressive behaviour would be useful for care providers. The care providers hence will be able to determine the best methods to reduce behaviour reoccurrence. Smartwatches are unobtrusive devices that can be used to assess upper limb movement and therefore they can be helpful to determine if a movement tends to be violent. The smartwatch wearable technology approach for human activity recognition will be explored to detect aggressive movements. This approach could be used to identify the person that initiated the aggressive behaviour if a confrontation occurs.

Objectives

The objectives are to determine watch sensors signals that are related to aggressive motions, identify the features that are useful for signal processing and pattern classification and combine smartphone and smartwatch signals to improve classification performance.

Methods

30 healthy and able-bodied participants wore a Microsoft Band 2 (MSB2) smartwatch on each wrist and performed an activity circuit that includes gentle and aggressive actions. Gentle actions correspond to regular activities such as opening/closing a door or typing on a computer whereas aggressive actions consist of movements such as slapping, shoving or punching.

Results and Conclusions

The TOHRC Data Logger Android app was modified for signal acquisition from the two MSB2, with Bluetooth communication between the MSB2 and a Nexus 5 smartphone. Video of the movement was used to extract gold standard comparator. Accelerometer and gyroscope data were imported into Matlab and the Waikato Environment for Knowledge Analysis (WEKA) for feature extraction and pattern classification. Following 5-fold and 10-fold cross validation, Random Forest, Neural Networks and J48 Decision Trees machine learning classifiers are evaluated to determine the best features and performance metrics for distinguishing aggressive movements from gentle actions. Random forests is the best classifier with accuracies up till 91%.

Laying the Foundation for Change: A survey of paramedic mental health in Ottawa

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BACKGROUND: First responders encounter significant work related stressors and higher rates of critical stress (CS) incidents than the general population. Not only are they at an elevated risk for the development of Post-Traumatic Stress Disorder (PTSD), regular exposure to CS incidents increases the risk of mental disorders such as: depression, anxiety, alcohol abuse and dependence symptomatology, and the development of suicidal thoughts. A review of the literature reveals a lack of research investigating the mental health of first responder populations, especially within the Canadian context. As an initial step in addressing this problem, we conducted a survey of Ottawa Paramedic Service (OPS) members.

OBJECTIVE: The objectives of the survey were to record barriers impeding help seeking during crisis; estimate the frequency of CS incidents; evaluate the proportion of OPS staff with mental health diagnoses including suicidal thoughts and behaviours; and to provide an evaluation of the OPS' Peer Support Group (PSG) program.

METHODS: The survey was administered by the OPS' Peer Support Group during routine mandatory training sessions.

RESULTS: A total of 512 OPS Paramedics, Communications and Logistics personnel completed the survey, a response rate of 84.5%. The two main barriers to seeking help were "not sensing a need" and not wanting mental health diagnoses "on their record". Seventy percent of respondents reported having experienced CS. Across their lifetime 18.3% of respondents had received a clinical diagnosis of depression and 10.2% a clinical diagnosis of PTSD. Nearly one in four respondents reported suicidal thoughts and 3.4% had made at least one previous suicide attempt. Respondents rated the helpfulness of the Peer Support Group service as 7 out of 10.

CONCLUSIONS: Rates of depression, suicidal thoughts, and PTSD were high compared to the general Canadian population. The results of the survey have contributed to a larger needs assessment of the City of Ottawa's Tri-Services and a pilot randomized controlled trial of prevention of mental health disorders.

Effects of the Unity Vacuum Suspension System on Gait Parameters for Uneven Grounds

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Adapting to surfaces encountered in everyday life requires amputees to continually adapt their movement patterns. Elevated vacuum suspension systems could benefit transtibial amputee gait for uneven grounds, but research is lacking to inform clinical practice. Twelve transtibial amputees were fitted with the Ossur sleeveless vacuum suspension system (Unity). After 1 month accommodation period, the CAREN-Extended system was used to evaluate gait on a self-paced treadmill with continuous perturbations (medial-lateral translations, rolling hills, simulated uneven ground), with vacuum active or inactive. The symmetry index (SI) between legs for step time and stance time was considered good for both vacuum conditions (SI<10%). Significant differences between active and inactive vacuum conditions ($p<0.05$) were found for some temporal spatial, kinematics, and kinetics gait parameters, but the differences were small and not clinically significant. The Unity suspension system can function properly even with vacuum pump failure; however, stump volume changes over time due to the removal of elevated vacuum may adversely affect socket fit.

Keywords: Amputee, prosthetic limb, prosthetic suspension system, elevated vacuum, gait, virtual reality, rehabilitation

Effects of the Pro-Flex Foot on Trans-Tibial Amputee Gait and Comfort During Level and Slope Walking

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Background: Choosing an appropriate prosthetic foot capable of improving gait, balance and user satisfaction is an important element during the amputee rehabilitation process. Ossur has recently released two carbon fiber split toe energy storing feet (i.e., Pro-Flex and Pro-Flex XC). The Pro-Flex XC was initially released with an emphasis on power generation during physical activity and the more recently available, Pro-Flex foot, with a focus on superior ankle range of motion.

Objective: As a result of the limited research available on the Pro-Flex foot, this study examined trans-tibial amputee gait with the Pro-Flex and compared outcomes with the Pro-Flex XC during level and slope walking.

Methods: Three people with trans-tibial amputation who were previously fitted with the Ossur Unity suspension system and Pro-Flex XC foot entered the study. Their mean age was 44.7 (SD=16) years, weight was 82.3 (SD=11) kg, and height was 171.0 (SD=5) cm. The same prosthetist changed their feet to the Pro-Flex foot and aligned their prosthesis. After 1 month accommodation period, 3D gait analysis was performed using CAREN-Extended virtual reality system. Participants completed two walking trials in a park virtual reality scenario on level ground, slope conditions, and continuous perturbations. 3D motion was processed using Vicon Nexus and subsequently, kinematic and kinetic analysis was processed using Visual3D software. Subjective feedback was also collected at the end of the gait evaluation.

Results: Prosthetic ankle range of motion increased during level (22 %) and slope conditions (34 %) with the Pro-Flex foot, compared to the Pro-Flex XC foot. Kinetic assessment demonstrated that both prosthetic feet are capable of generating similar push-off power during level (2.5 W/kg) and decline (2.3 W/kg) conditions, although more push-off power is generated (8 %) with the Pro-Flex XC during incline walking. Subjective feedback also revealed more ankle range of motion and improved comfort with the Pro-Flex foot compared to the Pro-Flex XC foot.

Conclusion: Increased ankle range of motion could be one of the main reasons explaining amputee's improved comfort with the Pro-Flex foot during incline walking, even though the Pro-Flex foot generated 8 percent less push-off power than the Pro-Flex XC foot. Future investigations with a larger sample size are needed to better understand the effects of these feet on amputee gait and comfort.

A Clinical Prediction Model for the Early Identification of the Need for Major Intervention in Patients with Traumatic Hemorrhage

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Background: The mainstay of treatment for traumatic hemorrhage involves the early recognition of patients at risk of shock in order to provide timely blood products and hemostatic interventions. However, there is a lack of well-validated clinical decision tools to assist clinicians with risk stratification.

Objective: This project derives and internally validates a simple clinical prediction score, based on predictors available within the first hour of assessment, in order to identify patients at high risk of requiring major interventions for traumatic hemorrhage, defined as a composite outcome of massive transfusion, embolization and surgery for hemostasis.

Methods: In accordance with the Prognosis Research Strategy Guidelines, we created a model based on the pre-specification of predictors. We conducted a systematic review and meta-analysis of clinical prediction models and a national survey of Canadian traumatologists in order to identify candidate predictors. Study findings were reviewed by an adjudication committee in order to inform predictor selection. The full pre-specified model was then refined using a cohort of adult patients with major torso trauma presenting to the Ottawa Hospital from September 2014 to February 2017. We utilized Frank Harrell's stepdown procedure for simplification and bootstrap resampling for internal validation. Using weighted regression coefficients, the model was converted to a simple score.

Results: We included 748 patients of whom 110 required a major intervention. The final multivariable model was comprised of five variables: systolic blood pressure, clinical exam suggestive of hemorrhage, lactate, FAST ultrasound and CT imaging. The c-statistic for the model was 0.953 (naïve) and was 0.952 following optimism-correction with bootstrap validation. At a cut-off of 3 points or greater, the simplified score demonstrates 98.2% (95% CI 93.6 to 99.8) sensitivity, 79.2% (95% CI 76.0 to 82.3) specificity. The median (Q1 – Q3) time to first major intervention was 2.0 (1.0 – 4.0) hours.

Conclusion: This project utilizes pre-specification of predictors in order to minimize reliance on small datasets and reduce potential for over-optimism. Pre-specification is based on the best existing knowledge available within the literature and clinical expert community. A simple Canadian Bleeding Score is proposed based on five variables in order to systematically identify high risk bleeding trauma patients, demonstrates excellent sensitivity and specificity for predicting the need for major intervention within 24 hours. Multi-centre refinement and external validation studies are required prior to implementation.

Age-related eye disease and participation in lifestyle activities in older adults

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Background: Studies have shown the benefits of living a cognitively active lifestyle in older age. However, the loss of vision may make it difficult for older adults to do so. There has been limited research comparing the number of cognitively stimulating lifestyle activities in older adults with and without eye disease.

Objectives: Investigate the relationship between having an age-related eye disease (such as age-related macular degeneration and glaucoma) and participation in number of lifestyle activities per month.

Methods: Cross-sectional hospital-based study of older adults (n=244) having either age-related macular degeneration (AMD) (n=75), glaucoma (n=74), or normal vision (n=95) in Montreal, Canada. Controls had no diagnosis of AMD or glaucoma, a visual acuity better than 20/30 in both eyes and a visual field mean deviation better than -3dB in both eyes. Those in the AMD group were diagnosed with late stage AMD in both eyes with a better eye visual acuity of 20/30 or worse. Finally, those in the glaucoma group had primary open-angle glaucoma in both eyes with a visual field mean deviation worse or equal to -4dB in their better eye. Participants had to be 65 or older and had to obtain a score of 10 or more on the Mini-Mental State Examination Blind (MMSE-Blind) to be included in the study. Lifestyle activities were measured with a 70-item questionnaire assessing the frequency of performing various lifestyle activities. The sum of activities done at least once per month was calculated and used as the outcome in a linear regression analysis.

Results: The mean number of activities done at least once per month was 22 (SD=7). Adults with AMD (β=-5.0, 95%CI=-7.1, -2.8) and glaucoma (β=-2.4, 95%CI=-4.3, -0.6) participated in fewer activities per month compared to adults with normal vision after adjusting for age, sex, education, diabetes, comorbidity level, and cataract.

Conclusion: Older adults with AMD and glaucoma participated in fewer activities per month compared to older adults with normal vision. Future research is needed to better understand how this reduced activity level affects other aspects of aging such as cognition.

The Structure and Function of Eukaryotic Condensin*

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Background: There are two kinds of eukaryotic condensin in eukaryotic cells, designated as condensin I and condensing II,respectively. Both of condensin I and condensin II are pentameric complexes consist of shared SMC 2/SMC4 heterodimer and three non-SMC subunits those play regulatory action(Structure maintenance of chromosomes,termed as SMC protein). Three non-SMC subunits in condensin I are CAP-D2, CAP-H, CAP-G,respectively. Three non-SMC subunits in condensin II are CAP-D3, CAP-H2, CAP-G2,respectively.

Objective: The main role of condensins is to regulate the chromosome behavior during cell cycle. They not only participate in mitotic chromosome condensation, but also they participate in chromosome dynamics in meiotic division and interphase.This paper is to review the most recent sudy progress of the structure and function of eukaryotic condensin.

Methods: Method of Literature Analysis

Results: This paper reviewed research progresses of the structure and function of eukaryotic condensin in recent years on the bases of briefly introduce of the founding of eukaryotic condensin in order to provide references to related study.

Conclusions: Great breakthrough has been made in the study of eukaryotic condensin.

Contribution of genomic copy number variations detected by microarray in fetuses with congenital heart defects

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Background: Congenital heart defect (CHD) is one of the most common birth defects, affecting 8 to 9 per 1000 live births. The etiopathogenesis of this disease has not been completely explained. Genomic copy number variations (CNVs) detected by microarray are considered to contribute greatly in uncovering the pathogenicity of CHD.

Objectives: To assess the detection rate and additional diagnostic yield of microarray analysis in fetuses with congenital heart defects (CHD), compared with karyotyping, and to evaluate the contribution of copy number variations detected by microarray in fetuses with CHD.

Methods: We studied 84 fetuses with various phenotypes of congenital cardiac defects. Cases were divided into isolated and non-isolated CHD groups according to whether there were extracardiac ultrasound findings. Both conventional karyotyping and chromosomal microarray analysis were performed. Detection rate and increased diagnostic yield were calculated, and compared with the pathogenic results detected by karyotyping.

Results: Pathogenic CNVs were detected in 20 cases by microarray with a detection rate of 23.8%. Eight cases were overlooked by conventional karyotyping but identified by microarray, indicating the additional value of 9.5%. Microarray improved diagnostic efficiency in both isolated and non-isolated CHD groups (10.3% vs. 11.5%). The incidences of trisomy and gross chromosomal structural anomaly were significantly higher in non-isolated than in isolated CHD. However, there was no statistical difference in prevalence of microscopic rearrangements between the two groups. We also found that 15q11.2 microdeletion was significantly enriched in CHD cases over controls. Two cases of Soto syndrome were detected and presented microdeletion of 5q35.2.

Conclusion: The present study demonstrated the improved detection rate of microarray in fetuses with CHD. The results suggest that microarray is recommended not only for isolated cases but also for non-isolated cases in prenatal CHD cases. We also propose 15q11.2 deletion, 5q35.2 deletion, and 4q terminal deletion as the potential CHD-causing genetic loci. Genes encompassed in these regions, including UIMC1, ZNF346, FGFR4, TRIML2, and ZFP42, might function as candidate genes to play important roles in human heart development.

Dynamics of the Gut Microbiome during the first six month of newborn with vaginal birth in China

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Background: The gut microbiota is essential to human health throughout life, yet the acquisition and development of this microbial community during infancy remains poorly understood.

Objective: This study aimed to characterize the gut microbiota of healthy infants in a Chinese population and describe the dynamics of gut microbiome during the first six months of life.

Methods: A total of 10 healthy full-term, vaginal birth, and mixed feeding infants were recruited in Changzhi, China. The meconium and faecal samples at 1 month and 6 months were collected. Bacterial DNA was extracted from stool samples using QIAamp DNA Stool Mini Kit. We characterized the microbiota composition using high-throughput DNA sequencing.

Results: Bacterial community was not detected in the baby's meconium. The profiles were generally dominated by Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria in the faecal samples at ages 1 and 6 months. The differences on genus level of gut microbiota at ages 1 and 6 months were evident (both between samples and groups). There were significant differences in gut microbiome on phylum and genus levels between the faecal samples collected at 1 month and 6 months. Partial least squares discriminant analysis showed a significantly difference of gut microbiome between the newborns at 1 month and 6 months of ages.

Conclusions: These findings advance our understanding of the gut microbiota in healthy infants and establish a framework for understanding the interplay between the gut microbiome and the human body in early life. In future study we will compare the gut microbiome profiles between vaginal birth and cesarean birth infants.

Exclusion criteria and adverse events in peri-operative trials of tranexamic acid: a systematic review

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Background: Numerous clinical trials and systematic reviews have demonstrated that tranexamic acid (TXA) is an effective and safe antifibrinolytic agent for minimizing blood loss peri-operatively for a wide variety of surgeries and patient populations. Nevertheless, TXA continues to be underutilized in many surgical areas, largely due to concerns of possible side effects, such as thromboembolic events and seizures.

Objectives: The primary purpose of this systematic review was to identify common reasons for excluding patients from peri-operative TXA trials, in order to determine patient groups for whom TXA safety data may be lacking, and conversely, which patient groups have sufficient safety data and for whom recommendations for TXA use can be made. A secondary objective was to determine if TXA was associated with an increased risk of adverse events for surgical patients.

Methods: The database Medline (Ovid) was searched from 2014 to 2017 for RCTs administering systemic TXA peri-operatively to any elective or emergent surgery patients. Outcomes of interest were exclusion criteria of RCTs, and adverse events from TXA use. A qualitative synthesis of eligible RCTs was performed, describing general study characteristics and identifying common exclusion criteria for peri-operative TXA trials. Meta-analysis using a random effects model was performed to determine the risk of adverse events with TXA use.

Results: Twenty six RCTs were included in this systematic review, spanning a variety of surgeries and patient populations. Common reasons for excluding patients from peri-operative TXA trials were a history of thromboembolic events (77% of studies), allergy or hypersensitivity to TXA (73% of studies), coagulopathy (73% of studies), renal dysfunction (65.4% of studies), co-treatment with blood thinners (50%), and cardiopulmonary disease (38.5% of studies). Meta-analysis showed that overall TXA was not associated with an increased risk of adverse events compared to placebo or no intervention (RR = 0.83, 95% CI: 0.64, 1.06).

Discussion/Conclusion: This systematic review demonstrates that TXA is a safe anti-fibrinolytic agent to use in a wide variety of surgeries. Further investigation of TXA use is needed in patients with a past history of thromboembolic events, but evidence informed recommendation for use of TXA in many surgeries can be made.

EMS Clinical Decision Tool for Diversion of Syncope Patients

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Background
Previously, we found that the majority of syncope patients (>80%) transported to the Emergency Department (ED) by Emergency Medical Services (EMS) do not suffer any serious adverse events (SAE) within 30-days. Furthermore, over 50% of patients are diagnosed with vasovagal syncope. There is a potential group of patients who are at very low-risk for SAE and could be diverted from the ED to alternate care pathways such as their family physician or rapid access clinics.

Objectives
The objective of this study was to derive a clinical decision tool to help EMS accurately identify adult syncope patients who are at very low risk for SAE within 30 days after EMS evaluation.

