Ottawa Hospital Research Institute
OHRI IRHO
Institut de recherche de l'Hôpital d'Ottawa

12th ANNUAL OHRI RESEARCH DAY

PROGRAM

Thursday, November 15, 2012
7:30 a.m. – 5:05 p.m.

St. Elias Centre
750 Ridgewood Ave.
Ottawa, ON

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COMMITTEE

The Ottawa Hospital Research Institute (OHRI) would like to express its appreciation to members of the OHRI 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. Duncan Stewart
Dr. Jay Baltz
Dr. Tim Ramsay
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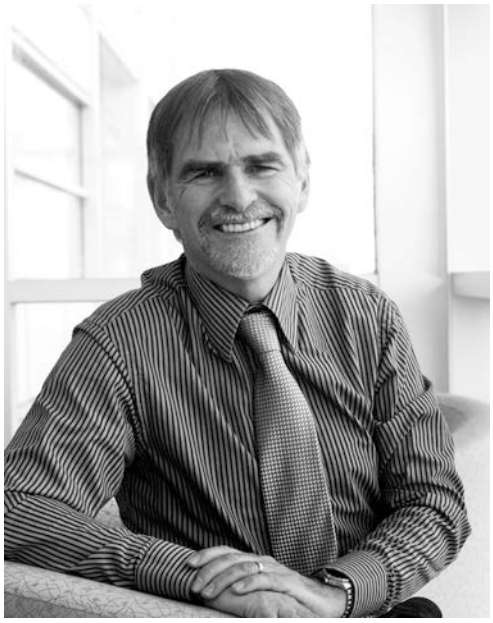
Dr. Jim Dimitroulakos
Dr. Angela Crawley
Dr. Cathy Tsilfidis
Ms. Alessandra Pasut
Dr. Anouk Fortin
Dr. Luc Sabourin
Dr. Dean Fergusson
Mr. Paddy Moore
Ms. Michelle Leleu-Evans

VOLUNTEERS

Greg Canham
Lynn Crosbie
Jane Canniff
Patricia Robb
Lindsay Armstrong

WELCOME TO RESEARCH DAY



It is with great pleasure that I welcome you to OHRI's 12th Annual Research Day!

Today is dedicated to celebrating and showcasing the outstanding work of our students and fellows. Our young researchers are vital to OHRI's lifeblood — providing insight, enthusiasm and dedication that is critical to our success as a top research institute.

We have a full program designed to promote scientific interaction and provide this experience to our trainees in a friendly environment. Whether or not you are formally involved in the judging, I strongly encourage you to ask questions of our trainees during the poster sessions and oral presentations. While serving as an important training exercise, it is also a fantastic opportunity for us all to learn about the exciting research projects taking place at our institute.

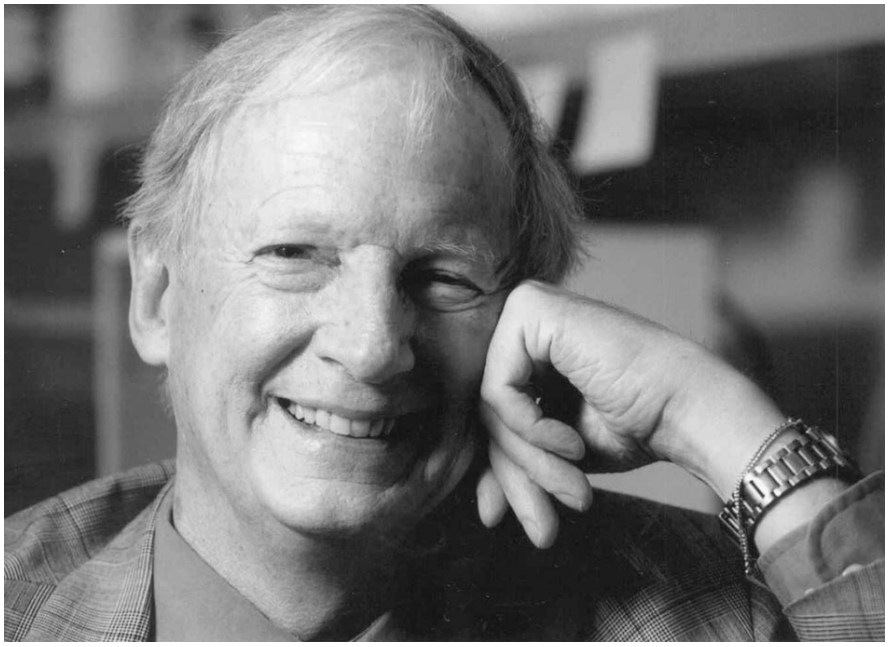
I would also like to draw your attention to the 2nd Annual OHRI IMPACT (Identification of Marketable Products, Applications and Commercializable Technologies) Award. This prize is designed to encourage trainees to consider how their research could lead to innovations and to identify technologies, products or services that stem from their work. The IMPACT Award is part of a larger effort at OHRI to foster a culture that is proactive in translating research into benefits for Canadians.