Methods
We conducted a prospective observational cohort study at 5 Canadian academic EDs from February 2012 to February 2016. Adults with syncope who were transported to the ED by EMS were enrolled. The exclusion criteria included previously enrolled, prolonged LOC (>5 min), mental status changes, obvious witnessed seizure, significant trauma, intoxicated - alcohol/illicit drug, or language barrier. Variables collected include demographics, EMS vitals, EMS variables, medical history, and the Canadian Syncope Risk Score predictors. Primary outcomes were any SAE occurring within 30 days after EMS evaluation including death, arrhythmias, MI, PE, severe pulmonary artery hypertension, subarachnoid hemorrhage or any significant hemorrhage. Univariate and multivariable logistic regression were used to derive the final model.

Results
61 variables were initially identified; 33 variables were found to be statistically significant ($p<0.05$) from univariate analysis. 14 variables were selected for the multivariable logistic regression model. Predictors were included in the final model and Canadian EMS Syncope Risk Score (CESRS) was derived from the model estimated coefficients. We derived the Canadian EMS Syncope Risk Score (CESRS) comprising of 9 predictors (c-statistic=0.9 and p-value=0.09 for Hosmer-Lemeshow Goodness-of- Fit).

Conclusion
Once prospectively validated, CESRS has the potential to: 1) Improve EMS syncope management 2) Risk stratify very low risk syncope patients for diversion to alternate care pathways and 3) Reduce EMS burden, ED overcrowding, and overall healthcare costs.

Resident competency in identifying seizures

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BACKGROUND: Nonconvulsive status epilepticus (NCSE) is a neurological emergency. Delayed diagnosis of NCSE by more than 24 hours is associated with increased mortality from 36% to 75%. An EEG is essential in the diagnosis of NCSE. Not all tertiary care centres have EEG access after daytime hours. Some centres have resorted to resident initiated studies to aid in the detection of NCSE. Residents should therefore be knowledgeable in the American Clinical Neurophysiology Society (ACNS)'s Standardized Critical Care EEG Terminology, which is the standardized language used for to identify important EEG patterns. However, it remains unknown if residents can become proficient in this terminology.

OBJECTIVE: This study has two phases. Phase 1 aims to evaluate whether residents can acquire competency in the commonly used ACNS Standardized Critical Care EEG Terminology, most importantly, evaluate residents' ability to identify seizure vs not. Phase 2 will determine feasibility of resident initiated EEGs during off hours. Phase 2 is currently underway.

METHODS: After self-guided pre-reading and a brief 2 hour session of didactic teaching on the ACNS terminology, 16 adult neurology residents (PGY 2-4) were asked to complete the web-based ACNS certification test. This test has 37 EEG samples, each consisting of 11 questions; this standardized examination was developed and validated at Harvard University. Residents were asked to report their comfort level using the terminology and performance scores were reported as average percent agreement (PA%) with a previously established 5 member expert panel. Data were analysed using Microsoft Excel.

RESULTS: The overall pass rate (for all ACNS terms) for our cohort was 50% and the average score was 65.9%. 100% of the cohort met the minimum score required to show proficiency in seizure identification (>/=70%). The reported levels of comfort with the terminology ranged from uncomfortable (50%) to neutral (31.25%), while only 18.75% reported feeling comfortable with this terminology.

CONCLUSIONS: With minimal training, adult neurology residents at various stages in their training can learn and become proficient in the ACNS critical care EEG terminology. 100% of residents demonstrated competency at identifying seizures from background rhythmic or periodic patterns. This is an important step towards developing resident initiated off-hour EEGs in the care of patients suspected of having NCSE. On-going reinforcement may be needed to improve self-perception in comfort level.

The Parkin protein: From its Function in the Brain to the Characterization of a New Mouse Model

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Background: Recessively inherited loss-of-function mutations in the *PARK2* gene cause early-onset Parkinson disease. The disease-relevant mechanisms by which Parkin is neuroprotective remain elusive. Furthermore, the lack of symptomatic mouse models for parkin deficiency has impeded progress in PD research and therapeutic advancement. Our studies build on work from our team that Parkin protects against oxidative stress and are informed by what is known about Parkin biochemistry in human brain.

Objectives: We hypothesize that Parkin neutralizes reactive oxygen species (ROS) produced by mitochondria, thus conferring a previously reported "pro-mitochondrial benefit" (LaVoie et al., 2007). We posit that this is mediated by its abundant, redox-sensitive thiol-containing residues, and in doing so, directly contributes to the available thiol pool in cells (Wood et al., 2007). Building on these finding, we set to create an enhanced oxidative stress marker phenotype in mice, and thus a mouse model with a PD-like phenotype.

Methods: We tested this hypothesis using *in vitro*, cellular and genetic mouse models and lysates of human tissue coupled with assays to monitor ROS levels.

Results: We have modeled the oxidation of Parkin that is observed in human brain using recombinant untagged Parkin proteins and cellular models. Parkin oxidation is reversible, occurs through a direct interaction with oxidants and is dependent on its thiol-containing cysteines. Upon oxidation, Parkin forms high molecular weight species, which affects its solubility and modulates its E3 ligase activity. These changes confer protection in that cells overexpressing wild-type human Parkin show lower ROS levels, as assayed by measurements of H_2O_2. This insight led us to establish an oxidative stress marker phenotype, as previously described by Palacino et al. (2004), which demonstrated significantly increased oxidative damage in lysates of murine brain and heart, i.e., elevated protein carbonylation and nitrotyrosination.

Conclusion: Parkin protects high energy-dependent organs against oxidative stress-induced damage. This effect is mediated through the oxidation of its thiol groups. Our findings suggest a related function for Parkin in human brain. Characterizing a novel mouse model of recessive parkinsonism, by combining parkin deficiency with sod2 haploinsufficiency, promises to allow for the development of better neuroprotective therapies.

The Wnt-β-catenin Pathway in Convergent Extension-Mediated Nerve Cord Assembly in C. elegans

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Background: Convergent extension (CE) is a morphogenetic process that acts to narrow and elongate tissues through the intercalation of cells along one axis and the consequent extension along the perpendicular axis. In C. elegans the DD, DA, DB ventral neuroblasts undergo a CE-based process during embryogenesis to form the ventral nerve cord (VNC). The canonical Wnt-β-catenin pathway is involved in many aspects of embryonic development including the regulation of cell fate , cell migration, cell polarity, and axon guidance. Here we link the canonical Wnt-β-catenin pathway to CE-mediated nerve cord assembly.

Objective: Our aim is to characterize Wnt-β-catenin signaling mechanisms in CE utilizing C. elegans ventral neuroblasts as model system.

Methods: We use the six evenly spaced DD neurons along the AP axis as a marker for proper VNC assembly. Time-lapse video microscopy, utilizing an unc-30p::gfp cnd-1p::PH::mCherry reporter will allow visualization of DD and DA neuroblasts, is conducted to assess cell intercalation defects during VNC assembly. Lastly, protein localization is examined using a transgenic line expressing a GFP translational fusion to BAR-1/β-catenin.

Results: We found that bar-1/β-catenin mutants display an anteriorly displaced DD2 whereas the β-catenin overexpression mutant pry-1/Axin display a posteriorly displaced DD1, which suggests β-catenin activity affects neuron positioning in a highly stereotypical manner. One possibility is that the position defects in β-catenin mutants reflect cell fate changes. However, as the DDs adopt AP positions independent of their sister cells (RIGR, RIGL) in both β-catenin mutants, we suggest that β-catenin is not acting to direct binary cell fate decision in these lineages. Exploring further, we found that defects in both β-catenin mutants arise during single-cell intercalations preceding AP axis elongation. Utilizing our BAR-1::GFP translational fusion we found DB4, which interfaces ventrally with DD2, is the only DD, DA, or DB neuroblast which exhibits recognizable cytoplasmic localization of β-catenin. This suggests that BAR-1 activity in DB4 may act to organize the spatial and temporal pattern of cell intercalations involving DD1 and DD2.

Conclusion: In summary, this work suggests Wnt-β-catenin signaling is involved in CE-mediated nerve cord assembly. Further work, which evaluates if a transgenic line expressing BAR-1::GFP driven by a DB neuroblast promotor rescues bar-1/β-catenin mutants defects, will allow us to determine DB neurons act instructively to regulate DD intercalation during VNC assembly.

Determinants of spinal hyperexcitability in a human ex vivo model of pathological pain processing.

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Background:
The dorsal horn of the spinal cord is a primary pain processing center. In rodent models of neuropathic pain (Hildebrand et al, 2016, Cell Reports) and inflammatory pain (Dedek et al abstract, CAN 2017), we have found that BDNF-mediated loss of synaptic inhibition primes a subsequent potentiation of excitatory NMDAR responses in lamina I neurons of the dorsal horn, resulting in amplified spinal pain signalling. The molecular determinants of lamina I hyperexcitability include downregulation of the KCC2 chloride transporter and STEP61 phosphatase by BDNF, followed by activation of Fyn kinase and potentiation of GluN2B-containing NMDARs.

Objective:
A critical question is whether similar spinal mechanisms of hyperexcitability are conserved during human pathological pain processing. Here, we developed a human ex vivo model of pathological pain using viable human spinal cord tissue that is collected one to four hours post-mortem.

Methods:
We treated lumbar human spinal cord segments with recombinant BDNF or oxygenated saline control and investigated superficial dorsal horn signalling using biochemical and immunohistochemical approaches.

Results:
Our preliminary western blot data suggests that BDNF treatment results in decreased KCC2 and STEP61 and increased Fyn activation and GluN2B selectively at superficial dorsal horn synapses, similar to that observed in rodent models of neuropathic and inflammatory pain.

Conclusions:
We propose that spinal mechanisms of BDNF-mediated hyperexcitability are conserved between rodents and humans and could lead to novel therapeutic approaches for the treatment of pathological pain.

Identifying cannabinoid receptor 1 in human spinal cord stem progenitor cells

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Background:
The role of the endocannabinoid system (ECS) in human spinal cord development, injury and repair is not well known and has been previously implicated in having a role in pathologies including following spinal cord injuries. Cannabis, which acts on the ECS, is widely used among spinal cord injury patients to help manage pain, spasticity, and inflammation. Neural stem progenitor cells (NSPC) are found in the subependymal zones of the spinal cord and have the potential for neural regeneration. Although cannabinoid receptor 1 (CB1) have been identified in rodent spinal cord NSPC, they have not been well characterized in the human spinal cord.

Objective:

We aimed to identify and characterize CB1 presence in human spinal cord NSPC and their progeny.

Methods:
Human spinal crod NSPC were isolated from the subependymal region of the human spinal cord from both thoracic and lumbar regions and grown as adherent monolayer cells. Populations were passaged once before performing immunocytochemistry to maintain a stem-progenitor population. NSPC populations were maintained in proliferative conditions containing epidermal and fibroblast growth factors in serum free medium while to induce progeny via differentiation, cells were exposed to 1% fetal bovine serum in serum free medium 4 days following cell seeding.

Results:
By specifically tagging CB1, cannabinoid receptor 2 (CB2), and neuronal beta-III tubulin with immunocytochemistry, we demonstrated no CB2 expression (0%, n=3 human donors) on NSPC or progeny populations. In cells maintained in proliferative conditions CB1 was expressed on all cells (100%, n=3 human donors). We found that in differentiative conditions 89.46% (±2.6%, n=3 human donors) of cells were immature neurons, and every immature neuron expressed CB1 (100%, n=3 human donors).

Conclusion:
The presence of CB1 receptors in both NSPC and their progeny may implicate CB1's possible involvement in neural differentiation. As such ECS involvement maybe necessary for the development of neurons from stem-progenitor cells in the spinal cord and understanding the role of CB1 receptors in the spine may give insight in possible future therapeutics.

In vitro stimulation of neural stem/progenitor cells with regeneration factor loaded scaffolds

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Differential contributions of NMDA receptor subtypes composition to lamina II synaptic responses across spinal cord development

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Chronic pain is a persistent and debilitating condition which increases in prevalence with age and is often associated with hyperexcitability in the spinal cord. The N-methyl-D-aspartate receptor (NMDAR) is a heteromeric complex composed of two GluN1 subunits and two GluN2 subunits. NMDARs are critical regulators of excitatory synaptic transmission, including at spinal synapses. The presence of specific subtypes of GluN2 (GluN2A, GluN2B, GluN2C or GluN2D) determines the kinetic properties of receptor activity, which drives divergent NMDAR roles in synaptic integration. GluN2A subunits show the quickest deactivation (channel closing) rates, with a progression of slower decays from GluN2B to 2C to 2D. Our previous research has demonstrated that GluN2B and GluN2D dominate NMDAR responses at lamina I spinal synapses, which is unlike the GluN2A-dominated synapses found throughout most of the mature CNS. Lamina II interneurons receive synaptic inputs from peripheral afferents in addition to descending inputs from the brain and are critical for spinal pain processing. However, the functional contribution of specific GluN2 subunits towards NMDAR responses at lamina II synapses has not been characterized. We therefore performed whole-cell patch clamp recordings of NMDAR-mediated miniature excitatory postsynaptic currents (mEPSCs) in the presence and absence of GluN2 subtype-specific pharmacological blockers. In contrast to adult lamina I synapses, our results suggest that both GluN2A and GluN2B prominently contribute to NMDAR responses at juvenile lamina II spinal synapses. We also find a differential role of GluN2D at lamina II synapses, and a potential change in GluN2 subunit contributions across development. These findings will provide key insights into the role of specific NMDAR subtypes during spinal cord development, which could underlie the increased prevalence of maladaptive pain signaling in aging organisms.

Electrophysiological Profile of Differentiating Spinal Cord Stem Cells

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Background: Following spinal cord injury (SCI), current treatments look to halt the spread of tissue damage to minimize pain, with limited effectiveness. At present, there is no widely used approach for promoting re-growth across the lesion site, a necessity for functional recovery. To that end, stem cell transplant therapies are a promising tool for repairing damage from SCI and other neurodegenerative conditions.

Objective: As most stem cell studies are based exclusively on animal models, we aim to address several issues that are fundamental for potential translation to humans. First, are differentiation processes and resultant neuronal properties the same for stem cells isolated from human spinal cord as compared to rodent models? Second, we aim to examine what factors/conditions best drive this neuronal differentiation, and what the properties of these differentiated neurons are. As immunohistochemistry alone cannot adequately determine whether stem cells have differentiated into mature neurons, we will use patch-clamp electrophysiology to assess the passive and active electrical properties of cells throughout the differentiation process.

Methods: Ependymal cells from the central canal of thoracic and/or lumbar spinal cords are collected and isolated from male human donors. These cells are cultured on small coverslips under either: FBS control conditions, where they are allowed to grow and differentiate naturally; or via application of FBS and Retinoic acid (RA), which acts to promote neuronal differentiation. Each culture is supplemented with BDNF and GDNF. After several weeks of culture growth, patch clamp electrophysiological recordings are performed on cells at different culture time points to examine: passive membrane properties (capacitance and resistance); resting membrane potential; presence or absence of action potentials; threshold, rate, and pattern of action potential firing; and characterization of spontaneous synaptic currents.

Preliminary Results: Ependymal stem cells growing under control FBS conditions for 2-6 weeks in culture have a resting membrane potential that is often consistent with the neuronal phenotype (-37 to -60mV), but do not exhibit spontaneous or induced action potential firing. Surprisingly, we observed spontaneous excitatory postsynaptic responses in recordings at +60mV from cells that had neuronal morphologies but lacked action potentials.

Future Directions: We are currently using the electrophysiological technique to investigate the effects of RA guided growth on the electrical properties of cultured human stem cells.

A role for dip-2 (Disco-Interacting Protein 2 homolog) in neuronal migration and maintenance of neuronal morphology.

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Background: Mechanisms that inhibit inappropriate neurite outgrowth in the mature nervous system are likely to play important roles in maintaining normal neuronal function. A genetic screen for ectopic neurites in the VC motor neurons identified mutations in *nde-5* (*neurite outgrowth defective-5*) (1).

Methods: Using three models of DIP2 expression including loss-of function alleles and a RNAi DIP2 knockdown, the mechanosensory and HSN neurons were examined using in vivo florecence at multiple time points under 25°C conditions. To examine expression of DIP2, transgeneic and CRISPR GFP knock-in reporters were created using standard lab protocols. These models were then subjected to genetic analysis and compared to EMS treated worms to identify novel mutations that affect maintenance.

Results: We now show that *nde-5* encodes the worm orthologue of Disco Interacting Protein-2 (Dip2) and has thus been renamed *dip-2*. DIP2 proteins belong to a highly conserved protein family that contain an N-terminal DNA methyltransferase-associated protein 1 (DMAP1) binding domain and two adenylate-forming enzyme family domains (AFDs). In addition to VC defects, we found that *dip-2* mutants show morphology defects in several other neurons. Most prominently in the touch neurons where morphology defects show an age-dependent increase suggestive of a role in neuronal maintenance. *dip-2* mutants also show HSN neuronal migration defects and bifurcation of the PLM neuron. A CRISPR/Cas9-mediated functional GFP insertion into the native *dip-2* locus revealed expression in many neurons including the touch neurons and HSN as well as epidermal cells. DIP2 localization in neurons was found to be predominantly cytoplasmic. In contrast, DIP-2 in epidermal cells was predominantly plasma membrane localized. Correct localization in the embryo required the AFDs, loss of either AFD resulted in cytoplasmic expression and *dip-2* ectopic sprouting. Finally, we performed a forward genetic screen as well as a candidate screen for DIP2-like ectopic sprouts. The candidate screen revealed that mutation of some members of the insulin signaling pathway resulted in partial suppression of the *dip-2* phenotype, suggesting that they might function in a pathway with DIP2. The forward genetic screen found several proteins which interact with the cytoskeleton.

Conclusions: These findings indicate that DIP2 proteins are important for the development and maintenance of neuronal connections; and that an important signalling pathway remains to be uncovered. (1) D. Carr et al., 2016 Plos One.

Development of a growth factor-releasing biomaterial to promote post-stroke endogenous stem cell repair of the brain

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THE PREDIGT SCORE: MODELING IDIOPATHIC PARKINSON'S AS A COMPLEX ILLNESS CAN INFORM INCIDENCE RATES IN HEALTHY ADULTS

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Background: Fifty years after dopamine replacement therapy was introduced, Parkinson disease (PD) remains an incurable neurological disorder. As of 2017, no disease-modifying therapeutic has been approved. The inability to predict PD incidence risk in still healthy adults is seen as one limitation in drug development, because by the time of clinical diagnosis >60% of dopamine neurons have been lost.