As always, the keynote address is sure to be one of the day's highlights. This year we are lucky to present Dr. Peter Liu, the recently appointed Scientific Director at the Heart Institute. Dr. Liu is renowned for his research contributions to our understanding of heart failure and cardiac inflammation. His talk, entitled " Innovation Through Collaboration: Research lessons from studying heart disease", promises to be both informative and thought provoking.

On behalf of everyone at OHRI, I would like to extend a special thanks to our guest speaker, who saved the day by stepping in at the eleventh hour. Thanks also to our presenters, judges, planning committee and volunteers for their contributions to this important day for our organization. In addition, I would like to thank the sponsors for helping to make it possible.

Duncan Stewart, MD, FRCPC
CEO & Scientific Director, and Senior Scientist in the Regenerative Medicine Program, and Evelyne and Rowell Laishley Chair, Ottawa Hospital Research Institute
Vice-President, Research, The Ottawa Hospital
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 OHRI 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 OHRI. He is also remembered through the Dr. J. David Grimes Research Chair at the University of Ottawa and through OHRI's Dr. J. David Grimes Research Career Achievement Award, which is awarded annually at The Ottawa Hospital Gala.

THE 2012 DR. J. DAVID GRIMES LECTURER

Dr. Peter Liu, MD, FRCPC

Dr. Liu is the Scientific Director at the University of Ottawa Heart Institute. He received his MD and completed his postgraduate training in Internal Medicine and Cardiology at Toronto General Hospital and Sunnybrook Hospital. Dr. Liu was the inaugural Director of the Heart & Stroke/Richard Lewar Centre of Excellence at the University of Toronto. He also held the position of Scientific Director of the Institute of Circulatory and Respiratory Health at the Canadian Institutes of Health Research (CIHR). Dr. Liu currently sits on the Science and Policy Council for the World Heart Federation and is the director of several large-scale international research programs.

Dr. Liu is well known for his contributions to heart failure and cardiac inflammation research, with more than 350 peer-reviewed publications — many in the most celebrated journals in the world — that have been cited more than 20,000 times. Dr. Liu discovered how viruses can enter the myocardium and trigger inflammation, and how innate and acquired immunity contribute to cardiac remodeling and heart failure progression following injury. His laboratory currently focuses on innovative proteomic biomarkers to detect early and personalize treatment for cardiac diseases, and elucidate novel mechanisms of disease.

In recognition of his outstanding commitment to research, Dr. Liu has received numerous awards, including the Research Achievement Award and the Life Time Achievement Award of the Canadian Cardiovascular Society, the Rick Gallop Award of the Heart & Stroke Foundation, and the Institute of Circulatory & Respiratory Health Distinguished Lecture Award of CIHR.

RESEARCH TRAINEE SALARY AWARDS

Feras Al-Ghazawi
Joseph Burns
Adam Davidson
Elliott Faller
Heather Goldthorpe
Ji Young Kim
Curtis McCloskey
Christopher Patrick
Nicholas Tokarew
Yu Xin Wang
Kai Xue
Khalkid Al-Zahrani
Hafsa Cherid
Simon Pierre Demers
Kyla Garbuio Baron
Alexander Gont
Serena Liu
Anne Millar
Scott Sugden
Nhung Vuong
Sarah Wassmer
Charles Best
Matthew Coyle
Sarah Dick
Andrea Giberson
Kendra Hodgkinson
Janet Manias Rothberg
Ariana Noel
Lee-Hwa Tai
Qi Wang
Emily Xu

GOODMAN COHEN SUMMER STUDENT AWARDS

Krista Quesnel
Oliver Varette

PROGRAM OUTLINE

OHRI RESEARCH DAY PROGRAM - November 15, 2012

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

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

8:30 AM **CANCER – THERAPIES AND SURVIVAL** (9 minutes plus 3 minutes discussion)
Moderator: Naomi De Silva

- **Rozanne Arulanandam** (John Bell Group) *VEGF sensitizes the tumor vasculature to attack by replicating viral therapeutics*
- **Lee-Hwa Tai** (Rebecca Auer Group) *Preventing post-operative metastatic disease by inhibiting surgery-induced dysfunction in natural killer cells*
- **Natasha Kekre** (Jason Tay Group) *Impact of prolonged storage of red blood cells on cancer survival*
- **Erin Bassett** (Valerie Wallace Group) *Norrie Disease Protein: A Novel Mediator of Early Tumorigenesis in Hedgehog Pathway-Induced Medulloblastoma*

9:20 AM **IMMUNITY AND DISEASE** (9 minutes plus 3 minutes discussion)
Moderator: Jason Fernandes

- **Scott Sugden** (Paul MacPherson Group) *HIV Tat protein down regulates CD127 from the surface of CD8 T cells by binding the cytoplasmic tail of CD127 via Tat's N-terminal domain and recruiting CIS to induce CD127 ubiquitination*
- **Charlene Young** (Nongnuj Tanphaichitr Group) *Sperm may act as a vector for HIV transmission to the genital epithelium*
- **Steven Hawken** (Kumanan Wilson Group) *The impact of the transition from whole-cell to acellular pertussis vaccine on health services utilization in Ontario, Canada*
- **Mahmoud Husseini** (Fraser Scott Group) *Induction of heme oxygenase-1 inhibits development of type 1 diabetes - potential involvement of antimicrobials and M2 macrophages*

10:10 AM **REFRESHMENT BREAK** (15 minutes)
Sponsored by: **Centre for Commercialization of Regenerative Medicine**

10:25 AM **POSTER VIEWING / JUDGING OF POSTERS PRESENTED BY POSTDOCTORAL FELLOWS, CLINICAL FELLOWS, RESEARCH ASSOCIATES AND RESIDENTS)** (60 minutes)

11:25 AM **TISSUE REGULATION AND REPAIR** (9 minutes plus 3 minutes discussion)
Moderator: Pamela Lagali

- **John Paul Michalski** (Rashmi Kothary Group) *Integrin-linked kinase regulates oligodendrocyte differentiation and myelination via control of the actin cytoskeleton*
- **Sarah Wassmer** (Catherine Tsilfidis Group) *Understanding the Optical Properties of the Regenerated Newt Lens*
- **Salina Teja** (Kashif Baig Group) *A comparative study of collagen crosslinking for keratoconus in below-threshold versus above-threshold thickness corneas*
- **Kenny Schlosser** (Duncan Stewart Group) *Circulating extracellular microRNAs in patients with pulmonary arterial hypertension*

12:15 PM **BUFFET LUNCH** (60 minutes)

1:15 PM **POSTER VIEWING / JUDGING OF POSTERS PRESENTED BY PhD, MSc, 4th YEAR HONOURS, CO-OP STUDENTS AND IMPACT AWARD APPLICANTS** (60 minutes)

2:15 PM **THE DR. J. DAVID GRIMES KEYNOTE LECTURE** (35 minutes plus 10 minutes discussion)
Moderator: Dr. Duncan Stewart

- **Dr. Peter Liu**, Scientific Director, The University of Ottawa Heart Institute (UOHI)

Innovation through Collaboration: Research lessons from studying heart disease

As we face the challenge of chronic diseases such as heart failure and atherosclerosis, new paradigms of disease pathogenesis, such as activation of innate immunity, and tissue remodelling and reprogramming are important factors. To solve pressing needs in health care at the bedside, we need to innovate solutions through lateral connections by taking advantage of novel technologies, such as proteomics, biomarkers and therapeutic targets.

3:00 PM **STEM CELL BIOLOGY AND REGENERATIVE PATHWAYS** (9 minutes plus 3 minutes discussion)
Moderator: Jennifer Collins

- **Lisa Gamwell** (Barbara Vanderhyden Group) *The ovarian surface epithelium contains a population of LY6A (SCA-1) expressing progenitor cells that are regulated by ovulation-associated factors and BRCA1*
- **Mehdi Shafa** (Bernard Thébaud Group) *Efficient and expedited cellular reprogramming in stirred suspension bioreactors: a new tool for tissue replacement therapies*
- **Soji Sebastian** (Jeff Dilworth Group) *Alternative exon usage in Mef2D allows myocytes to evade the repressive effects of PKA signalling to complete the myogenic differentiation program*
- **Vahab Soleimani** (Michael Rudnicki Group) *Molecular regulation of muscle stem cell function*

3:50 PM **REFRESHMENT BREAK** (15 minutes)

4:05 PM **THE SCIENCE BEHIND THE HEADLINES** (45 minutes)
Moderator: Paddy Moore

- **Dr. Dean Fergusson**, Program Director and Senior Scientist, Clinical Epidemiology, OHRI
  Fresh blood not better, clinical trial shows
- **Dr. Lauralyn McIntyre**, Clinician Scientist, OHRI
  Ottawa researchers to lead world-first clinical trial of stem cell therapy for septic shock
- **Dr. Rashmi Kothary,** Senior Scientist/Associate Director OHRI *New approach for treating genetic muscle-wasting disease shows promise in mice*

4:50 PM **POSTER / ORAL PRESENTATION AWARDS AND CLOSING REMARKS**
Moderators: Dr. Duncan Stewart and Dr. Fraser Scott

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

ORAL PRESENTATIONS

Cancer – Therapies and Survival (8:30 to 9:20)

Moderator: Naomi De Silva

1-1. VEGF sensitizes the tumor vasculature to attack by replicating viral therapeutics

Rozanne Arulanandam, Cory Batenchuk, Carolina Ilkow, Christina Addison, John C. Bell

Background: Resistance to therapies targeting the tumor vasculature usually develops through the upregulation of alternate pro-angiogenic pathways and increased tumor invasiveness. Therefore, new strategies aimed at inhibiting the tumor blood supply are needed. Oncolytic viruses (OVs) were originally selected or designed to specifically infect and destroy tumour cells while leaving normal tissues unaffected. However, beyond direct cytolysis of malignant cells, it is becoming apparent that OVs can exert their anti-neoplastic activity by altering the tumour microenvironment. Indeed, results from our lab have shown that intravenous administration of an oncolytic version of the rhabdovirus, vesicular stomatitis virus, can directly infect the tumor associated vasculature resulting in decreased tumor perfusion and acute vascular shutdown in murine colon carcinoma models. In a recently completed clinical trial, we have extended these observations to patients treated with oncolytic vaccinia virus.