Objective: We have designed an incidence prediction model founded on the concept that the pathogenesis of PD is similar to that of other disorders in ageing humans, i.e., a complex, multifactorial disease.

Methods: Our model considers five factors to determine the cumulative incidence rate for PD in healthy adults: 1) DNA variants altering susceptibility (D); 2) Exposure history to select environmental factors including xenobiotics (E); 3) Gene-environment interactions that initiate pathological tissue responses (I), e.g., a rise in ROS levels, misprocessing of select proteins (foremost, alpha-synuclein) and dysregulated inflammation; 4) Sex (or gender; G); and importantly, 5) Time (T) encompassing ageing-related changes, latency of illness, and propagation of disease.

Results: We have proposed, successfully modeled and published that the incidence rate for PD (P_R) in neurologically healthy adults can be calculated using the formula: $P_R=1-\text{baseline}^{((E+D+I)\times G\times T)}$, where the probability falls between 0 and 1 (Schlossmacher et al., Eur J Neurosci 2017)

Conclusion: Validation of the discriminative and calibrating powers of a transformed P_REDIGT score with an easy-to-use scoring system holds the promise to improve subject recruitment in future intervention trials. These validation studies will be carried out with support from Parkinson Canada and the Michael J Fox Foundation.

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 SNCA expression.

Methods: We employed viral and bacterial infection paradigms using genotypically modified mice and primary macrophages with no endogenous Lrrk2, carrying wild-type Lrrk2 (2), or with mutant G2019S knock-in Lrrk2 (3).

Results: First, we inoculated newborn mice with a respiratory-enteric-orphan virus ('reovirus', serotype-3) applied to the nose pad (4). 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, where Lrrk2 deficiency led to more colony forming units in solid organs of adult mice (p=0.05). In contrast, the Parkinson's-linked mutant, Lrrk2 G2019S, lowered Salmonella typhimurium burden and reovirus titres in acutely infected organs (including brain) from knock-in mice. Despite the 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). Further, we identified Lrrk2 dependency in phosphorylation of S727-STAT1, a major signaling component in response to viral and bacterial infection. Namely, REO infected macrophages and brains with G2019S Lrrk2 background have significantly increased pool of phosphorylated STAT1 at S727, while the Lrrk2 KO counterparts with significantly less phosphorylation of S727-STAT1.

Conclusions: We identified a systemic, anti-microbial effect for Lrrk2. Paradoxically, both Lrrk2 deficiency and the Parkinson's-linked G2019S mutant worsened the outcome of a viral brain infection that was naturally acquired; the former reduced the host's innate immune responses, whereas 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 SYNERGY MOUSE: A BI-GENIC MODEL OF HUMAN SYNUCLEINOPATHY

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Objectives: Mutations in GBA increase the risk for developing Parkinson disease and dementia with Lewy bodies. There, they are associated with earlier age of onset, worse clinical progression, greater risk of cognitive impairment and more extensive synucleinopathy. These neuropathological findings are consistent with studies in murine and cellular models in which altered GBA1 activity affected alpha-synuclein (SNCA) levels. The SYNERGY mouse was created to restage two significant risk factors in a preclinical model: human SNCA burden and mutant Gba1 expression.

Methods: SYNERGY mice carry 4 insertions of a PAC-derived human SNCA (A53T) locus on a murine Snca null background and two Gba1 D409V knock-in mutations. Age-dependent analyses of behaviour, SNCA and lipidomic metabolism and neuropathology have been performed up to 18 months of age. SYNERGY mice were compared to humanized SNCA littermates (wild-type Gba1) and wild-type controls.

Results: Humanized SNCA mice express 2.5- to 4- fold higher levels of SNCA in the brain. We observe significant SNCA-dependent cognitive impairment and progressive motor deficits, accumulation of SNCA-associated neuropathology and a loss of TH-positive neurons. Unexpectedly, in this context of a strong SNCA-dependent phenotype, the Gba1 mutation did not confer a worsened motor phenotype by 18 months of age. Age-dependent analysis of SNCA metabolism and lipid metabolism are ongoing.

Conclusions: The SYNERGY mouse recapitulates key aspects of human synucleinopathies. It represents a valuable preclinical research tool that can inform disease pathogenesis and be used in the testing of new therapeutic approaches.

The development of astrocytes and their interactions with the cerebrovasculature

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Background

In the central nervous system, neurons and blood vessels are linked together in a multicellular complex called the neurovascular unit (NVU). The coupling between neuronal activity and hemodynamics (neurovascular coupling) allows blood vessels to respond to elevated neural activity by increasing blood flow. A key component of the NVU is the astrocyte. Its processes encompass neural synapses and express neurotransmitter receptors, allowing these glial cells to detect synaptic activity. They also surround blood vessels with their endfeet and play a role in controlling vasodilation and vasoconstriction. The mechanisms that control these interactions are currently an area of active research. However, very little work has been done to explore the role of astrocytes in the development of the NVU.

Objective

It is known that the NVU is not functional at birth but matures a few weeks after. We explored how this maturation occurs and what role astrocytes play in vascular network expansion (i.e. angiogenesis).

Methods

We used a mouse model (in which astrocyte are genetically labeled with GFP; Aldh1L1-eGFP) to explore what role astrocytes play in proper cerebrovascular development and how they mature and incorporate into the NVU. To initiate this investigation, we used immunohistochemistry to visualize astrocytes (eGFP), endothelial cells and neurons. The stepwise recruitment of astrocytes around blood vessels was systematically charted throughout postnatal development (from birth to adulthood), allowing for a novel and precise morphological description of NVU formation.

Results

We show that, 7 days after birth (P7), astrocyte density is high in the cerebral cortex, but these glial cells do not contact blood vessels yet. At P14, while parenchymal astrocyte density progressively decreases, we began to detect astrocyte endfeet around blood vessels. Endothelial coverage of endothelial cells by astrocyte endfeet increased significantly from P14 to P50. Interestingly, while all “fibrous” (i.e. white matter) astrocytes expressed GFAP as expected, protoplasmic cortical astrocytes (eGFP+) also expressed GFAP when close to or in contact with blood vessels. These novel observations allow for a more precise morphological description of NVU formation.

Lack of Early Improvement Predicts Poor Outcome Following Acute Intracerebral Hemorrhage

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***VISTA-ICH Steering Committee:**
D.F. Hanley (Chair), K. Butcher, S M. Davis, B. Gregson, K.R. Lees, P. Lyden, S. Mayer, K. Muir and T. Steiner.

Background: Intracerebral hemorrhage (ICH) is the most devastating stroke subtype and is a major cause of morbidity and mortality. Predictors of poor long-term outcome in spontaneous ICH include: baseline volume, anticoagulation pretreatment, and spot sign presence. Even with these predictors, prognosis remains difficult. Patients with ICH are subject to rapid changes in clinical status, which adds additional uncertainty to prognostication. This creates difficult management decisions for both patients' families and medical staff alike, and has led to the premature withdrawal of care in certain cases. The effects of early neurological change and its potential relationship to long term outcome have not been well explored.
Objective: To use a data-driven approach to determine the degree of early post-ICH change that best predicts clinical outcome.
Methods: We analyzed 552 patients with ICH from the Virtual International Stroke Trials Archive (VISTA). We generated a receiver operating characteristic (ROC) curve for the association between 24-hour NIH Stroke Scale (NIHSS) change and clinical outcome. The primary outcome was a modified Rankin score (mRS) of 4-6 at 90 days; secondary outcomes were other mRS ranges (mRS 2-6, 3-6, 5-6, 6). We employed Youden's J Index to select optimal cut-points and calculated sensitivity, specificity, and predictive values. We determined independent predictors via multivariable logistic regression. The derived definitions were validated in the prospectively collected PREDICT cohort (275 patients).
Results: 24-hr NIHSS change was strongly associated with 90-day outcome with an area under the ROC curve of 0.75. Youden's method showed an optimum cut-point at -0.5, corresponding to NIHSS change of ≥0 (a lack of clinical improvement), which was seen in 46%. Early neurological change accurately predicted poor outcome when defined as ≥0 (Sens 65%, Spec 73%, PPV 70%, aOR 5.05 [CI:3.25-7.85]). For every 1 point increase in NIHSS, the odds of poor clinical outcome increased by 1.4 (95% CI: 1.28 – 1.50). All definitions reproduced well in the validation cohort.
Conclusion: Lack of clinical improvement at 24 hours robustly predicted poor outcome and showed good discrimination for individual patients who would do poorly. These findings are useful for prognostication and may also present as a potential early surrogate outcome for future intracerebral hemorrhage treatment trials.

Optogenetic activation and inhibition of 5-HT neurons in vivo

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Background: Depression is ranked among the top four medical conditions with the greatest worldwide disease burden. Serotonin (5-HT)-specific reuptake inhibitors (SSRIs) are the first line of current antidepressant treatment and act to increase 5-HT neurotransmission by blocking its reuptake. However, SSRIs require 2-3 weeks to elicit improvement and induce remission in only 30% of patients.

Objective: We hypothesized that direct activation of 5-HT neurons could lead to a much quicker antidepressant response and we used optogenetic mice and light stimulation of raphe 5-HT neurons to bypass the delay period of SSRIs.

Methods: The transgenic mice, Pet-Cre x ROSA-flx-STOP-ChR2-GFP (Pet-ChR2) or Arch-GFP (Pet-Arch) mice, were surgically implanted with a light fibre at the left directed at the dorsal raphe nucleus. Mice went through validated tests for depression such as the forced swim test and tail suspension test. The Pet-ChR2 mice were acutely stimulated with blue light to activate 5-HT neurons while the Pet-Arch mice were exposed to green light to inhibit 5-HT neurons during behavioural assays, for 5 min, then sacrificed after 90 min. Immunohistochemistry was done on raphe brain slices after stimulation to evaluate the expression of Fos and FosB, markers for acute and chronic neuronal activation, in TPH-positive 5-HT cells.

Results: Opsins were detected using anti-GFP and were strongly and specifically expressed in 5-HT neurons of the dorsal raphe nucleus. Upon acute stimulation of the dorsal raphe nucleus, all regions of the dorsal and median raphe nucleus were activated. Importantly, after stimulation of Pet-ChR2 mice, the number of Fos-activated neurons increased, with a significant rise in Fos-positive 5-HT neurons in all dorsal raphe nucleus regions. Stimulation of Pet-Arch mice attenuated the activation of Fos neurons, with a significant decrease in Fos-positive 5-HT neurons in all raphe nuclei regions. The number of FosB-activated cells and FosB-positive 5-HT cells was not significantly different compared to wildtype mice in both Pet-ChR2 and Pet-Arch mice, suggesting that acute light stimulation did not produce chronic changes in 5-HT activity.

Conclusions: Overall, this study will provide a better understanding of how serotonin cell activation or deactivation can lead to an anti-depression like phenotype. It also establishes a new mouse model for studying depression using optogenetics. Supported by CIHR and uOBMRI.

Characterization of a mouse model of Börjeson-Forssman-Lehmann syndrome (BFLS)

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Plant homeodomain finger protein 6 (Phf6) is a chromatin adaptor protein structurally defined by its zinc-knuckle PHD domains. Mutations in human PHF6 are associated with Börjeson-Forssman-Lehmann syndrome (BFLS), a rare X-linked intellectual disability characterized by mental retardation, obesity, and distinct physical abnormalities. Little is known about how PHF6 mutations lead to BFLS. To provide more insight into PHF6 and BFLS, we have generated a transgenic mouse line with a patient-related nonsense mutation (R342X) in the mouse Phf6 gene using CRISPR-Cas9 technology. Mutant mice were viable, healthy and born at normal Mendelian ratios. Throughout maturation and into adulthood, the mutant mice were significantly smaller. Organ weight to body weight ratios showed no differences from control littermates. Analysis at the cellular level demonstrated that Phf6 transcript levels were drastically reduced, protein levels were ablated and that the animals were essentially null for Phf6. Female mutants recapitulate the total loss of Phf6 expression, demonstrate a similar decreased body mass and were healthy. Previous investigation has suggested that during embryogenesis Phf6 is highly expressed in the cortex, and is involved in cortical development and neuronal migration. Immunohistochemical analysis of brains from mutant mice demonstrated no obvious structural defects. Volumetric analysis of brain regions (cerebral cortex, cerebellum, hippocampus, hypothalamus, and pituitary gland) is underway using MRI. Immunofluorescence experiments suggest a cell cycle defect in the neuronal progenitor population during embryogenesis. Further experiments will characterize the complete neurogenesis and neuronal migration phenotype in the mutant mice. Abnormal pituitary gland development and function has been well documented in BFLS patients. Immunohistochemical analysis of pituitary glands from mutant mice reveal significantly smaller anterior pituitary glands. Preliminary behavioral testing implies that the mice have a learning deficiency, have weaker muscle strength and may have an ADHD phenotype. Additional behavioral testing and gene expression studies will further enhance the characterization of these animals.

Hedgehog signaling regulates satellite cell function.

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Background: In 2015, the laboratory of Dr. Rudnicki made a seminal discovery that dystrophin is expressed in newly activated muscle stem cell where it regulates the establishment of PAR-mediated polarity and its subsequent asymmetric division. Accordingly, we observed a loss of asymmetric divisions and a decreased numbers of differentiated cells, leading to failure of regenerative myogenesis to keep pace with disease progression in *mdx* mice, a mouse model for Duchenne Muscular Dystrophy where the dystrophin is absent. Therefore, to uncover other molecules that may control and establish polarity in muscle stem cells, we conducted expression data analysis between wild-type and *mdx* mice, which revealed that cilia-mediated Hedgehog (Hh) signaling pathway is repressed in *mdx* muscle stem cells.

Objectives: Knowing its role during embryonic muscle development, I undertook a molecular characterization of Hh signaling in regulating muscle stem cell function, in order to assess to what extent restoring Hh signaling could improve the regeneration in dystrophic muscle.

Methods: Our project requires several *in vivo* experiments and molecular biology methods to ultimately define the role of cilia-mediated Hh signaling for muscle stem cell function.

Results: In a normal context, I showed that stimulating cilia-mediated Hh signaling pathway with Hh agonist treatments activates *Myf5* expression in the committed satellite cell following an asymmetric stem cell division. By contrast, primary cilia removal in satellite cells inhibits Hedgehog signaling and leads to a loss of both asymmetric divisions and *Myf5* expression, highlighting the essential role of the primary cilium for Hedgehog transduction in satellite cells. The primary cilium regulates the processing of both Gli2 and Gli3 transcription factors, the direct effectors of the Hh signaling pathway. Using mice allowing for tamoxifen-inducible genetic deletion of *Gli2* and *Gli3* in satellite cells, we found that *Gli2*-specific deletion trends decreasing the number of Pax7-expressing cells while *Gli3*-specific deletion in satellite cells dramatically decreases the number of myogenin-expressing cells. Given that both Gli2 and Gli3 can transcriptionally activate Hh signaling but only Gli3 is a transcriptional repressor, our data support the idea that active Hh signaling enhances proliferation and early commitment of satellite cells, whereas its repression allows for their differentiation. Altogether, these preliminary results demonstrate the requirement of cilia-mediated Hh signaling for regulating satellite cell function.

Conclusion: Therefore, we propose that stimulating the activity of Hh signaling in *mdx* satellite cells will restore their function and their ability to regenerate a dystrophic muscle.

Differences in metabolic determinants associated with right heart failure in the SU5416 chronic hypoxia model of pulmonary arterial hypertension

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Introduction: Pulmonary Arterial Hypertension (PAH) is a progressive, incurable disease of the microvasculature of the lung. In PAH, the progressive loss of microvasculature is accompanied by an increase in pulmonary vascular resistance leading to right ventricular hypertrophy. Initially adaptive, RV hypertrophy progresses to maladaptive hypertrophy, heart failure and ultimately death. Alterations in cardiac energy metabolism have been observed in PAH but the mechanisms linking heart failure, energy metabolism and PAH remain unknown. The objective of this study is to determine early RV metabolic alterations in two rat models of PAH showing highly divergent abilities for RV adaption.

Methods and Results: Severe PAH was induced by a single subcutaneous injection of the SU5416 in age-matched Sprague-Dawley (SD) or Fischer rats (125-200g), followed by a 3-week exposure to chronic hypoxia (SUHx). Both SD and Fischer rats develop severe PAH; however, only Fischer rats exhibit maladaptive RV remodeling progressing to right heart failure and death (100% by 7 weeks). Immunohistochemical analysis revealed similar levels of cardiomyocyte hypertrophy in both SD and Fischer rats. However, in response to SUHx capillary density was significantly lower in Fischer rats. Microarray analysis of the RV at 4 weeks revealed 318 genes uniquely regulated in Fisher rats, 41 uniquely regulated in SD and 82 genes similarly regulated in both strains. Of the genes uniquely regulated in Fischer rats, gene ontology analysis identified biological processes involved in NK cell-mediated innate immunity, immunity and defense, fatty acid metabolism, regulation of vasoconstriction and dilation, and blood circulation and gas exchange. Notably, key regulators of fatty acid oxidation (long-chain FATP1, acyl-CoA synthetase long-chain family member 1, hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase) and mitochondrial biogenesis (PGC1-α) all were downregulated in Fischer rats. These data are in agreement with recent studies showing a metabolic imbalance in the RV pointing to a role of energy metabolism in the progression of maladaptive RV hypertrophy into heart failure.

Conclusion: Our data implicate both perfusion mismatch and energy metabolism as determinants of RV adaptation in PAH. Current experiments aim at characterizing the metabolic and mitochondrial adaptations in both SD and Fischer rats in response to SUHx.

Uncovering synthetic lethal interactions in acute myeloid leukemia

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Background

Acute myeloid leukemia (AML) is a disease characterized by the expansion into the bone marrow and blood of abnormally differentiated hematopoietic cells known as blasts. While AML is the most common form of adult leukemia in North America, the five-year survival rate is disappointingly below 20%. Dismal outlook among patients is partly the result of the vast heterogeneity in the molecular etiology of AML rendering a single treatment regimen unlikely to suit all those affected by the disease.
Using a unique panel of molecular prognostic indicators identified by our lab, I will perform a whole genome synthetic lethal screen in matched AML cell-lines to identify new gene targets that can be inhibited to better treat AML.