Objective: Our in vitro studies strongly suggest that overexpression of VEGF in the tumour microenvironment may be the critical factor that sensitizes vascular endothelial cells to infection. We propose that the key antiviral cytokine, interferon (IFN), and pro-angiogenic growth factors may have evolved antagonistic signaling properties and therefore predict that VEGF treatment of endothelial cells could suppress interferon responses leading to productive OV infection.

Methods: To examine this possibility, human umbilical vein (HUVEC) and dermal (HMVEC) endothelial cells were pretreated with VEGF, followed by a low (therapeutic) infectious dose of OV. Viral titers, as well as type I IFN production, were compared over time in the presence or absence of VEGF.

Results: Our results reveal that OV replication and transgene expression (eGFP) could be enhanced ~10 fold by VEGF in subconfluent cultured endothelial cells. Confluent monolayers of HDMEC were resistant to infection, demonstrating the selectivity of OVs towards actively proliferating endothelial cells, characteristic of the angiogenic phenotype. Further data reveal that VEGF treatment of endothelial cells could dramatically upregulate levels of a key transcriptional repressor of the interferon-ß promoter, thereby reducing the ability of the treated cell to activate its antiviral program.

Conclusions: These findings elucidate a mechanism explaining the preferential infection of the tumor vasculature by OVs. Furthermore, while the role of IFN in suppressing angiogenesis is fairly well understood, our results identify VEGF as a novel inhibitor of IFN production, pointing towards a potential bi-modality between the two signalling molecules. Understanding and harnessing these microenvironment alterations will allow the optimization and customization of the next generation of OV therapy.

1-3. Preventing post-operative metastatic disease by inhibiting surgery-induced dysfunction in natural killer cells

Lee-Hwa Tai [1], Christiano Tanese de Souza [1], Simon Bélanger [2], Lundi Ly [12](1,2), Almohanad A. Alkayyal[12], Jiqing Zhang[1], Julia L. Rintoul[12], Abhirami A. Ananth[12], Tiffany Lam [1], Caroline J. Breitbach [3], Theresa J. Falls[1], David H. Kirn[3], John C. Bell[123], Andrew P. Makrigiannis[2], and Rebecca A. Auer[14]

Background:

Surgical resection remains the most effective treatment of solid cancers, but preclinical studies indicate that surgical stress itself facilitates the dissemination and formation of metastases. Natural killer (NK) cells are innate immune cells that play a critical role in the clearance of tumour metastases. NK cell suppression following surgery has been linked to the development of postoperative metastases but mechanistic studies are lacking.

Objective:

In this study, we definitively establish the central role of NK cell suppression in the prometastatic effect of surgery and characterize postoperative NK cell dysfunction, using both in vitro and in vivo assays. We also establish the therapeutic potential of preoperative NK cell activation with Oncolytic Viruses (OV) to prevent postoperative metastases.

Methods and Results:

An implanted (B16 melanoma) and a spontaneous (4T1 breast) lung tumor model were used to address these objectives. Surgical stress was induced with a laparotomy and nephrectomy. In both models, surgery increases the number of metastases by > 2-fold. When NK cells were depleted, this effect was no longer seen. When NK cells from surgically stressed mice were transferred into NK-deficient mice, a significant increase in metastases was observed, as compared to mice who received NK cells from mice that did not undergo surgery. Surgery markedly reduced NK cell total numbers in the spleen and affected NK cell migration, with increased NK cells observed at the site of surgery and in vitro and in vivo tumor cell killing and IFN? secretion by NK cells was significantly impaired following surgery. Furthermore, secreted cytokines (e.g. IL-6 and TGF-ß) and regulatory immune populations (Myeloid Derived Suppressor Cells) were altered in surgically stressed mice. Perioperative administration of oncolytic ORFV and vaccinia virus completely reversed NK cell

suppression and this effect correlated with a reduction in postoperative metastases. In human studies, cancer surgery patients had reduced NK cell cytotoxicity postoperatively and we demonstrated, for the first time, that oncolytic vaccinia virus markedly increases NK cell activity in cancer patients.

Conclusions:

Postoperative global NK cell dysfunction is, in large part, responsible for the increase in metastases observed following surgical stress. Perioperative cancer treatments aimed at enhancing postoperative NK cells function, such as OV, can attenuate the prometastatic effects of surgery thereby reducing recurrences and improving survival in surgical cancer patients.

1-4. Impact of prolonged storage of red blood cells on cancer survival

Natasha Kekre[1] , Ranjeeta Mallick[2] , David Allan[2], Alan Tinmouth[2], and Jason Tay[2]

1 University of Ottawa

2 Ottawa Hospital Research Institute

Background: The duration of storage of red blood cells has been associated with poor clinical outcomes amongst those receiving red cells. Patients with cancer are at risk of red cell transfusions and the effect of red blood cell storage is unknown in this population. We investigate the influence of duration of storage of red blood cells and clinical outcomes in patients diagnosed with cancer who received red blood cells. We also describe the transfusion-related outcomes in this large cohort of patients.

Methods: Patients diagnosed with cancer at The Ottawa Regional Cancer Centre between January 01, 2000 and December 31, 2005 were included. Transfusion data was collected from The Ottawa Hospital transfusion database and linked to the cancer database. Units of blood were categorized as "new" if stored for less than 14 days, "intermediate" if stored for 14 to 28 days and "old" if stored for greater than 28 days. Baseline characteristics between the comparative groups were compared with ANOVA. Further, categorical variables and continuous variables were compared using Chi-squared and Wilcoxan rank-sum tests respectively. Kaplan-Meier analyses were used to examine differences in unadjusted survival while Cox-regression analyses were applied to adjust for potential confounding variables.

Results: There were n=27,591 patients diagnosed with any cancer at the ORCC between 01 Jan 2000 and 31 Dec 2005 with 1,929 (7.0%) of patients receiving red cell transfusions within 1 year of the diagnosis of cancer. Of the patients transfused within the first year from diagnosis, 1335 (69.2%) received exclusively one "aged" category of red blood cells. The median overall survival (OS) was worse for patients who were transfused versus those not transfused (1.1 vs 7.5 years respectively, $p<0.0001$). The number of units transfused was also significantly associated with overall survival (median OS for 1-2 units was 1.2 years, 3-5 units was 1.05 years and 6 or more units was 1.1 years; $p=0.0017$) in univariate analysis. Overall survival was not however associated with the transfusion of only new, only intermediate and only old blood transfused (1.2, 1.7, 1.1 years respectively, $p=0.36$). Cancer recurrence, defined as recurrence of original malignancy or new metastatic disease, was significantly higher in patients who received an RBC transfusion ($p<0.0001$). Time to cancer recurrence was not however affected by the age of blood transfused ($p=0.06$). In multivariate analysis, age as a continuous variable, lung cancer, chemotherapy, radiation, cancer-related surgery and cancer recurrence impacted overall survival ($p<0.05$). The number of units of blood transfused and age of blood transfused were not significantly associated with overall survival in multivariate analysis.

Conclusion: Patients with cancer who are older, receiving chemotherapy, radiation or surgery are more likely to require an RBC transfusion. Although RBC transfusion has an effect on median overall survival, the length of time the RBC unit is stored does not impact survival. This indicates that current RBC storage policies are adequate for patients with malignancy requiring an RBC transfusion.

1-2. Norrie Disease Protein: A Novel Mediator of Early Tumorigenesis in Hedgehog Pathway-Induced Medulloblastoma

Erin Bassett[1], Nicholas Tokarew [1], Brian McNeill [1,3], Chantal Mazerolle [1], Alan Mears [1,2], Carolina Perez-Iratxeta [4], Kim Paes [5], Dennis Rice [5], Adrian Dubuc [6,7], Paul Northcott [6,7], Michael Taylor [6,7], Valerie Wallace[1,2,3]

1Vision Program, Ottawa Hospital Research Institute

2 Department of Ophthalmology, University of Ottawa

3 Department of Biochemistry, Microbiology and Immunology, University of Ottawa

4 Regenerative Medicine Program, Ottawa Hospital Research Institute

5 Ophthalmology Department, Lexicon Pharmaceuticals Inc., The Woodlands, TX, USA

6 Program in Developmental and Stem Cell Biology and The Arthur and Sonia Labatt Brain Tumour Research Center, Hospital for Sick Children, Toronto, ON

7 Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON.