Objective

To conduct a whole genome CRISPR/Cas9 synthetic lethal screen in matched THP1 and KG1 cell-lines to identify genes that can be inhibited to kill AML blasts with genotype specificity. Following the screen, top hits will be validated in primary samples from both healthy and diseased patients.

Methods

We are combining high throughput sequencing with lentiviral delivery of Cas9 and pooled short guide RNA cassettes to knock-out every gene in the genome. Drop-out of viral integrations from sequencing data are then compared between matched cell-lines to identify genotype specific liabilities.

Results

Thus far, THP1 acute myelomonocytic leukemia cells have been engineered to express Cas9 nuclease while lentiviral infection of these cells has been optimized to avoid dual transduction of guide sequences upon screening.

Conclusion

This approach will not only generate a low incidence of off-target effects, but a decreased false negative rate due to high specificity and elimination of variable knock-down efficiencies which have been problematic in past shRNA-based synthetic lethal screens. The CRISPR-based strategy will allow us to dissect the molecular pathways underlying AML, as well as identify potential drug targets that can be harnessed for improved therapy.

Role of ATRX in neuronal circuitry and behaviour

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Background: ATRX is a nuclear protein of the SWI/SNF family with chromatin remodeling activity. Its association with DAXX facilitates the deposition of histone variant H3.3 into telomeric, pericentromeric and interstitial chromatin. ATRX binding studies have suggested a role in DNA stabilization by preventing the formation of secondary structures, which perturb gene replication and transcription. Germline mutations to the *ATRX* gene can lead to ATR-X syndrome (Alpha-Thalassemia X-Linked Intellectual Disability Syndrome), which is classified as a congenital disorder with abnormal craniofacial features, genital anomalies, severe developmental delay and hypotonia. Furthermore, conditional ablation of *Atrx* using *Nestin*-cre and *Foxg1*-crex mouse lines resulted in early postnatal lethality as well as a reduction in cortical size.

Objective: Investigate the role of ATRX and its associated proteins in the development of neuronal impairments that are associated with ATR-X syndrome. Additionally, we want to determine the cognitive effect of ATRX ablation in the forebrain.
Method: We used the *Emx1*-cre driver to conditionally remove *Atrx* in the forebrain to avoid lethality. Also, another mouse line with a frequent mutation found in the ATR-X syndrome, the *Atrx*-ΔE2 mice, was used to assess the effect of a truncated protein.

Results: The *Atrx*$^{f/y}$ *Emx1*-cre mice survived to adulthood and showed no weight difference. Furthermore, *Atrx*-conditional knockout mice exhibit a strong hyperactivity phenotype which was detected during the beam break test. This hyperactivity was not found in the *Atrx*-ΔE2 mice. The *Atrx*$^{f/y}$ *Emx1*-cre mice phenotype correlates with the daily light/dark cycle which suggests that they need more time to adjust to new stimuli. Also, this could suggest that *Atrx*-conditional knockout mice have an ADHD phenotype but more tests need to be performed.

Conclusion: These results have allowed for the preliminary behavioral characterization of *Emx1*-cre conditional knockout mice lacking the *Atrx* gene and *Atrx*-ΔE2. Further behavioral tests as well as morphological and cellular characterization of both mouse lines will be done.

p38-gamma MAPK regulation of Carm1 mediates asymmetric cell fate of muscle stem cells

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The regenerative capacity of skeletal muscle is dependent on the robust activation of resident muscle stem cells. Muscle stem cells undergo asymmetric cell division in order to ensure the simultaneous maintenance of the stem cell pool and to generate the myogenic progenitors required to repair damaged muscle tissue. These asymmetric cell divisions are regulated by the establishment of cell polarity within the stem cell, a process that is mediated by the dystrophin glycoprotein complex (DGC). Dystrophin expression is restricted to the self-renewing daughter stem cell. In contrast, the methyltransferase Carm1 promotes the epigenetic activation of myogenic commitment genes exclusively in the committed daughter cell. The molecular mechanisms that link the DGC to the regulation of Carm1 activity, however, have remained elusive. We have identified p38-gamma MAPK as a Carm1 regulatory kinase. Moreover, p38-gamma activity is regulated through its interaction with the DGC via direct protein binding with beta-1-syntrophin. Phosphorylation of Carm1 by p38-gamma restricts Carm1 localization to the DGC thereby preventing Carm1 activation of myogenic commitment genes within the stem cell. Importantly, this pathway is dysregulated in dystrophin-deficient muscle stem cells. In the absence of a functional DGC, Carm1 is hyper phosphorylated, resulting in decreased epigenetic activation of myogenic commitment genes. Our findings uncover a novel signaling pathway mediating asymmetric cell fates of muscle stem cells that is directly affected by the loss of dystrophin. These results ultimately provide insight into the stem cell dysfunction and ineffective muscle repair that contributes to the severe degeneration observed in Duchenne Muscular Dystrophy.

Protracted endothelial cell apoptosis leads to lung microvasculature loss and elevated pulmonary arterial pressures preceding the appearance of occlusive proliferative lesions in the rat SU5416-Hypoxia model of severe PAH

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Background: The mechanism that leads to elevation in pulmonary vascular resistance in pulmonary arterial hypertension (PAH) is still uncertain. Dysregulated cell growth has emerged as a major target for novel therapies, and is thought to result in arterial narrowing and occlusion through medial hypertrophy intimal hyperplasia, respectively. However, recent studies have suggested that severe and complex atrial remodelling may be a consequence, rather than a cause, of sustained elevation in pulmonary pressures. Evidence from experimental models have implicated endothelial cell (EC) apoptosis as an initial trigger of PAH, although how this leads to the hemodynamic and typical pathological features of this disease is unclear.

Hypothesis: EC apoptosis results in the direct loss of microvasculature through degeneration of small lung arterioles as a primary mechanism producing increase in pulmonary arterial pressure and resistance in PAH.

Methods and Results: Sprague Dawley rats were given a single subcutaneous injection of the VEGFR2 antagonist, SU5416 (SU; 20 mg/kg), with or without 3-week chronic hypoxia (CH; 10% O_2). EC apoptosis was markedly increased in the pulmonary microvaculature for at least 16 weeks after SU as assessed by immunohistochemistry, western blotting and activity assay for cleaved caspase-3. Marked increases in right ventricular systolic pressure (RVSP) were seen as early as 4-weeks post-SU (76±11 mmHg) with no significant progression at 7-weeks (100±15 mmHg) or 16 weeks (88±15 mmHg). The lung microcirculation was assessed by microCT at 4 and 7 weeks post-SU after perfusion with barium at the relevant arterial pressures. There was a marked reduction in arterial volume as early as 4 weeks post SU ($1.6x\ 10^{11} \pm\ 1.8x10^{10}\ \mu^3$), which showed no further progression at 7 weeks ($2.1x\ 10^{11} \pm\ 4.2x10^{10}\ \mu^3$), compared to control rats lungs ($4.2x\ 10^{11} \pm\ 5.4x10^{10}\ \mu^3$; $p<0.001$ vs. 4 and 7 weeks). Functional arterial loss was nearly exclusively restricted to the small arterioles (<200μ), and paralleled the increases in RVSP; whereas evidence of occlusive arteriopathy was seen only after the 7-week time-point. Further, treatment with estradiol, a female sex hormone with potent anti-apoptotic effects on ECs, was able to prevent the SU-induced increase in cleaved caspase-3 activation and completely inhibit development of PAH.

Conclusions: These results support degeneration of fragile precapillary arterioles, occurring as a direct result of EC apoptosis, as a primary mechanism for the hemodynamic abnormalities in severe PAH.

Modeling Defective epigenetic inheritance in Hutchinson-Gilford Progeria Syndrome vascular smooth muscle cells

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Background: During replication, the chromatin state is inherited by daughter cells in a process known as epigenetic inheritance. Defective epigenetic inheritance occurs in aging and cancer; however, little is known about the process. Abnormal epigenetic inheritance is also a hallmark of skin fibroblasts isolated and cultured from patients with the accelerated aging disorder Hutchinson-Gilford Progeria Syndrome (HGPS). Children affected by HGPS develop numerous aging-related pathologies and succumb to vascular complications in their teens due to deterioration of vascular smooth muscle cells (VSMCs) leading to atherosclerosis, heart attacks and stroke. HGPS is caused by a mutation in the *LMNA* gene that activates a cryptic splice site, leading to the accumulation in the nuclear lamina of a mutant Lamin A protein termed Progerin. Pathological studies have shown that oxidative stress and DNA damage are hallmarks of normative and Progeria atherosclerotic lesions.

Objective and Hypothesis: As the DNA damage response has been shown to recruit epigenetic complexes, we hypothesize that unresolved epigenetic modifications at sites of DNA damage or repair underlies defective epigenetic inheritance in Progeria. Our goal is to study the molecular basis of defective epigenetic inheritance in Progeria and aging VSMCs using patient derived induced pluripotent stem cells (iPSCs).

Methods and Results: First, we reprogrammed HGPS fibroblasts that demonstrated loss of heterochromatin and found that despite abnormal epigenetic inheritance manifest in the fibroblasts, the process of reprogramming returned HGPS cells to a normal pluripotent state and extinguished Progerin expression (Chen et al., *Aging Cell* 2017). Upon differentiation of the HGPS iPSCs into VSMCs, Progerin expression is initiated and oxidative stress and DNA damage are observed. We have found that ROS scavengers decrease DNA damage in HGPS VSMCs, suggesting that oxidative stress drives DNA damage and replicative stress. Furthermore, we determined that the DNA damage response machinery and chromatin remodelers accumulate at sites of nascent replicated DNA, suggesting that HGPS VSMCs are undergo replicative stress. Using a proteomics-based approach, we have found that epigenetic complexes and DNA repair proteins are differentially recruited to sites of DNA damage in HGPS VSMCs versus control VSMCs. We are currently defining the impact of these protein complexes in epigenetic inheritance.

Identification of Small Molecule Modulators of Satellite Stem Cell Asymmetric 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. A subset of satellite cells has been shown to recapitulate the muscle stem cell reservoir by symmetric expansion and asymmetric self-renewal via cell polarity pathways such as Wnt, JAK/Stat and p38α pathways. In the context of Duchenne Muscular Dystrophy (DMD), dystrophin-deficient satellite cells are unable to maintain cell polarity during asymmetric self-renewal, resulting in increased mitotic errors and decreased regeneration potential. The importance of asymmetric self-renewal has also been demonstrated in aging muscle, where decline in stem cell self-renewal leads to a reduced capability for muscle regeneration. Unfortunately, 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 satellite cell asymmetric self-renewal and cell polarity pathways will restore the muscle regeneration capacity in mdx mice. With our findings, we aim to characterize additional molecular signaling pathways that participate in muscle regeneration, and uncover novel therapeutic targets for the treatment of DMD.

Objective

1. Identify novel regulators of satellite stem cell division.
2. Elucidate the mechanisms of identified molecular pathways involved.
3. Confirm therapeutic effects in vivo using the MDX (DMD) transgenic mouse model.

Methods

We have developed a high content analysis platform using myofibers derived from Myf5-Cre R26YFP mice to screen small molecule compounds previously characterized in clinical trials. Western blot analysis, qPCR and siRNA knockdown will be used to determine receptor expression. We will employ Co-IP and PLA assays to determine signaling pathway partners. Floxed alleles will be used to perform lineage trace experiments, force assays, and regeneration engraftment assays.

Results

Our screen has successfully identified previously characterized receptors and pathways involved in stem cell division. More importantly, we have also identified a number of novel modulators of asymmetric stem cell division.

Conclusion

We have validated the effect of our inhibitors by siRNA KD and drug explosure in both wild-type and DMD satellite stem cells. We have also validated the expression of our targets of interest by immunofluorescence, lineage tracing, flow cytometry, qPCR, RNA-seq, and ATAC-Seq.

Correcting peak-calling biases to accurately quantify ChIP-seq signals

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Background

Chromatin immunoprecipitation followed by sequencing (ChIP-seq) is a powerful tool used to identify cistromic interactions between protein transcription factors (TFs) and genes. The technique relies on cross-linking DNA with cellular proteins and isolating the DNA bound to a TF of interest using highly specific antibodies. Isolated DNA is typically recovered in small fragments called reads which are aligned to a reference genome to determine candidate TF binding sites. Compared to microarray-based technologies, ChIP-seq enjoys greater sensitivity and specificity in identifying TF binding sites. However, accurately quantifying ChIP-seq data still presents a major challenge in bioinformatics due to difficulties modeling the null distribution of reads in control data.

Objective

Evaluate the bias of three peak-callers and propose an algorithm to correct their statistical analyses.

Methods

Artificial ChIP-seq datasets were simulated with known TF binding sites. Datasets were analyzed by peak callers diffReps, MACS, and SICER at various p-value thresholds to determine the number of identified enriched regions. We proposed a re-mixing procedure to correct software biases corresponding to poorly defined null hypotheses. This empirical procedure mixes treatment and control ChIP-seq datasets to generate a null distribution of reads. After peak-calling, the p-values obtained from re-mixed datasets were used to adjust those obtained from normal treatment and control datasets.

Results

Against various types of simulated data, diffReps, MACS, and SICER consistently identified more binding sites than expected. Using our re-mixing procedure, all three software yielded well-calibrated p-values corresponding to the probability of a correct candidate binding site. Therefore, the peak callers returned the expected number of TF-enriched regions at nearly any user-defined p-value threshold, resulting in fewer falsely identified candidate binding sites in the simulated data.

Conclusions

We have successfully developed an algorithm to model the null distribution of ChIP-seq reads and generate unbiased estimates of potential TF-targeting elements. This method is shown to work with three popular peak callers and may be extended to other ChIP-seq software or even RNA sequencing tools which may be affected by similar biases in statistical analyses.

Enhancing adenovirus replication for treatment of cancer

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Background

Oncolytic viruses selectively replicate in and kill cancer cells while leaving healthy cells unharmed. Although conditionally-replicating, oncolytic adenoviruses (CRAds) have shown promising results in preclinical studies, they have had limited therapeutic efficacy in human clinical trials.

Preclinical studies suggest the spread of CRAd throughout a tumor mass is a major limitation for effective tumor regression. Typically, CRAds only diffuse a few millimeters from the injection tract, leaving much of the tumor unaffected. Increasing the level of the Ad death protein (ADP), which is required for efficient killing of infected cells, and incorporating the p14FAST protein, which causes host cell-cell membrane fusion, may help overcome these physical barriers.

Objective

Our objective is to investigate whether inclusion of ADP and p14FAST in a CRAd vector enhances viral spread throughout the tumor mass, thereby increasing its ability to replicate in and kill cancer cells in tissue culture and animal models of cancer.

Methods

CRAds that variably include ADP and p14FAST were constructed, and their ability to replicate in, fuse, and kill cancer cells was evaluated. Expression of viral proteins was evaluated through immunoblot analysis. Cellular metabolic activity was measured by MTS assay.

Results

We previously developed a fusogenic CRAd, CRAdFAST, in which the early region 3 (E3) of the viral genome, which encodes ADP, was removed and replaced by a p14FAST expression cassette, and placed under regulation by the Ad major late promoter (MLP). CRAdFAST efficiently fused cancer cells and improved oncolytic activity compared to a non-fusogenic CRAd in tissue culture and mouse models of cancer, but had only a modest effect on overall tumor growth and mouse survival. To evaluate the effect of including ADP in a fusogenic CRAd, we developed two vectors, CRAdRC109 (E3+) and CRAdRC111 (E3-). In these viruses, the p14FAST expression cassette was relocated downstream of the viral late 5 (L5) region, allowing reinsertion of the entire E3 region including ADP. Relative to CRAdFAST, these viruses had a 400-fold reduction in p14FAST protein expression and did not fuse cells, nor did they show improvements in reducing cellular metabolic activity. However, inclusion of ADP in CRAdRC109 increased viral plaque-size.

Discussion

Our results suggest placing the p14FAST expression cassette downstream of L5 drastically reduced p14FAST protein expression, thereby reducing fusogenic and anti-cancer activity. We are currently evaluating alternative vector design strategies to achieve high-level ADP and p14FAST protein expression from CRAd vectors.

Agarose single-cell cocooning of endothelial progenitor cells to improve cell retention and therapeutic efficacy in pulmonary arterial hypertension

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Background: Late outgrowth (L) endothelial progenitor cells (EPC), also called endothelial colony forming cells, are derived from circulating blood mononuclear cells. Compared to early outgrowth EPCs, they represent a uniform, highly endothelial-like progenitor cell population; however, their therapeutic benefits in models of pulmonary arterial hypertension (PAH) are limited by poor cell persistence, due to rapid cell loss by apoptosis (anoikis) and redistribution to non-target organs. Temporary microencapsulation (i.e. cocooning) provides a portable stem cell niche, that can promote cell survival and retention in animal models of organ lung and heart injury.

Hypothesis: We hypothesize that microencapsulation of L-EPC with an agarose hydrogel supplemented with integrin-binding proteins will increase survival and retention of L-EPCs injected into the jugular vein and result in greater therapeutic benefits compared to non-cocooned cells in a rat monocrotaline (MCT) model of PAH.

Methods: L-EPCs were encapsulated using various concentrations of agarose, together with fibronectin and fibrinogen, by vortex-emulsion and capsule size, cell viability and time to cell egress were assessed. Encapsulated and non-encapsulated L-EPCs transduced with luciferin were administered three days post MCT and tracked by bioluminescence to assess cell persistence and bio-distribution for up to 3 weeks post cell injection. At end-study, right ventricular systolic pressure (RVSP) and right ventricular hypertrophy (RVH) were assessed for therapeutic efficacy.

Results: Varying the agarose concentration between 2 and 5% resulted in a shift in the size of cell cocoons from 31.7±8.5 µm to 43.7±12.5 µm, respectively. Increasing agarose concentrations had a modest inverse effect of cell viability at 0h between (86.9±4 vs 76.4±6.5 %, respectively; p = 0.029), but no detectable effect on cell viability at 24h assessed by WST assay or CalceinAM intake. Cell egress was reduced by higher agarose concentration, with 221±3 vs 78±36 cells per field of view, respectively exiting the capsules over 24 hrs. Cocooned L-EPC were detectable up to 24h *in vivo* compared to non-encapsulated cells which could not be detected even 4h post cell injection. Encapsulation improved the ability of L-EPC to improve RVSP (40.5±7.4 vs 77.4±5.4 mmHg, p = 0.009, respectively) and RV/LV+septal weight (47±7 vs 69±5 %, p = 0.049, respectively) compared to non-encapsulated cells.