Medulloblastoma (MB), a tumor of the cerebellum, is the most common malignant brain tumor in children. A significant proportion of MB patients remain incurable, and current therapies often result in severe side effects, indicating a strong need for novel treatments. One third of all human MB exhibits a gene expression signature of Sonic Hedgehog (Shh) signaling, a pathway that regulates development. These Shh-MBs have responded to Hedgehog (Hh) pathway inhibitors in clinical trials; however, tumors develop resistance to these compounds, highlighting the need to identify additional therapeutic targets. To this end, we recently identified a novel target of the Hh pathway in neural progenitors, Norrie Disease Pseudoglioma (Ndp). Ndp encodes a secreted protein that is best known for its role in angiogenesis; however, its role in brain development and cancer is unexplored. We therefore studied the role of Ndp in the cerebellum and in Shh-MB. We determined that Ndp is expressed in Shh-MB precursor cells and in actual Shh-MBs that develop in mice

heterozygous for the tumor suppressor Patched (Ptch+/-), which models human Shh-MB. NDP is also upregulated in human Shh-MB relative to all other MB subgroups. To investigate the requirement for Ndp expression in MB, we compared tumor frequency and latency as a function of Ndp expression in the MB-prone Ptch+/- mouse by generating NdpKO;Ptch+/- (DKO) mutants. Loss of Ndp caused extreme acceleration of MB in the Ptch+/- model, dramatically increasing incidence and decreasing latency. This accelerated MB in DKOs was associated with earlier onset, higher frequency and increased vascular invasion of pre-neoplastic lesions in the cerebellum, suggesting that Ndp functions during MB initiation and/or early progression. Loss of Ndp also disrupts cerebellar blood brain barrier integrity. Whole genome expression profiling revealed that DKO and Ptch+/- tumors have clearly separable gene signatures, particularly with respect to extracellular matrix genes.

We have uncovered a novel Hh-regulated gene in the cerebellum with a strong tumor suppressive role in Shh-MB. Our data show that Ndp normally inhibits early stages of tumorigenesis, and implicates a possible role for Ndp in modulation of the early tumor microenvironment.

Immunity and Disease (9:20 to 10:10)

Moderator: Jason Fernandes

2-1. **HIV Tat protein down regulates CD127 from the surface of CD8 T cells by binding the cytoplasmic tail of CD127 via Tat's N-terminal domain and recruiting CIS to induce CD127 ubiquitination**

Scott Sugden, Paul MacPherson

HIV Tat protein down regulates surface expression of the interleukin-7 receptor alpha-chain (CD127) on CD8 T-cells resulting in impaired T-cell proliferation and cytolytic capacity. Once taken up by CD8 T-cells, Tat binds directly to the cytoplasmic tail of CD127 inducing receptor internalization and degradation. Given CD127's important roles in proper immune function, I characterized the interactions and mechanisms required to induce receptor loss from the surface of CD8 T cells.

Tat deletion mutants were generated and CD8 T-cells isolated from HIV negative volunteers were exposed to exogenous or intracellular Tat proteins before surface CD127 expression was analyzed by flow cytometry. To characterize Tat-CD127 physical interactions, wild type Tat and Tat mutants were incubated with Jurkat-CD127 lysates followed by CD127 co-immunoprecipitation. The effect of Tat on CD127 post-translational modifications was also investigated.

Deletion of the first 10 N-terminal residues or deletion of residues 17-21 prevented Tat from down regulating CD127 and prevented Tat from binding CD127 via co-immunoprecipitation. Deletion of the basic domain (aa 48-59) also prevented Tat from down regulating CD127 but did not prevent Tat from interacting physically via co-immunoprecipitation. Strikingly, endogenously expressed basic domain-deleted Tat acted as a dominant negative mutant, causing an accumulation of CD127 at the cell surface. Furthermore, Tat encourages the ubiquitination of CD127 by recruiting the cytokine-inducible socs (CIS) protein to the receptor, possibly causing accelerated CD127 internalization and proteasomal degradation.

This work suggests that novel HIV therapeutics targeted against the N-terminal or basic regions of Tat may help to preserve CD8 T cell CTL functions during HIV infection.

2-2. **Sperm may act as a vector for HIV transmission to the genital epithelium**

Charlene Young [1], Duriya Fongmoon[1], Kessiri Kongmanas[1,2], Kym Faull[3], Jonathan Angel[1,2,4], Nongnuj Tanphaichitr[1,2,5]

1 Chronic Disease Program, Ottawa Hospital Research Institute, Ottawa, Ontario

2 Dept of Biochemistry/Microbiology/Immunology, University of Ottawa

3 The Pasarow Mass Spectrometry Laboratory, University of California Los Angeles, CA.

4 Dept of Medicine (Division of Infectious Disease),

5 Dept of Obstetrics/Gynecology, Faculty of Medicine, University of Ottawa, Ontario

Background: HIV-1 infection in women occurs primarily through vaginal intercourse. The exact mechanisms of viral transmission from semen through the vaginal mucosa are still poorly understood. Several lines of research indicate that gp120 can interact with non-CD4 T-cells via its affinity for cell surface sulfoglycolipids, sulfogalactosylceramide (SGC) and sulfogalactosylglycerolipid (SGG). Since SGG is abundantly and selectively present on the sperm surface, we hypothesize that sperm may be able to capture HIV-1 via interaction between gp120 and SGG, and the gametes can act as vectors transferring the captured HIV-1 to female genital epithelial cells.

Objective: To determine whether: 1. HIV-1 can bind to sperm in vitro; 2. HIV-1 bound to sperm is infectious; and 3. Sperm can act as a vector transmitting HIV to vaginal/cervical (V/C) cells.

Methods: HIV-1 binding to sperm was shown by co-incubation of HIVcs204 with live sperm, followed by p24 ELISA. Infectivity of HIV bound to sperm was demonstrated using TZM-bl reporter cells or by co-culture of HIV bound sperm with activated peripheral blood mononuclear cells. Following exposure of V/C cells to HIV-associated sperm, infection of V/C cells was shown by the presence of HIV-1 DNA in V/C cells by nested PCR of HIV-gag gene. The presence of SGG on sperm was determined by thin layer chromatography and mass spectrometry of extracted cellular lipids.

Results: HIV-1 virions bind to sperm and retain their infectivity. In addition, HIV virions that were captured by sperm were transmitted to PBMCs and V/C cells. The potential of SGG as an alternative receptor for gp120 has been implicated, since SGG was localized to the sperm surface

Conclusion: Sperm can capture and transmit HIV to cells of the vaginal mucosa. Understanding alternate mechanisms including sperm-associated HIV transmission would allow better and inclusive approaches for preventing transmission. This research could lead to new approaches for microbicides by including dual targets one for preventing cell free HIV and one for sperm- associated HIV.

2-3. The impact of the transition from whole-cell to acellular pertussis vaccine on health services utilization in Ontario, Canada

Steven Hawken, Kumanan Wilson[1]

1 OHRI, ICES@uOttawa, Department of Medicine

Background: Acellular pertussis vaccines(acP) have a better safety profile than whole-cell pertussis vaccines(wcP). Canada switched to acP in 1997-1998, and the safety benefit was confirmed in surveillance studies. However, only specific events were considered (convulsions, hypotonic-hyporesponsive episodes (HHEs)), not overall health services utilization (HSU). We examined the impact of the transition to acP in terms of HSU (admissions and emergency room (ER) visits).

Methods: We included children born between April 1994 and March 1996 (wcP used) and between April 1998 and March 2000 (acP used) in Ontario, Canada using health administrative data housed at the Institute for Clinical Evaluative Sciences (ICES). Using the self-controlled case series design, we examined the relative incidence (RI) of ER visits and admissions in the first 3 days post-vaccination versus an unexposed period from days 9-18 following 2,4,6 and 18 month vaccinations. We compared the RI of events before versus after introduction of acP by calculating relative incidence ratios (RIRs).

Results: We observed a striking reduction in events immediately before and after each vaccination, which we attributed to a healthy vaccinee effect, which muted the RI observed in the immediate post-vaccination period. However, by comparing the wcP period to the acP period using RIRs we demonstrated a highly statistically significant reduction in RI after introduction of acP. RIRs at ages 2,4,6 and 18 months were 1.82(95%CI:1.64,2.01), 1.91(95%CI:1.71,2.13), 1.54(95%CI:1.38,1.72) and 1.51(95%CI:1.34,1.69) respectively, for the wcP compared to acP, translating into the avoidance of about 90 ER visits and 9 admissions per month after switching: a 38-fold higher impact than when we only considered admissions for convulsions.

Conclusion: Future vaccine safety analyses should examine both specific end-points as well as all-cause HSU. The RIR allows comparison of relative incidence in a baseline versus a comparator group, overcoming the effect-masking properties of the healthy vaccinee effect.

2-4. Induction of heme oxygenase-1 inhibits development of type 1 diabetes - potential involvement of antimicrobials and M2 macrophages

Mahmoud Husseini[12] , Wang, G-S., Crookshank, J.A., and Scott, F.W.[12]

1 Chronic Disease Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada;

2 Department. Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada

Background: Type 1 diabetes (T1D) is an autoimmune disease that is characterized by a T-cell mediated attack on insulin-producing ß-cells in the pancreas, possibly related to an inappropriate immune reaction to dietary antigens and/or microbes in the gut lumen. We previously observed a deficit in gut-resident CD163+ M2 anti-inflammatory macrophages in BioBreeding diabetes-prone (BBdp) rats compared with control rats. Heme oxygenase-1 (HO-1) is the rate-limiting enzyme involved in the CD163 pathway and through the breakdown of toxic heme, releases potent antioxidants and suppresses inflammatory immune responses. Microarray analyses of mesenteric lymph node and rat insulinoma beta-cells incubated with diabetes-promoting wheat peptides (WP) revealed an upregulation of HO-1. Furthermore, initial flow cytometry analyses showed that isolated lamina propria mononuclear cells from BBdp rats failed to upregulate HO-1 in response to lipopolysaccharide and WP compared with control animals.

Hypothesis/Objective Because HO-1 response is impaired and there is a deficit of CD163 macrophages in BBdp rats, this suggested a key a role for HO-1 in diabetogenesis. We therefore hypothesized that animals treated with CoPP, a heme analogue which induces HO-1 expression, would inhibit development of T1D and increase expression of CD163+ in macrophages.