C**onclusions:** These results demonstrate that single-cell cocooning can increase retention of L-EPCs, potentially enhancing the therapeutic effects of L-EPCs in the rat MCT model of PAH.

Abnormal fatty acid metabolism is a feature of spinal muscular atrophy

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Introduction

Spinal muscular atrophy (SMA) is a devastating neuromuscular disorder characterized by paralysis and muscle weakness. Recent evidence suggests that SMA is a multi-organ disease. We have previously shown abnormal glucose homeostasis due to altered pancreatic islet cell distribution in SMA mouse models and in human patients. Some studies have suggested that SMA patients may have fatty acid metabolism defects.

Objective

Investigate whether liver and/or skeletal muscle lipid metabolic defects are present in SMA.

Methods/Results

A thorough characterization of gross, histological and molecular changes in the liver and skeletal muscle from SMA model mice was performed. Progressively paler livers were noted in *Smn*$^{2B/-}$ mice as they aged, a possible sign of fatty liver. Haematoxylin and eosin staining showed a dramatic decrease in cellular density in P19 *Smn*$^{2B/-}$ livers, potentially because of fatty infiltration. Fatty acid quantification and profiling highlighted a 25-fold increase in triglycerides, a 6-fold increase in cholesterol esters, and misregulation of all other lipids classes, albeit to a milder extent. Of note, fatty acid profiles in each lipid class were altered. Microarray analysis of fatty acid metabolism pathways revealed major changes in mRNA of both liver and muscle of symptomatic mice. About 50% of the 84 genes analysed were misregulated, 25 of which were changed in both muscle and liver, while 15 and 17 changes were specific to the muscle and the liver, respectively.

Conclusion

Taken together, these results provide further evidence in metabolic defects in SMA. Further investigation will be required to establish the primary mechanism of these lipid metabolic defects and understand whether they lead to additional co-morbidities in SMA patients.

Expression of the p14 Fusion Associated Small Transmembrane Protein Improves the Oncolytic Efficacy of a Conditionally Replicating Adenovirus

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Background

Recent advances in the field of oncolytic virotherapy have sparked a number of studies aiming to design oncolytic adenovirus (Ad) that can specifically replicate in and kill cancer cells. Unfortunately, Ad has been shown to have difficulty spreading throughout a significant proportion of the tumor mass following intratumoral injection. Multiple studies have highlighted the effectiveness of viral fusogenic membrane glycoproteins (FMG) in improving the efficacy of oncolytic Ad through cell to cell fusion within the tumor and the generation of multinucleated syncytia. However, the size of typical viral FMGs makes their inclusion into an oncolytic Ad genome difficult due to viral DNA packaging size constraints and prohibits the use of other genes used to "arm" Ad, such as the tumor suppressor p53 or immune-stimulating cytokines. The p14 Fusion Associated Small Transmembrane (p14 FAST) protein is a non-structural viral fusogenic protein originating from reptilian reovirus, which is significantly smaller than typical FMGs.

Objective

Our objective is to evaluate whether expression of p14 FAST from an oncolytic Ad vector can enhance virus spread and efficacy in a multi-dose xenograft model of cancer.

Methods

We have expressed the p14 FAST protein in a conditionally replicating Ad (CRAdFAST) and tested its anti-cancer capacity in both immune competent and immune deficient mouse models of cancer.

Results

Our current results show that CRAdFAST provides some anti-cancer effect in an immune-competent mouse model of cancer using the 4T1 cell line, limited by Ad's capacity for replication in this mouse cell line. In the A549 human lung adenocarcinoma xenograft model of cancer, p14 FAST expression significantly improved CRAd's oncolytic ability in vitro and in vivo.

Conclusions

Further refinements to the CRAdFAST treatment regime based on viral burst kinetics are underway to improve overall anticancer efficacy, including a multi-dose regime to improve viral replication and spread.

Investigating the Ability of Wnt7a to Ameliorate Duchenne Muscular Dystrophy

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Background:

Duchenne muscular dystrophy (DMD) is a progressive muscle wasting disease caused by an X-linked mutation in the DMD gene encoding dystrophin. Loss of functional dystrophin leaves muscle susceptible to contractile damage and in affected individuals results in death during the second or third decade of life. Muscle stem cells termed satellite cells (SCs) are responsible for the maintenance and regeneration of skeletal muscle. Using *Myf5-Cre:ROSA-YFP* mice, where Myf5 expression leads to a permanent labelling by yellow fluorescent protein (YFP) , our laboratory showed that while most SCs are committed to the muscle lineage and have expressed Myf5 during development, a subpopulation has never expressed Myf5 and remains YFP negative (YFP-). Notably, YFP- SCs can divide symmetrically to replenish the stem cell pool or asymmetrically giving rise to one self-renewing stem cell and one YFP positive (YFP+) committed cell expressing Myf5. However, in DMD, loss of dystrophin impairs the polarity required to drive asymmetric division, decreasing the overall number of committed progenitors. Wnt7a has been demonstrated to activate the planar cell polarity (PCP) pathway by binding to its receptor Frizzled 7, to promote motility and symmetric expansion of YFP- satellite stem cells in wild-type myofibers.

Objective

Accumulating evidence supports a potential therapeutic role of Wnt7a for the treatment of DMD. Here we investigate whether the ability of Wnt7a to drive YFP- symmetric division is sufficient enough to overcome the progenitor deficit observed in DMD.

Methods

This study utilises *ex vivo* cultured extensor digitorum longus (EDL) myofibers from both wild type and *mdx* mice. Upon isolation fibers are treated with either 100ng/ml of wnt7a or vehical. Myofibers are then fixed 36h, 42h, and 72h and immunostained to determine the division orientation, type of division and proportion of differentiating cells respectively.

Results and Conclusions

We confirm that *mdx* myofibers treated with Wnt7a demonstrate an increase in the YFP- cell proportion. We the sought to determine if this increase of YFP- cells was able to increase the number of committed progenitors thus, myofibers were cultured up to 72h. Wnt7a treatement increased the number of commited progenitors by 2 fold, however desipite this increase the observed number of progenitors was still below that in untreated wild type mice. To deterimine whether this wnt7a induced progenitor increase is sufficient enough to ameliorate DMD, requires further *in vivo* studies.

Studying the role of DNA methylation on satellite cell function

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Background

The adult skeletal muscle stem cells (Satellite stem cells) is a heterogeneous population based on the expression of the transcription factors Pax7 and Myf5. Using Myf5-cre/R26R-YFP mice, it has been confirmed that a pool of satellite cells expressing both Pax7 and Myf5 undergo final muscle differentiation. In contrast, the expression of Pax7 and the absence of Myf5 expression correlate with higher self-renewal and engraftment capacity of these cells. Importantly, myogenic factor Pax7 directly activates Myf5 gene expression in satellite cells in response to Carm1 and recruitment of the histone methyltransferase complex ASH2L–MLL2 at Myf5 regulatory elements. However, the molecular mechanisms mediating the repression of Myf5 gene within the $Pax7^{+}/Myf5^{-}$ population of satellite stem cells remain unexplored.

The recent discovery of the Tet (1-3) enzyme family capable of demethylate DNA during embryonic development and the presence of the 5hmC intermediary of the DNA demethylation pathway in a variety of tissues including skeletal muscle, lead us to interrogate whether active DNA demethylation process regulates Myf5 transcription during the transition of satellite stem cells to myogenic commitment.

Objectives

To determine the DNA methylation levels of the *Myf5* locus during asymmetric division of sateliite cells and determine if Tet enzymes play a role in the *Myf5* locus demethylation pathway.

Methods

The DNA methylation levels will be assessed by methyl-DNA immunoprecipitation (MeDIP) using specific antibodies against 5mC or 5hmC. Enrichment of these marks will be analyzed at the *Myf5* locus in satellite stem cells and satellite myogenic cells previously isolated by FACS. In addition, knock-down assays of TET1, TET2 and TET3 will provide insight relative to the demethylation process of Myf5 locus during asymmetric divisions in single myofibers cultured *ex-vivo*.

Results

Preliminary data shows that the *Myf5 locus* in primary myoblast is depleted of DNA methylation. In contrast, in mouse embryonic fibroblasts, a cell-type that do not expresses *Myf5* gene, the promoter region is highly methylated. Additionally, immunostaining of Pax7, GFP and 5hmC in cultured myofibers show the presence of 5hmC within both satellite stem cells and satellite myogenic cells

Conclusions

DNA demethylation is a dynamic process occurring in proliferating satellite cells in adult skeletal muscle.

The Role of VGF in Post-stroke Neurogenesis and Recovery

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Background

The high incidence of stroke worldwide as well as the poor efficacy of neuroprotective drugs has shifted the focus of research towards therapies targeting stroke recovery and rehabilitation. VGF (non-acronym), a neuropeptide that is processed into several secreted peptides, has been identified as a post-stroke repair molecule playing a role in neurogenesis and the modulation of neuroinflammation.

Objective

In the present study, we assess the role of VGF and its secreted peptides during post-stroke neurogenesis and brain circuitry repair after ischemic brain injury.

Methods

Photothrombosis induced ischemic model: Using 1% Rose Bengal (photosensitive dye) and a collated beam of green lazer light, we induced stroke in the left frontal cortex of C57BL6 mice. VGF or a control vehicle was then adenovirally delivered to the mice two days post-stroke. The mice were euthanized for tissue collection at various time points ranging from two days to two weeks post injection.

Role of VGF in post-stroke neurogenesis: The number of newborn neurons in the periinfarct area of coronal sections were quantified using immunofluorescence. Doublecortin (DCX) labelling was used to distinguish newly generated neurons, indicative of neurogenesis.

Effects of VGF on inflammation in vivo: Neuroinflammation around the periinfarct area was also assessed using immunofluorescence. Markers of gliosis such as Allograft inflammatory factor 1 (AIF-1/Iba1) and glial fibrillary acidic protein (GFAP) were used to label microglial/macrophages in the periinfarct area.

Results

VGF treated mice displayed a greater number of DCX labelled neurons in the periinfarct area in comparison to their control littermates, indicating that there is increased neurogenesis post-stroke. VGF treated mice also displayed a greater number of $Iba1^{+}$ labelled microglia/macrophages in the periinfarct area in comparison to their wildtype littermates, which could suggest an increase in pathogen clearance

Conclusions

VGF treated mice show an increased production of newborn neurons as well as a greater recruitment of $Iba1^{+}$ microglia/macrophages to the periinfarct area post-stroke. This suggests that VGF stimulates the generation of new neurons as well as pathogen and tissue clearance through neuroinflammation. However, further investigation into the mechanism of VGF in the mediation of stroke recovery is necessary.

Investigating the Chromatin Remodeling Contributions of Snf2l in Cerebellar Cell Fate Determination

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Background and Objective

Chromatin remodeling complexes are responsible for temporal regulation of targeted genes, a subclass of which act by displacing or restructuring nucleosomes to alter DNA accessibility for transcription and expression. The Snf2h and Snf2l ISWI chromatin remodelers play a critical role in embryonic and post-natal brain development. Their importance is made evident by their involvement in many X-linked mental retardation and potential contribution to autism spectrum disorders. Murine models lacking functional Snf2h or Snf2l point to complementary activities of these remodelers; Snf2h cKO mice present a significantly reduced cerebellum, while Snf2l Ex6DEL cerebella are larger than their wild-type counterparts'. Our objective is to determine the involvement of Snf2l in regulating neurogenesis of granule neuron progenitors (GNP) through gene regulatory programs. We also aim to begin to explore their role within a number of ISWI-specific nucleosome remodelling complexes which has yet to be characterized as an additional level of ISWI chromatin remodeling throughout GNP differentiation.

Methods

Our study focuses on GNPs isolated from P5 murine cerebella. Isolated cerebella are minced, plated, and enriched for GNPs. These primary cultures are then maintained in either a proliferating progenitor state or allowed to spontaneously differentiate over a 3 day period into granule neurons. Cells are harvested at different stages for analysis by western blot or immunofluorescence. In addition, we are employing next generation sequencing (NGS) (RNA-seq and ATAC-seq) in order to dissect the role of Snf2l in maintaining the chromatin landscape.

Results

We are able to observe GNP terminal differentiation into granule neurons within 3 days *in vitro* using wild-type mouse GNPs. Immunofluorescence for Ki67 and NeuN (markers of proliferating progenitors and postmitotic neurons, respectively) as well as BrdU incorporation assays suggest that cultured GNPs from Snf2l Ex6DEL display delayed cell cycle exit and hindered terminal differentiation compared to wild-type over the course of the first 48h in culture. Furthermore, the expression of a number of ISWI interacting partners appears to be modified when active Snf2l is lost.

Discussion and Conclusion

In vivo cerebellar development abnormalities are able to be modelled and studied *in vitro* using GNP cultures. Initial observations of Snf2l Ex6DEL GNP cultures suggest a cell intrinsic differentiation defect likely contributing to Snf2l Ex6DEL cerebellar hyperplasia *in vivo*. This model is currently being used with NGS methodologies to further understand the role of the ISWI proteins in promoting neurogenesis and regulating cerebellar development.

Exosomes as a vector for Wnt7a systemic treatment in Duchenne Muscular Dystrophy

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Background: Duchenne Muscular Dystrophy (DMD) is a genetic myopathy characterized by the lack of dystrophin, evoking skeletal muscle debilitation and ultimately death. To date, no successful therapies exist to cure DMD. Our group discovered that Wnt7a, a secreted glycoprotein, represents an intrinsic mechanism for skeletal muscle regeneration. Local muscle injection of Wnt7a into dystrophic mice restores muscle function by stimulating muscle regeneration. However, given its hydrophobic nature, Wnt7a cannot be delivered systemically.

Objective: Improve the therapeutic potential of Wnt7a on Duchenne Muscular Dystrophy by validating a novel drug delivery system that will allow the systemic distribution of Wnt7a to skeletal muscle body-wide.

Methodology: Exosomes were isolated from human transfected cells using a novel method designed by our laboratory that allows for the separation of exosomal secreted Wnt7a and non-exosomal secreted Wnt7a. Endogenous secretion of Wnt7a via exosomes upon injury was studied by isolation of exosomes from muscle explants. Suitability of exosomes for Wnt7a delivery was tested by assaying exosome uptake by Fzd7-deficient muscle stem cells.

Results: After transfection of expression plasmids into human cells, Wnt7a is specifically secreted on the exosomal surface. Unexpectedly, exosomal secretion of Wnt7a is not impaired upon specific mutation of the palmitoylation sites in Wnt7a. Therefore, unlike other Wnts, palmitoylation is not required for exosomal secretion of Wnt7a. Furthermore, endogenous secretion of Wnt7a through exosomes confirmed a new intrinsic compensatory mechanism for Wnt7a delivery due to its known limitation to travel via the circulation. Results also showed that Wnt7a in human exosomes can be uptaken by murine primary myoblasts without expressing Frizzled-7 specific receptor.

Conclusions: Our experiments demonstrated that Wnt7a is efficiently delivered through exosomes into myogenic cells to elicit the regenerative response in skeletal muscle through a mechanism independent of Frizzled-7 interaction. Moreover, we found that palmitoylation is not required for secretion of Wnt7a in exosomes. This finding suggests that different mechanisms are involved in short- versus long-range Wnt signaling. Taken together, our data indicates that delivery of Wnt7a via exosomes represents a promising therapeutic avenue for system delivery for treating DMD.

Characterization of the EGFR-STAT3 signaling during muscle stem cell division in aging

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Background: Adult muscle stem cells, or satellite stem cells (SSCs), are crucial for muscle homeostasis and regeneration. Through asymmetric division, SSCs are capable of self-renewal to replenish the stem cell pool, and also produce more committed myogenic progenitors. SSCs can also perform symmetric division, giving rise to two daughter SCs of equal stemness. With aging, muscle tissue homeostasis is progressively disrupted and the regenerative capacities of SSCs markedly. Our group has identified JAK-STAT3 signaling as one of the intrinsic mechanisms underlying SSCs dysfunction in aging. In addition, in human brain tumor stem cells, EGFR-STAT3 signaling was identified to drive the expression of *Osmr* gene. Interestingly, OSMR was found to interact with EGFR and orchestrate a feed-forward signaling mechanism with EGFR and STAT3 to drive tumorigenesis.

Objective: To investigate whether EGFR-STAT3 signaling promotes asymmetric cell division through regulation of *Osmr* gene.

Methods and Results: To examine the role of OSMR in satellite stem cells, we first assessed the mRNA and protein expression of OSMR in primary myoblasts and myofibers using qPCR and immunohistochemistry, respectively. Our preliminary analysis using proximity ligation assay (PLA) showed an interaction between OSMR and EGFR in activated satellite cells. We will examine this interaction in myofibers isolated from young versus old mice. We hypothesize that the phosphorylation of the EGFR plays a role in its interaction with OSMR. Thus, we will then investigate the interaction in satellite cells with or without OSM, the OSMR ligand. Immunohistochemical analysis using phosphospecific EGFR and STAT3 antibodies will help us to determine whether OSM induces activation of EGFR and its downstream target STAT3 in young versus aged satellite cells. Furthermore, will elucidate the role of OSRM in regulating satellite cell division using *Myf5-Cre:ROSA-YFP* mice. Finally, will evaluate the role of OSMR as a regulator of EGFR-STAT3 signalling on muscle repair program *in vivo* by examining the satellite stem cell-conditional *Osmr* knock-out (KO) mice, and double *Osmr* and *Stat3* conditional KO mice. ?

Conclusions: This project will allow us to have a more comprehensive understanding of the intrinsic mechanisms underlying satellite stem cell behavior during aging and muscle dystrophies.

Defining the epigenetic regulation of satellite cells identity.

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Background. Duchenne Muscular Dystrophy (DMD) is a devastating genetic muscular disorder of childhood manifested by progressive debilitating muscle weakness and wasting, and ultimately death in the second or third decade of life. Different studies support the hypothesis that epigenetic changes due to dystrophin deficiency perturb the function of the transcriptional networks that control activation and proliferation of satellite cells and the subsequent growth and differentiation of their daughter myoblasts. Satellite cells express the paired box transcription factor Pax7, which lie genetically upstream of the myogenic regulatory factors (MRFs) MyoD, Myf5, Myogenin and MRF4. Concerted action of these factors during myogenesis, ensure proper modulation of target genes during skeletal muscle regeneration. Increasing evidence suggests that long-range interactions between enhancers and promoters as well as modifications in chromatin structure contribute to global regulation of gene expression and the execution of genetic programs. Elucidating the interactions between enhancers and their target promoters during myogenesis will allow us to address whether abnormal epigenetic regulation occurs in satellite cells isolated from mdx mice, a model for DMD.

Objectives. In this study, our aim is to identify the repertoire of enhancer elements and their target genes, which are responsible for the maintenance of cell identity in satellite cells isolated from wild-type and mdx mice.

Methods. By using a promoter capture Hi-C method and ChIP-seq; we are studying the genome-wide chromatin contacts between active enhancer elements with their target promoters in satellite cells and primary myoblasts.

Results. Preliminary data in primary myoblasts shows that promoter capture Hi-C assay on limited number of cells, provides sufficient and informative interactions between known genomic contacts. Likewise, our ChIP-seq method to identify H3K4me1, H3K4me3, H3K27Ac and myogenic transcription factors on satellite cells works successfully to generate informative analysis of global enhancer-promoter interactions during myogenesis.

Conclusions. Our initial experimental strategy, suggests that promoter capture Hi-C analysis is feasible to perform in satellite stem cells and primary myoblasts with deep resolution in order to interrogate how three-dimensional organization of chromatin affects transcription and gene expression that determines satellite cells self renewal and final differentiation.

EFFICIENT REVASCULARIZATION OF DECELLULARIZED LUNG SCAFFOLDS BY CO-DELIVERY OF iPSC-DERIVED ENDOTHELIAL AND SMOOTH MUSCLE CELLS

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Background: Patient-specific bioartificial lungs represent an alternative for organ transplantation. However, inadequate revascularization limits *in vivo* function and survival of recellularized lung scaffolds. We hypothesized that combined delivery of induced pluripotent stem cells (iPSCs) derived, endothelial cells (iECs) and smooth muscle cells (iSMCs) will increase efficiency and robustness of revascularization by taking advantage of beneficial cell-cell interactions.

Methods/Results: iSMCs were derived using an embryoid body differentiation protocol. iSMCs or pulmonary artery SMC (PASMC) were co-cultured with human umbilical vein ECs (HUVECs) in the Matrigel *in vitro* angiogenesis assay. Co-culture with either SMC type improved the persistence of EC capillary-like networks, with an optimal EC:SMC ratio of 1:3. While capillary-like networks in EC monoculture collapsed after 12 hours, they persisted for at least 36 hours in co-culture with PASMCs and more than 72hrs with iSMCs. Immunoflourescence imaging revealed a close apposition of iSMCs along the entire extent of the EC capillary-like networks. Compared to PASMCs, iSMCs exhibited higher expression of pericyte markers (CD146, PDGFR) (>95% vs. <15%, respectively) as assessed by flow cytometry. When separated by $CD144^+$ immunomagnetic-selection, ECs showed a time-dependent increase expression in endothelial (eNOS, KLF2, TIE2, CD34) and pro-angiogenic genes (VEGFA, ANGPT1), after 24-72 hours of co-culture, which was more significant with iSMCs (~4-fold) compared with PASMCs (~2.5-fold). Moreover, iPSC-derived iECs exhibited very similar cell-cell interactions in co-culture with iSMCs in the Matrigel model, again resulting in more durable capillary-like network formation. Therefore, combined delivery of iECs and iSMCs, (1:3 ratio) was used in a decellularized rat lung scaffold model, this strategy resulted in rapid and extensive revascularization which was nearly complete in 3 days.

Conclusion: Pericyte-like iSMCs interact with ECs to promote the stabilization of neovessels through structural support and upregulation of pro-angiogenic genes. Their combined delivery may thus enhance revascularization of decellularized lung scaffolds.

AAV-Sftpb Gene Therapy Rescues Respiratory Distress and Improves Survival in a Mouse Model of Surfactant Protein B Deficiency

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Background: Surfactant is a mixture of lipids and proteins produced by alveolar type 2 epithelial cells in the lung. This mixture reduces surface tension so that alveoli remain open and proper gas exchange can occur. A critical protein component of surfactant is surfactant protein B (SPB). Genetic mutations leading to SPB deficiency manifests as severe respiratory distress leading to death within 3 to 6 months following birth. Currently, no treatment for SPB deficiency exists besides lung transplantation. In this study, we target SPB deficiency in a mouse model using adeno-associated virus (AAV) gene therapy.

Hypothesis: AAV gene transfer of SPB alleviates respiratory distress and increases survival in SPB-deficient mice.

Methods: AAV serotype 6 with capsid coat 2 was generated for gene transfer of the SPB protein (AAV6.2-*Sftpb*), as this vector is effective in transducing lung epithelial cells. We utilized transgenic mice that conditionally express SPB under the control of doxycycline-supplemented feed as our animal model of SPB deficiency. In this pilot study, a 'low-dose' of 1*10^11viral genomes (vg) per mouse was delivered intratracheally to ten *Sftpb*-/- mice. Ten control mice were sham surgeries with 1XPBS injected intratracheally. The mice were maintained on doxycycline for 1 month following AAV injection to allow for maximal expression of SPB before doxycycline removal.

Results: Five days following the removal of doxycycline, all sham control mice had succumbed to respiratory failure and death or were euthanized. AAV6.2-*Sftpb* treated mice demonstrated varying degrees of improved survival with the longest surviving animal living to 39 days post doxycycline removal. Median survival of control mice was 93.3hrs compared to 121.3hrs in AAV6.2-*Sftpb* treated mice (Log-rank p=0.0067; Gehan-Dreslow-Wilcoxon p=0.0134). Lung tissues sampled from euthanized control or treated animals demonstrated normal Pro-SPC expression and almost a complete absence of SPB expression by immunofluorescence.

Conclusion: AAV6.2-*Sftpb* gene therapy represents a novel strategy to treat genetic disorders of SPB deficiency.

Future Directions: A high dose (1*10^12vg/mouse) of AAV6.2-*Sftpb* will be tested for improved survival and efficacy in alleviating respiratory distress. AAV6.2-*Sftpb* treated animals will also be harvested at various time points to determine exogenous SPB expression, improvements in lung structure, and changes to lung function in comparison to control mice.

The role of the mixed lineage leukemia mutant MLL-PTD in leukemogenesis

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Background: Acute myeloid leukemia (AML), the most common acute leukemia that affects adults, is a genetically heterogeneous disease with a poor prognosis. A mutation in the mixed lineage leukemia (MLL) gene that results in partial tandem duplication (PTD) of the N-terminal region is found in 5-10% of AML cases. MLL-PTD has been shown to be a gain of function mutation and transform committed progenitor cells into leukemic cells.

Objective: Determine the molecular mechanism through which MLL-PTD induces leukemic transformation. We hypothesize that the PTD mutation will cause deregulated binding to both co-factors and to gene-loci as a result of its two additional DNA binding domains and increased size compared to wild type MLL (MLL-WT).

Methods and Results: In order to facilitate a comparative study between the wild type and mutant MLL, we have transfected HEK293T cells with FLAG and HA-tagged MLL-WT or MLL-PTD. Expression of the tagged MLL has been validated by western blot and RTqPCR. We have performed mass spec analysis on samples from both a FLAG and HA IP and have identified candidate proteins for future analysis.

Conclusion: Following further genomic and proteomic analysis we hope to identify MLL-PTD's role in leukemogenesis and provide new candidate targets for the design of antileukemic drugs.

MiR-145-5p negatively regulates oliodendrocyte differentiation and myelin production – insights into remyelination failure in progressive multiple sclerosis

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Background/Objective: Progressive multiple sclerosis (pMS) causes formation of demyelinated lesions in the central nervous system (CNS). Demyelination leads to recruitment of oligodendrocyte progenitor cells (OPCs), which normally differentiate into mature oligodendrocytes (OLs) that regenerate lost myelin. However in pMS, recruited OPCs fail to remyelinate, ultimately leading to neurodegeneration. One characteristic of pMS lesions is abnormally high expression of miR-145-5p. In OPCs, miR-145-5p is also expressed at high levels but is strongly downregulated as differentiation is initiated. Importantly, miR-145-5p is predicted to target myelin gene regulatory factor (MYRF), a transcription factor critical for OL differentiation and myelination. The downregulation of miR-145-5p likely plays a key role as OPCs transition to OLs; thus high levels may underlie the OL differentiation block in pMS lesions. We aimed to determine if miR-145 directly targets *MYRF* and how altering its normal expression affects OL maturation, to better understand how its upregulation may contribute to remyelination failure in the pMS lesion.

Methods: CNS RNAseq database Brain RNAseq and miR targeting algorithm TargetScan were used to predict miR-145-5p targets in differentiating OLs. MYRF targeting by miR-145-5p was assessed by dual luciferase assay. Lentiviral vectors were used to overexpress and knockdown miR-145-5p in primary OPCs and OLs. Transduced cells were assessed for morphological and molecular changes by immunofluorescence. Molecular changes were further assessed by qRT-PCR and Western blot.

Results: Expression of 15 OL-specific miR-145-5p targets was assessed following both overexpression and knockdown of miR-145. Amongst these, MYRF was the most enriched factor in OLs by ~400X. Further, overexpression of miR-145-5p resulted in reduced *MYRF* expression while knockdown resulted in increased expression. MiR-145-5p was shown to directly target MYRF at two distinct binding sites. Differentiating OLs overexpressing miR-145-5p showed significant defects in branching and myelin protein expression, including MYRF. Conversely, miR-145-5p knockdown OLs displayed enhanced differentiation evidenced by increased expression of MYRF and greater myelin membrane area. Finally, miR-145-5p knockdown in OPCs led to spontaneous differentiation, underpinned by increased expression of MYRF and extension of more complex branches.

Conclusions: Taken together, these data suggest that miR-145-5p negatively regulates OL differentiation through direct targeting of *MYRF*, preventing its translation in OPCs until an appropriate differentiation signal is perceived. Overexpression of miR-145-5p, as is observed in pMS lesions, severely stunts OL differentiation, while knockdown of miR-145-5p enhances OL differentiation and leads to spontaneous differentiation of OPCs. Thus, the overabundance of miR-145-5p in the lesion microenvironment may contribute to remyelination failure in pMS, and targeting miR-145-5p might serve as a therapeutic strategy to overcome this failure.

Characterization of histone modifications associated with MyoD-bound enhancers in regenerating muscle

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Myogenesis defines the cellular processes involved in muscle formation and repair of damaged muscle tissue. The fate of satellite cell differentiation into muscle tissue is likely reliant on histone marks found at muscle-specific enhancer regions. MyoD plays an important role in establishing active enhancer regions and several of the histone modifications that define these regions are known. However, it is likely that this list is not comprehensive as the methods for discovery have thus far been limited to histone-specific antibodies. To address this gap in knowledge, an unbiased approach, the BioID2 (biometric identification) technique, will be utilized to identify histone modifications adjacent to MyoD-bound DNA to reveal the full set of epigenetic marks that define muscle-specific active enhancers. The BioID2 technique allows for the screening of protein-protein interactors and their identification through mass spectrometry (MS). The technique uses a promiscuous biotin ligase (BirA) which is fused to a protein of interest, MyoD here. In the second iteration of BioID, the BirA used (from *A.aeolicus*) naturally lacks a DNA-binding domain which minimizes biotinylation background noise. It is expected that MyoD-BirA will biotinylate histones in nucleosomes that are near MyoD binding sites throughout the genome. Histone marks, associated with biotinylated nucleosomes, will be analyzed using native Chromatin Immunoprecipitation (ChIP); the biotinylated histones will be separated using a streptavidin-bead column and identified using MS. Characterization of novel histone marks can be further explored by also identifying MyoD interacting partners. Experiments will be conducted in a mouse myoblast C2C12 FRT/TO cell line that has been transformed to overexpress the MyoD-BirA fusion protein. A mutant MyoD (A114P) that is unable to induce differentiation is used as a control. Along with a MyoD-linker (13 repeats of 'GGGGS') -BirA fusion protein which will increase the range of the BirA in order to determine if the biotinylation radius of the MyoD-BirA is appropriately capturing the entirety of MyoD interactions. This investigation will uncover key components of muscle differentiation.

Analysis of Retinal Ganglion Cell and Post-Retinal Phenotypes in Atrx- Deficient Mice

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Background: Chromatin remodeling proteins are important for retinal neuron function and survival. Our previous work has demonstrated that multiple retinal cell types are compromised when these proteins are deleted from the mouse retina as a result of targeted genetic ablation or germline genetic mutation modeling human disease. Mice with retinal Atrx deficiency exhibit impaired retinal interneuron function and cell death. However it is unknown if these defects impact visual signaling at the level of the output neurons of the retina, namely the retinal ganglion cells (RGCs), and subsequent processing or transmission of visual signals to the brain.

Objective: Here we describe our analysis of the RGC phenotype and electrophysiological function of the visual cortex in Atrx-deficient mice to gain insight into the scope of visual function mediated by Atrx-dependent chromatin remodeling and the extent of visual deficits that may affect individuals with ATR-X Syndrome.

Methods: Conditional genetic knockout approaches were used to selectively remove the Atrx chromatin remodeling factor from different retinal cell populations *in vivo*. Transgenic mice harbouring a mutation found in ATR-X Syndrome patients represent a model of disease-associated constitutive Atrx deficiency. Immunohistochemistry and fluorescence microscopy of Atrx-deficient retinal tissues was performed to evaluate and enumerate the RGC population. Retinal function was assessed by electroretinography. Visual evoked potentials were measured to examine cortical responses to visual stimuli.

Results: We observed equivalent numbers of RGCs among all models of Atrx deficiency assessed and when compared to control retina tissues. RGC viability did not depend on intrinsic Atrx expression or on extrinsic Atrx expression in pre-synaptic retinal bipolar cells. However, RGC function was diminished upon pan-retinal knockout of Atrx. Furthermore, loss of Atrx in the retina resulted in reduced visual evoked potentials elicited from the visual cortex.

Conclusions: Atrx is not required for the survival of excitatory retinal neurons, including RGCs. However, excitatory visual neurotransmission through the inner retina and onto the visual cortex is compromised in the absence of Atrx. Therefore, Atrx-mediated chromatin remodeling plays a role in the proper function of retinal bipolar cells and RGCs that impacts the propagation of visual signals within the retina and onto higher visual centres in the brain. Our findings expand the range of visual deficits that may affect ATR-X Syndrome patients and suggest that cortical dysfunction caused by Atrx deficiency extends to visual processing centres of the brain.

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).

Methods

Primary OPCs were isolated from the cortex of P0 wild-type and C3aR-KO 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. Wild-type 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 ($p<0.05$). However, after 4DIV the treated and control cultures showed no significant difference in membrane area or MBP expression by western blot. C3aR-KO cultures produce myelin earlier than their wild-type counterparts, and pro-differentiation effects of TLQP-21 neuropeptide on wild-type cultures were further amplified in C3aR-KO cultures, 2DIV ($p<0.05$). Similarly, after 4DIV cultures showed no significant difference in cell complexity, membrane area, and MBP expression by immunofluorescence

Conclusion

TLQP-21 neuropeptide treatment may accelerate the differentiation process of OLs. Further studies will aim to confirm these results and define the pathways involved.

Therapy with Exogenous Endothelial Colony-Forming Cells Prevents and Rescues Monocrotaline-Induced Pulmonary Hypertension and Hypoplasia in Neonatal Rats

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Background and Objective: Congenital diaphragmatic hernia (CDH) is a life-threatening malformation occurring in 1-4/10,000 live births. The degree of pulmonary hypoplasia and hypertension - resulting from the impaired development of the lung's parenchyma and vasculature - determines outcome. Current treatment strategies are insufficient in promoting overall lung growth. Evidence suggests that angiogenic growth factors drive lung development. Endothelial colony-forming cells (ECFCs) represent a subset of vascular progenitors capable of self-renewal and de novo vessel formation. We hypothesized that exogenous supplementation of endothelial colony-forming cells to CDH lungs will restore their disrupted vascular - and intertwined alveolar - growth.

Methods and Results: Treatment of neonatal rats with the phytotoxin monocrotaline (MCT) at postnatal day 6 (PN6) resulted in disrupted alveolar and lung vascular growth as well as pulmonary hypertension. This was associated with an impaired function of lung-resident ECFCs as evidenced by their reduced proliferation, colony forming capacity and endothelial network formation. Umbilical cord blood-derived ECFCs were intravenously injected into immunocompromised neonatal MCT rats at PN7 (prophylaxis) or PN14 (rescue). Both strategies resulted in a significant improvement of alveolar and vascular growth and attenuated pulmonary hypertension.

Conclusion: The impaired function of lung-resident ECFCs in growing rats upon MCT-treatment may contribute to the pulmonary hypertension and arrested alveolar development. Therapy with exogenous ECFCs may offer new treatment strategies for CDH patients or other patients suffering from PH -both neonates and adults. Future research will provide mechanistic insight in their – presumably - paracrine mode of action.

Investigating heterogeneity in the muscle satellite stem cell population

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Background: Muscle stem cells provide the regenerative potential for skeletal muscle throughout adult homeostasis, while deficiencies in its maintenance may be the etiology of muscle diseases, such as Duchenne Muscular Dystrophy. Understanding the regulators of this stem cell population is therefore essential for cell therapies. One such regulator is Pax7, which uniformly marks muscle stem cells and is essential for muscle regeneration. However, the bulk population of Pax7+ muscle stem cells is highly heterogeneous. For instance, our laboratory has shown that within the Pax7+ population, the majority expresses *Myf5* (Pax7+/Myf5+), while a small subset (Pax7+/Myf5-) does not and retains greater stem cell characteristics. Moreover, stratifying the population between $Pax7^{Hi}$ vs $Pax7^{Low}$ expression reveals differences in quiescence. Therefore, my project is to uncover other differentially expressed genes that provide further understanding into stem cell maintenance and quiescence by using single cell sequencing technologies.

Objective: Uncovering subpopulations within the heterogeneous population of Pax7+ muscle stem cells and identifying their regulators.

Methods: Pax7+ muscle stem cells were sorted by fluorescence-activated flow cytometry (FACS) and captured with the Fluidigm C1 platform. Single cell cDNA libraries were assayed on the Fluidigm BioMark HD platform by qPCR.

Results: We have optimized the protocol for the Fluidigm C1 platform for proper capture of live single Pax7+ cells. Using 21 prepared cDNA libraries, we tested 94 candidate TaqMan qPCR probes on the BioMark HD platform. We have detected some differentially expressed genes, such as *bmp4* and *foxo4*. More cells will need to be captured and processed to gain bioinformatic statistical power.

Conclusion: As previously reported, within the bulk population of Pax7+ muscle stem cells, heterogeneities exist. Future directions include proceeding with mRNA sequencing of single cell libraries captured on the Fluidigm C1 platform.

Identification of cellular proteins involved in regulating the adenovirus nucleoprotein structure

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Background and Objectives: Human Adenovirus (HAd) infections are common worldwide and typically manifest as infections of the respiratory tract, gastrointestinal tract and mild infections of the eye (conjunctiva). Most infections are self-limiting in immunocompetent individuals, but certain populations – such as children and immunocompromised patients, are more susceptible to severe disease. To better understand HAd infection and elucidate new treatment options, we are investigating the protein-nucleic acid structure of its genome in the host cell. Within the infecting virus particle, a virus-encoded protein called protein VII tightly condenses the HAd DNA. Once the viral DNA enters the infected cell nucleus, protein VII is removed and replaced by cellular histones, an event that is crucial for efficient virus replication. However, the mechanism in which this gene modeling occurs is not well understood. We believe that elucidating the cellular proteins involved in this process may identify new therapeutic targets to attenuate HAd replication, and thus limit HAd-induced disease. As a first step, we will identify novel cellular proteins that interact with protein VII.

Methods: HAds containing epitope tags on protein VII were constructed in the lab. These tags were used to easily purify the protein along with interacting cellular proteins. Mass spectrophotometry was used to determine the identity of protein VII-interacting proteins. Bioinformatics analysis was used to generate a protein VII interactome.

Results: A total of 2000 cellular proteins were identified that co-purified with protein VII. These proteins were involved in normal cellular functions such as post translation modifications, nuclear import and DNA replication. We are currently validating several of these interactions, and evaluating the significance of these cellular proteins in HAd replication to determine if they represent novel targets for therapeutic intervention.

Conclusions/Discussion: We have identified a number of cellular proteins that interact with HAd protein VII that may play a role in transitioning the viral DNA into its active replicative state within the infected cell nucleus. Elucidating the function and importance of these proteins in the Ad lifecycle may identify novel targets to attenuate Ad-induced disease in patients.

Genetic and phenotypic evaluation of two dystonia musculorum alleles of varying disease severity.

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Recently it was discovered that the cause of a newly identified lethal sensory neuropathy (HSAN-VI) is a mutation in the human dystonin gene. These patients present with joint contractures, problems eating and breathing, motor deficits, and autonomic irregularities that ultimately leads to death before the age of two. Similarly a mouse disease known as *dystonia musculorum* (*dt*) also arises due to mutations in the mouse dystonin gene. Sensory neurons are particularly vulnerable to the loss of neuronal dystonin, as these are the primary cell type to show molecular defects and undergo degeneration. As such, the mice exhibit ataxia around P7-P10, and death results around 3 weeks of age. Several different alleles of *dt* exist, though the mains ones that we work with in our lab are $Dst^{dt\text{-}27J}$ and $Dst^{dt\text{-}Tg4}$. The $Dst^{dt\text{-}27J}$ mouse has typically been known as the more severe, as the mutation likely affects all 3 neuronal dystonin isoforms and the mice die before P21. Conversely, $Dst^{dt\text{-}Tg4}$ mice have a mutation that affects only the first two isoforms of dystonin, leaving the third dystonin isoform intact, and these mice typically live a few days beyond P21.

Here we characterize differences in phenotype and pathology between the $Dst^{dt\text{-}27J}$ and $Dst^{dt\text{-}Tg4}$ alleles. Defects in microtubule stability, which were previously thought to be central to *dt* pathology, were found to only be present in $Dst^{dt\text{-}27J}$ sensory neurons. This likely indicates that microtubule instability contributes to disease severity, but is not central to pathogenesis. We then explored the compensatory potential for the third neuronal dystonin isoform in $Dst^{dt\text{-}Tg4}$ sensory neurons. Surprisingly we did observe a tissue dependent increase in mRNA expression for this isoform, which highly suggests that the third neuronal dystonin isoform compensates for the loss of the other two by perhaps slowing disease progression. Further analysis will be required to establish a direct link between this isoform and microtubule stability.

Exosomes, a novel biomarker for spinal muscular atrophy

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Background: Spinal muscular atrophy (SMA) is a rare but devastating neuromuscular disorder that is often fatal in children. Hallmarks of SMA include the loss of motor neurons and progressive atrophy of muscle, but more recently has shown to effect peripheral organs such as the liver, kidneys and pancreas. While Spinraza, a newly FDA approved treatment for SMA, offers a potential treatment option for patients, there is currently a lack of an efficient, inexpensive tool to measure disease state or treatment progress. Cells release a variety of extracellular vesicles ranging in size including microparticles and exosomes. Research has shown that serum-derived vesicles can be an indicator for an assortment of disease states including various forms of cancer, neurodegenerative diseases and organ failure.

Objective: To investigate exosomes as a potential biomarker for SMA.

Methods: Exosomes were isolated from cell culture, serum from an intermediate mouse model of SMA (WT, $SMN^{2B/+}$, $SMN^{2B/-}$), and human serum using the commercial kit Exoquick, or differential centrifugation. Size and concentration of exosomes were determined using Zetaview nanoparticle tracking and electron microscopy. Exosome markers (Alix, Flotillin and Tsg101) and exosomal SMN protein were examined using immunoblot.

Results: Investigation of exosomes derived from various cell culture models, mouse and human serum, demonstrated that SMN protein was not only found to be located within exosomes but the levels of exosomal SMN protein was dependent on the cell of origin and the disease state of the cell, animal or patient. Furthermore, cell culture and animal models of SMA, in addition to patients of SMA display higher levels of circulating exosomes within media or serum in comparison to their normal controls.

Conclusion: SMN protein exists in exosomes from all cell sources tested to date, and the levels of SMN protein are decreased in cell culture and animal models of SMA, in addition to patients of SMA. The SMA disease state also leads to enhanced levels of available exosomes within media and serum, suggesting potential deficits in exocytosis or endocytosis machinery. Future work will examine the ability to correct the exosome defect by delivery of SMN.

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.)

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TAL1 interferes with the T cell regulatory function of BCL11B to mediate leukemic transformation in T-ALL

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The transcription factor TAL1 is a master regulator of hematopoiesis where it is essential for specification and self-renewal of hematopoietic stem cells and differentiation of erythroid cells and megakaryocytes. In contrast, TAL1 is turned off early in T-cell progenitors and when aberrantly expressed, it induces T-cell acute lymphoblastic leukemia (T-ALL). It has been previously shown that the leukemic function of TAL1 entails inhibition of both T-cell differentiation and apoptosis. However, the molecular mechanism(s) through which TAL1 induces and maintains a leukemic phenotype is not entirely clear. While TAL1 has been shown to directly regulate the expression of genes that control proliferation, apoptosis and differentiation, other non-mutually exclusive models have been proposed to explain the oncogenic role of TAL1, including TAL1-mediated interference with critical T-cell regulators (e.g. E proteins). Through a proteomic screen for TAL1-interacting proteins in T-ALL we identified the transcription factor BCL11B, a major T-cell regulator for lineage commitment, survival and differentiation of T cells, as a new interacting partner of TAL1. This suggested that the oncogenic function of TAL1 might be mediated, at least in part, through interference with the T cell regulatory function of BCL11B. To study this hypothesis, we combined knockdowns of TAL1 and BCL11B with ChIP- and RNA- sequencing analyses, which revealed that TAL1 directly activates expression of BCL11B and over 60% of BCL11B binding sites are also targeted by TAL1. Although TAL1 and BCL11B display some antagonistic functions in the regulation of their common target, the two transcription factors act together to directly block T cell differentiation and functions, and apoptosis, while activating cell cycle. Indeed, TAL1-induced leukemia cells undergo apoptosis upon BCL11B knock down in vitro and fail to engraft and expand in xenograft mouse models, in vivo, demonstrating that in T-ALL, the oncogenic factor TAL1 subverts the function of the T-cell regulator BCL11B to maintain leukemic cell proliferation. To address the question whether BCL11B is also required for TAL1-mediated arrest of differentiation in early T-cell progenitors, we developed a human-derived ex vivo T-cell differentiation protocol to ectopically express TAL1 in early T-cell progenitors. Consistent with our hypothesis, aberrant expression of TAL1 led to an arrest of T-cell differentiation in early progenitors. Surprisingly, when BCL11B is knocked down in T-cell progenitors, TAL1 is no longer able to block their differentiation, revealing that BCL11B is required for TAL1-mediated arrest of T-cell differentiation. In conclusion our results show that in T-ALL, TAL1 deregulates the function of a transcription factor that is essential for the commitment of hematopoietic progenitors to the T-cell lineage, highlighting an important contribution of the transcriptional interference model in the overall mechanism of TAL1-mediated leukemogenesis.

Generating Human Satellite-like Cells from Induced Pluripotent Stem 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

Episomal reprogramming of DMD patient and healthy donor cells was used to generate iPSC lines that were subsequently differentiated down the myogenic lineage to generate satellite-like cells. The gene and protein expression profile of cells undergoing myogenesis were assessed at several stages of the differentiation protocols with immunofluorescence staining and qRT-PCR. Muscle progenitors from embryonic stem cells and donor iPSCs were transplanted into cardiotoxin-injured tibialis anterior (TA) muscles of immunocompromised mice. Human cell contribution to engraftment and localisation to the muscle satellite stem cell niche were assessed by immunohistochemistry on TA cryosections. Mouse hind limb irradiation was performed to reduce the inherent satellite cell pool and providing a more favourable environment for human progenitor cells to engraft and contribute towards regeneration and the satellite cell pool.

Results

Myogenic progenitors were generated using three 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 somitic and 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.

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.

Investigating the Role of Negative Elongation Factor in Muscle Regeneration

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Progression of skeletal muscle degenerative diseases such as muscular dystrophy and sarcopenia greatly reduce the quality of life of affected individuals, where a gradual increase in muscle wasting results in increased weakness and associated complications. Although there are no current therapies to treat these diseases, one possibility is the development of drugs that induce endogenous satellite cell to promote regeneration of damaged tissue. Doing so requires that we understand how to regulate these changes in cellular fates that modulate conversion of muscle stem cells into functional myofibers.

Extensive analysis in transcriptional regulation has shown that Negative ELongation Factor (NELF)-mediated Promoter Proximal Pausing of RNA Pol II plays a key role in regulating gene expression in response to specific stimuli. Here, an active RNA Pol II is maintained in a paused state near the transcription start site by association with NELF and DRB Sensitive Inducing Factor (DSIF). Pause release occurs upon phosphorylation of NELF, DSIF, and the RNA Pol II CTD by the positive Transcription Elongation Factor b (pTEFb). This allows rapid gene expression in response to specific stimuli, as studied in many systems ranging from heat shock proteins to promote the release of immediate early genes in neurons. However, the role of NELF in regulating muscle regeneration has never been examined. Our goal here is to determine the role for NELF-mediated transcriptional pausing in regulating satellite cell mediated muscle regeneration.

To achieve this goal, I am using a satellite cell specific, inducible NELF-B knockout mouse model to study regeneration in the absence of NELF. Cardiotoxin induced injury of the TA muscle have shown that NELF is required for efficient muscle regeneration. Using cultured NELF-B KO satellite cells further shows that NELF plays a critical role in regulating transitions of myogenic cellular states, which is characterized by a proliferation defect seen through an inability to effectively expand their population when compared to control populations. In addition, I observed that knock-down of NELF-B in cultured myoblasts demonstrate a reduced ability to differentiate into myotubes. Based on these studies, my results suggest that NELF plays a critical role at several stages of satellite cell mediated regeneration.

MYST1 acetyltransferase plays a role in regulating 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 essential for its transcriptional activity and function in satellite cells. We are interested in understanding if PAX7 displays other post-translational modifications necessary for its function. By mass spectrometry using immunoprecipitated FLAG-PAX7, we identified two lysine residues (K105 and K193) within the PAX7 protein that are acetylated. 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 created Pax7 mutants (K105R, K193R) were introduced. This suggests that acetylation plays a role in PAX7 transcriptional activity.

Objective

To identify PAX7 acetyltransferase and characterize the function of PAX7 acetylation.

Methods

Co-immunoprecipitations, siRNA knockdown, qPCR and myofiber experiments.

Results

To find PAX7 acetyltransferase, we used a candidate approach. Nine acetyltransferase we chosen according to their interaction with PAX7 known partners or their ability acetylate other PAX proteins. We detected an interaction between PAX7 and MYST1 one of the candidates, by co-immunoprecipitation using overexpressed proteins. MYST1 is expressed in muscle satellite cells and is known for its interaction with Wdr5, a known PAX7 partner. MYST1 also interacts with PAX7 in primary myoblasts using co-immunoprecipitation. MYST1 knockdown decreases PAX7 acetylation status, suggesting that MYST1 could acetylate PAX7. MYST1 siRNA knockdown negatively impacts PAX7 target genes expression, as well as primary myoblasts proliferation, is decreased by half compared to scrambled control, suggesting the importance of MYST1's role on PAX7 activity. Moreover, primary myoblasts treated with siMYST1 express higher levels of later myogenic transcripts (MyH1, MyH2, MyoD). Interestingly, MYST1 seems to play a role in satellite cell proliferation. MYST1 depletion through siRNA knockdown causes a 5 fold reduction of satellite cell number as well as a slight increase in MyoD positive cells at 72hrs, compared to scrambled control.

Conclusion

In all, MYST1 modulating PAX7 activity through acetylation represents a novel mechanism in muscle stem cell biology.

ATAC-Seq Reveals Differential Chromatin Landscape in mdx Satellite Cells

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Background

Satellite cells (SCs) exist as a heterogeneous population where the self-renewing stem cells express the transcription factor Pax7 but not Myf5 and committed progenitors express both Pax7 and Myf5. Induction of Myf5 expression 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. MDX SCs, used as a model for Duchenne Muscular Dystrophy, show reduced Myf5 expression while MDX myoblasts display reduced H3K4me3 marks at the Myf5 promoter region. Furthermore, studies on satellite cells using electron microscopy revealed a vastly different chromatin state between WT and MDX SCs, whereby MDX SCs displayed higher amount of euchromatin compared to WT.

Objective

Given the differences in H3K4me3 and the higher order chromatin structure between WT and MDX, we want to discover the differences in chromatin accessibility between both in myoblasts and satellite cells.

Methods

To investigate genome-wide chromatin accessibility, we used the Assay for Transposase-Accessible Chromatin with high throughput sequencing (ATAC-Seq). ATAC-Seq was performed in duplicates on satellite cells isolated from the hind limbs of 6-8 weeks old WT and MDX mice. ATAC-Seq was also performed in duplicates on WT and MDX primary myoblasts cultured for less than 6 passages. ATAC-Seq libraries were quality controlled by agarose gel as well as qPCR looking for enrichment of known open regions. Around 65 million reads were obtained per sample on average using Illumina's Next-Seq platform. Reads were mapped to mm10 using Bowtie. PCR duplicates, non-mappable reads and reads mapped to mitochondrial DNA were filtered. Peaks were called using MACS2. Furthermore, DESeq2 was used to calculate fold enrichments between peaks of different samples.

Results

ATAC-Seq revealed enormous differences between WT and MDX SCs and myoblasts, with only around 30% of peaks being common across all conditions. Interestingly, very few of the differentially accessible regions are at TSS. Most of the variation are in distal regions. Performing region-gene association analysis, we can identify potential pathways which are affected. Preliminary analysis indicates MDX SCs display increased accessibility at regions associated to genes involved in phosphorylation. Among the regions displaying reduced accessibility in MDX satellite cells are genes involved in satellite cell differentiation, supporting years of research indicating impaired muscle regeneration in MDX mice.

Conclusion

This research will allow us to better understand the global dysregulation in MDX mice and possibly find novel mechanisms by which to ameliorate the DMD phenotype.

Identification of novel pharmacological targets and small molecules inhibiting adenovirus replication

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

During an infection, the structural proteins are removed from the Ad virion and the viral DNA enters the host cell nuclei. Within a few hours, Ad DNA associates with cellular proteins including histones. This assembly of the viral genome into a protein-associated, nucleosome-like structure is required for efficient expression of viral 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 viral genome infected cells.

Objective: To identify novel small molecules inhibiting Ad replication and to investigate the underlying molecular mechanism of the inhibition, including effects on viral DNA association with host proteins.

Methods: We have generated a wild-type-like Ad construct encoding the red fluorescent protein (RFP) within the viral genome. RFP from this construct is only expressed following Ad DNA replication, which allows us to effectively monitor virus replication. Using this construct, an efficient method was designed to screen small-molecule libraries to identify novel inhibitors of Ad. Compounds that significantly reduce RFP levels in the screen are further investigated.

Results: Through a preliminary screen, we identified the histone deacetylase (HDAC) inhibitor vorinostat (an FDA-approved anticancer drug) as a potential inhibitor of Ad. Further assays revealed that vorinostat significantly delays the onset of virus replication, and that this is likely mediated through the inhibition of HDAC2 activity. Further elucidation of the underlying mechanism and *in vivo* studies are underway. We also screened the Prestwick Chemical Library and the Cayman Epigenetics Screening Library. Several positive hits identified from these libraries have been verified to decrease Ad gene expression and DNA replication in follow-up studies.

Conclusion: We have identified several FDA-approved compounds as potential inhibitors of Ad and we are conducting follow-up studies to verify their effect on virus replication, cell viability, etc. 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.

PAX7 function in satellite cells is regulated by acetylation

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Satellite cells are the stem cells responsible for muscle regeneration upon injury. The understanding of 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. However, how PAX7 itself is regulated remains unclear. By mass spectrometry, we determined that PAX7 is acetylated on two conserved lysine residues. Acetylation positively regulates PAX7 transcriptional activity and chromatin binding, as demonstrated by luciferase assays using a *Myf5* reporter, as well as by chromatin immunoprecipitation. Acetylation does not impact PAX7 protein stability, its nuclear localization or its capacity to mediate protein-protein interactions. We are now analyzing the effect of PAX7 acetylation on satellite cell and muscle biology, using mice in which PAX7 acetylation site is mutated (CRISPR/Cas9-mediated lysine to arginine mutation). Preliminary results from luciferase assays also suggest that acetylation regulates PAX7-FOXO1 activity, a fusion oncogenic protein detected in rhabdomyosarcomas. We identified one deacetylase (SIRT2) involved in PAX7 regulation. Its interaction with PAX7 was confirmed by immunoprecipitation in myoblasts. Decreasing Sirt2 levels by RNA interference influences the expression of different PAX7 target genes including *Myf5*. In keeping with our hypothesis, immunoprecipitation experiments suggest that SIRT2 regulates the acetylation status of PAX7. Finally, muscle fibers treated *ex vivo* with siSirt2 show an increase in satellite cell commitment compared to control. Interestingly, siPax7 has the opposite effect on satellite cells, concordant with Sirt2 being a negative regulator of PAX7. Therefore, SIRT2 appears as a strong candidate for the regulation of PAX7 acetylation and function in satellite cells.

Determination of immunomodulatory bioactivity biomarkers in Wharton's Jelly MSCs

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Background
Detrimental immune and inflammatory responses contribute to various debilitating and potentially fatal conditions, including Crohn's disease, myocardial infarcts, and sepsis. Yet, effective treatments for many of these disorders are lacking. Mesenchymal stromal cells (MSCs) are emerging as a promising cellular therapy for various immune and inflammatory disorders because of their immunomodulatory functions, including T cell, NK cell, and B cell suppression. However, clinical trials that have employed MSC cellular therapies to treat inflammatory diseases produced inconsistent results. These discrepancies could stem from the heterogeneity within MSC populations, as well as the loss of immunosuppressive properties during the culture of MSC samples. Moreover, the lack of reliable biomarkers to characterize MSC immunomodulation complicates the identification and bioprocessing of effective therapeutic MSC populations for cellular therapies. We hypothesize that the immunomodulatory function of Wharton's Jelly MSCs (WJ-MSCs) can be accurately predicted through the detection of correlative immunomodulatory biomarkers.

Objective
To identify MSC immunomodulatory biomarkers and gain mechanistic insights into their immunomodulatory bioactivity. Ultimately, we plan to integrate metrics for immunomodulation into a pipeline to identify, isolate and manufacture WJ-MSCs with robust immunomodulatory function for use as a cellular therapy for immune and inflammatory diseases.

Methods
We performed an unbiased omics analyses on WJ-MSC lots with strong and weak immunomodulatory function. Mass spectrometry was then conducted on cell surface and secreted proteins from differing immunomodulatory WJ-MSCs lots. RNA sequencing was also conducted on three types of RNA: mRNA, microRNA (miRNA), and exosome isolated miRNA. Correlation and enrichment analysis using tools such as GSEA and Gene Ontology was conducted to determine biomarkers, as well as molecular and biological functions associated with immunomodulatory activity. Statistically significant biomarkers will be validated using qPCR and Western blotting.

ResultsThrough MLR and macrophage assays, we found WJ-MSC primary cell lines with greatly varying immunomodulatory abilities. Transcriptomic and proteomic analyses were conducted on these lines to identify potential biomarkers and gain mechanistic insights into MSC immunomodulation. Moreover, we have established a protocol to isolate and examine the secretome of immunomodulatory MSCs to select candidate targets that drive immunomodulatory function. Statistically significant biomarkers and functional terms have been identified and are currently being characterized and validated.

Conclusions
MSC populations possess differing immunomodulatory ability. Moreover, this heterogeneity in MSC immunomodulation may be correlated with differential protein expression.

Periostin is required for pancreatic stellate cells to induce proliferation in human islets.

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Background
Previously we found the secreted protein periostin was highly expressed in the mouse pancreas following partial pancreatectomy. Periostin expression was localized to regenerating areas containing mesenchymal stroma and proliferating tubular complexes. Moreover, periostin deficient mice exhibited impaired mesenchymal formation and reduced regeneration specifically within the pancreatic β-cell compartment. Notably, injection of periostin directly into the mouse pancreas stimulated proliferation of pancreatic stellate cells to form a mesenchymal stroma and induce pancreatic regeneration.

Objective
To determine if periostin and pancreatic stellate cells induce regeneration in human islets as we have observed in the mouse.

Methods
Pancreatic stellate cells isolated from periostin deficient and wildtype mice were co-cultured with human islets. Proliferation was quantified by assessment of the incorporation of EdU. Human islet cells were identified by expression of human nuclear antigen (HNA), beta cells by insulin, alpha cells by glucagon and ductal cells by cytokeratin 19. Co-cultures were blinded prior to quantification of proliferative clusters.

Results
Proliferation was increased in insulin expressing human beta cells when co-cultured with human or mouse pancreatic stellate cells. When whole human islets were co-cultured on pancreatic stellate cells proliferative clusters were present that did not express the alpha cell marker glucagon but did express low levels of insulin and the pancreatic ductal marker cytokeratin 19. Moreover, proliferation was reduced when the co-cultures were performed with pancreatic stellate cells that did not express periostin. Periostin-deficient pancreatic stellate cells had reduced expression of the activation marker CD140a. Furthermore, CD140a+ pancreatic stellate cells purified by fluorescent-activated cell sorting (FACS) had an increased ability to induce proliferation in human islets.

Conclusion
Pancreatic stellate cells induce proliferation in human pancreatic ductal and beta cells. In addition, periostin increases the activation of pancreatic stellate cells to increase proliferation within human islets.

A Novel Model of Pulmonary Arterial Hypertension Induced by Endothelial-Targeted Diphtheria Toxin Injury

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Introduction

Endothelial cell (EC) apoptosis is increasingly recognized as a central trigger for pulmonary arterial hypertension (PAH), although the mechanisms by which endothelial injury leads to the development of the characteristic hemodynamic and pathological features of this disease are not well understood. Therefore, we developed a model to induce selective endothelial injury in response to diphtheria toxin (DT) in mice by driving expression of the DT receptor, which is not normally expressed in rodents, using an EC specific promoter. This model was used to test the hypothesis that EC apoptosis is sufficient to induce the full PAH phenotype, and to explore the downstream consequences of endothelial injury.

Methods and Results

Mice expressing CRE under the VE-Cadherin promoter were crossed with animals harboring the human DT receptor (DTR) gene flanked by LoxP sites. Exposure of 8-week old binary transgenic (BT) animals to DT (1-10ng/g) resulted in rapid lethality with all animals succumbing within 3 days of single IP injection, whereas no mortality was seen in single transgenic (ST) mice receiving up to 10 ng/g of DT. Necropsy examination of DT-treated BT mice showed extensive hemorrhage and edema involving mainly the lung and the gastrointestinal tract. Administration of DT at doses of ≤0.1ng/g either as a single dose, or 4 weekly IP injections, was well tolerated with no mortality. At 5 weeks, DT treatment resulted in increased RVSP and RVH in BT mice (n=19) when compared to ST control animals (n-22): 27±0.9 vs. 23±0.6 mmHg ($p<0.01$) and 0.251±0.005 vs.0.238±0.004 ($p<0.05$), respectively. Interestingly, the increase in RVSP in response to DT was greater in male compared to female BT mice (29±1 vs. 24±1 mmHg, $p<0.01$) and weekly dosing of DT had no additive affect compared to a single injection (27±1 vs. 27±2 mmHg).

Conclusions

We have demonstrated that direct EC injury and apoptosis is sufficient to induce a PAH phenotype in BT-DTR mice. This model will be used to define the role of subsequent degenerative, proliferative and inflammatory sequalae in the development of progressive PAH, and thereby provide novel insights into mechanisms and potential therapeutic targets for the treatment of this disease.

Epigenetic regulation of lipid metabolism in determining neural stem cell fate

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Background: Epigenetic modifications integrate environmental signals to determine stem cell identity and/or its lineage commitment. However, how these epigenetic pathways are triggered, remains largely unknown. To this end, we recently identified an atypical protein kinase C- CREB binding protein (aPKC-CBP) pathway, as a homeostatic compensatory mechanism that helps maintain a sustained rate of hippocampal neuronal differentiation and maturation, and hippocampus-associated memory during normal aging. Previously, we have also shown that the aPKC-CBP pathway, which can be stimulated by metformin, a FDA-approved drug, selectively promotes neuronal differentiation of adult neural stem/precursor cells (NPC) *in vitro*. We have now identified monoglyceride lipase (Mgll) as the direct downstream target of this pro-differentiation pathway.

Objective: The objective of our current research is to elucidate a novel epigenetic pathway that regulates Mgll expression, and consequently lipid composition, to alter NPC fate in the adult brain.

Methods/Results: We used *in vitro* neurosphere culture assay with sub-ventricular zone (SVZ) derived NPCs from the *cbp*S436A transgenic mice, which lack a functional aPKC-CBP pathway, and observed significant neuronal differentiation deficits, that occurred concurrently with elevated Mgll levels, in differentiating NPCs. These differentiation deficits could be rescued by genetic knockdown of Mgll, as well as, by a blockade of its activity. We were also able to successfully rescue hippocampal neurogenesis deficits in the *cbp*S436A mice *in vivo*, through Mgll inhibition.
Additionally, we found that successive neurosphere/NPC passaging, was able to activate the aPKC-CBP pathway, independent of metformin stimulation, thus establishing another *in vitro* model to study the pathway. Interestingly, we have also observed that the late passage *cbp*S436A NPCs, mimic early passage NPCs from 3xTg-AD mice, a human AD mouse model, in terms of both, aPKC activity and Mgll levels.

Conclusion: In summary, the aPKC-CBP pathway is an epigenetic switch that directly regulates the expression of Mgll to control lipid metabolism and NPC fate in the adult brain.

XIAP gene therapy in a mouse model of glaucoma

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Background: Glaucoma is a prevalent retinal neurodegenerative disease that is characterized by visual field loss and eventual blindness. The primary cause of glaucoma is elevated intraocular pressure (IOP) which results in the damage and death of retinal ganglion cells (RGCs) as well as their axons. The endpoint of the disease is the death of these cells by apoptosis, therefore, blocking the activation of apoptosis may be an effective therapy.

Objectives: We plan to target apoptosis through the administration of X-linked inhibitor of apoptosis (XIAP), a potent caspase inhibitor that has previously been shown to be neuroprotective in various in vitro and in vivo models of retinal cell death.

Methods: We will be assessing the effects of XIAP in a previously established magnetic microbead model of glaucoma. In this model, microbeads are injected into the anterior chamber of the eye, blocking the trabecular meshwork and preventing the aqueous humour outflow, leading to an increase in IOP. We will test the effects of XIAP (with GFP as an injection control) on glaucoma progression. Control groups will be normal or glaucomatous animals. Following glaucoma induction, the mice will be monitored to detect changes in IOP, retinal morphology and visual function. After six weeks, eyes and optic nerves will be sampled to analyze RGC survival and conduct axon counts respectively.

Results: We have conducted two preliminary experiments in order to refine our experimental procedure, both monitored for six weeks as described above. In the first experiment, seven animals were unilaterally injected solely with beads. We saw some trends in functional decline in the beads-injected animals compared to the controls, but our experimental groups were not large enough to come to any significant conclusions. In the second experiment, five animals were unilaterally injected with both beads and either GFP or XIAP. We noticed a significant increase in IOP in the GFP+beads injected eyes two weeks post-beads surgery that remained elevated until four weeks post-surgery. Although not statistically significant, XIAP+beads injected eyes displayed a declining trend, recording lower IOPs than their control counterparts, suggesting that XIAP may be able to lower IOP.

Conclusions: The previous experiments have helped to fine-tune our techniques, but the sample sizes were too small to come to any definitive conclusions. We are currently optimizing the beads-injection technique and upon optimization, we can assess XIAP's neuroprotective effects in a series of definitive experiments with larger experimental groups.

Role of LYL1 in the maintenance of early T-cell acute lymphoblastic leukemia

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Background:
Early T-cell Precursor Lymphoblastic Leukemia (ETP-ALL) is considered to be a subtype of T-cell Acute Lymphoblastic Leukemia, which shows poor prognosis and high-risk side-effects under the traditional chemotherapy. Recent studies indicated that in ETP-ALL the tumor-initiating cells had a higher expression of oncogenic transcription factor gene LYL1, and its mechanism still need further investigation. Epigenetics is possible to regulate the epigenome through pharmacological approaches to eliminate cancer cells or sometimes to enhance the properties of particular stem cells, which shed light on the epigenetic treatment on specific type of T-ALL.

Objective:
By targeting the transcription factors LYL1 at the origin of leukemia through inhibition of their associated epigenetic cofactors, we may be able to eradicate leukemia cells.

Methods:
Human T-ALL cell lines were purchased from American Type Culture Collection and cultured in RPMI 1640 supplemented with 10% FBS and 100 U/L penicillin and 100 mg/ml streptomycin. For mass spec (MS) identification, silver-stained proteins were excised from the gel, digested with trypsin, and analyzed by liquid chromatography (LC)-tandem MS (MS/MS) on a linear trap quadrupole -Orbitrap XL mass spectrometer with a nanospray source and Surveyor high-performance liquid chromatography. GSK-J4 (UTX inhibitor) was obtained from Cayman Chemicals (no. 12073) and used at the concentrations indicated.

Results:
The expression of LYL1 is high in the development of loucy cells compared with other types of cell lines. MS-spec results show that LYL1-IP in Loucy cells has identified 1534 interacting proteins, and 272 proteins are supposed to be uniquely interacted with LYL1. Among 272 proteins, UTX is identified to be interacted with LYL1, inhibition of UTX by GSK-J4 can inhibit the normal growth of loucy cells.

Conclusions:
The preliminary data indicates that by using MS we have identified 272 proteins which are uniquely interacted with LYL1 in Loucy cells, it still need to find another interesting proteins interacting with LYL1. Base on the transcription factors LYL1 in the ETP-ALL model, the epigenetic mechanism is significant to be clarified for the targeted treatment in further investigation.

The role of the endocannabinoid system in human spinal cord ex vivo

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There is little known about the endocannabinoid system's (ECS) implication in spinal cord (SC) injury and repair, yet cannabis is commonly used in SC injury patients to manage pain, spasticity and inflammation. We are hoping to determine if cannabinoid receptor activation could change the proliferation and determination of the neural stem progenitor cells found in the subependymal zones of the human SC and we will be studying SC tissue *ex vivo* hoping to determine the role of cannabinoid receptor activation in inflammation. The tissues will be treated with either THC or IL-6/TNF-α or in combination and incubated for 7 days. After being harvested from the donor, the spinal cord will be sectioned into 3 mm thick sections and then incubated in serum free media as well as the different treatment conditions. Although there is little literature on this topic, we expect to find reduced inflammation and decreased cytotoxicity, and expect the activated cannabinoid system to maintain the integrity of the spinal cord. The ECS provides a diverse range of mediated cellular effects, and understanding its role in the SC allows for exploring therapeutic potential.

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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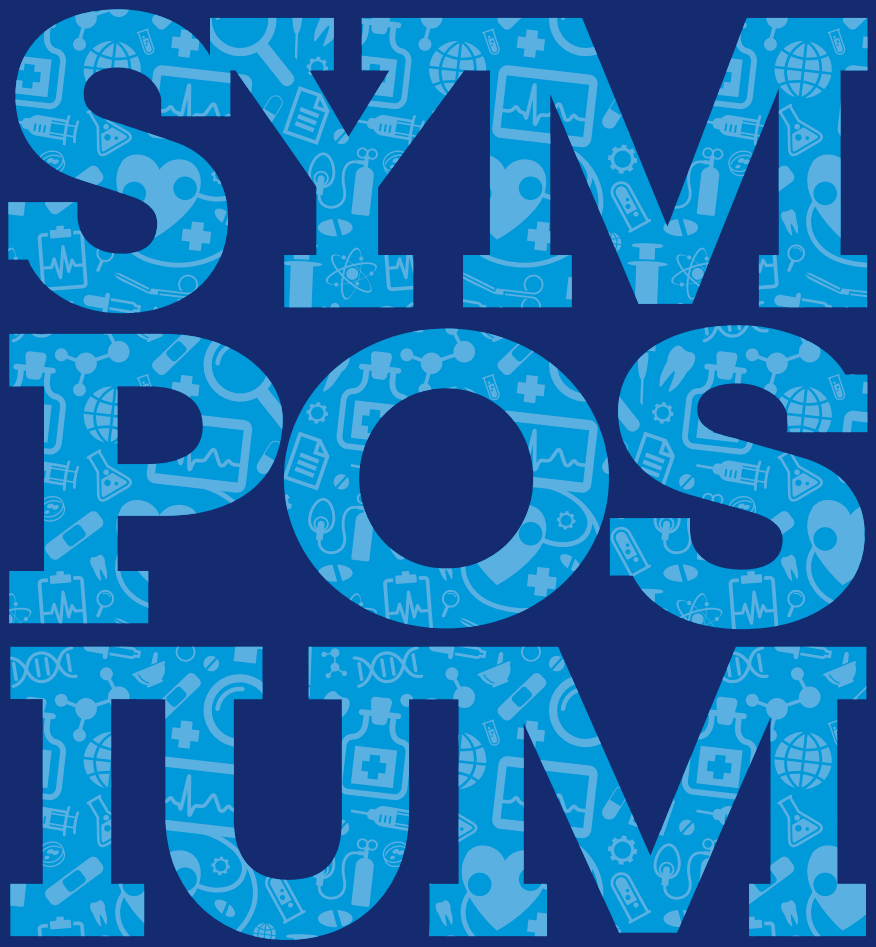

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