Methods: Beginning at 30 d, BBdp rats were injected i.p. with CoPP (6.5 mg/kg, n=33) or saline (n=31) twice per week for three weeks to induce HO-1. Animals were killed at 51 d (n=16/group) to harvest and fix organs of interest (pancreas, jejunum) for immunohistochemistry and RNA analyses. Gene expression analysis in the pancreas and gut was performed using Affymetrix Rat Gene 1.0 ST chips and PCR arrays, respectively. Remaining rats (15-17/group) were monitored for development of T1D until ~120 d.

Results: T1D incidence was inhibited in CoPP-treated rats (CoPP, 27% vs. saline, 65%, p=0.01, log rank; p=0.03, Fisher's exact test). CoPP-treated animals (51 d, n=8/gp) showed a marked increase in HO-1 expression associated with islets as well as the lamina propria compared with controls. The majority of these cells co-localized with the pan-macrophage marker CD68 and to a lesser extent with the M2 (anti-inflammatory) marker, CD163. Confocal analyses revealed a distinct population of CD68+CD163+HO-1+ cells in the pancreas and jejunum. In the pancreas, there was a striking increase in the a-defensins and Reg family of genes which confer anti-microbial and growth promoting properties. Reg3ß area fraction was significantly higher in the islets of CoPP-treated animals compared with controls. Jejunal PCR array analysis revealed a significant increase in the Camp gene which encodes the cathelicidin anti-microbial peptide. There was a significant increase in CAMP+ cells in the jejunum of CoPP-treated animals compared with controls and these cells co-localized

with M2 CD163+ macrophages. A transcription factor which regulates the polarization of M2 macrophages, KLF4, was significantly increased in the pancreas and jejunum of CoPP-treated BBdp rats compared with controls.
Conclusion: HO-1 induction prevented T1D possibly through modulation of the gut immune system and recruitment of a distinct population of anti-inflammatory M2 macrophages in gut and pancreas. HO-1 associated upregulation of antimicrobials in pancreas suggests important pleiotropic roles for these molecules in T1D, possibly linked with the CD163/HO-1 pathway.

Tissue Regulation and Repair (11:25–12:15)

Moderator: Pamela Lagali

3-1. Integrin-linked kinase regulates oligodendrocyte differentiation and myelination via control of the actin cytoskeleton

John-Paul S Michalski[1,2] , Ryan O'Meara[1,2] , Rashmi Kothary[1,2,3]
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The interplay between oligodendrocyte (OL) and its local extra-cellular matrix (ECM) is critical to the proper maturation of this unique cell type. Recent work has highlighted the ß1 integrin-signalling pathway, a mediator for ECM/OL interactions, as an essential component of myelin sheath formation in the central nervous system (CNS). A major downstream effector of ß1 integrin is integrin-linked kinase (ILK), an adaptor and structural platform protein. To assess the importance of ILK in OL-mediated myelination, we conditionally ablated ILK in primary OLs, both in isolation as well as in co-culture with dorsal root ganglion neurons (DRGNs). Loss of ILK in either system resulted in delayed morphological and differentiation marker maturation, though to a lesser degree when in the DRGN microenvironment. As well, accumulation of filamentous actin was observed in the processes and cell body of ILK-null OLs. To further study the role of ILK in CNS myelination we generated a conditional knockout mouse model (ILK cKO) employing the Cre-loxP system. The Cre gene was placed under the control of the proteolipid protein (Plp) promoter, allowing for OL specific ILK ablation. Ultrastructural analysis of the optic nerves (ONs) from ILK cKO mice revealed an increased number of amyelinated nerve fibers at P14 with subsequent recovery by P28. The observed transient defects are due neither to a loss nor a gain in total number of mature or progenitor OLs as assessed across multiple developmental time points. To account for the possibility of OL turnover, we measured the number of cleaved-caspase 3 positive cells in ONs of P14 ILK cKO and WT mice. No overt cellular turnover was observed in ILK deficient mice. Taken together, our data suggests a role for ILK in regulating the morphological maturation of OLs both in vitro and in vivo, the loss of which results in defective OL branching and membrane formation with subsequent recovery dependent upon niche complexity. Future work is directed towards identifying downstream signalling pathways that are perturbed in the ILK-deficient OLs.

3-2. Understanding the Optical Properties of the Regenerated Newt Lens

Sarah Wassmer, Margaret Beddaoui, Payman Rajai, Réjean Munger, Catherine Tsilfidis
Background: Urodele amphibians such as the red-spotted newt, Notophthalmus viridescens, have the unique capability to regenerate lost or damaged structures of the eye as an adult, such as the lens, retina and optic nerve. Despite extensive research on the histological and molecular aspects of lens regeneration, studies on the optical properties of the regenerated lens and the restoration of functional vision are non-existent.
Objective: The purpose of this project is to compare the optical properties of original and regenerated lenses to better understand recovery of vision in the regenerated lens.
Methods: The lenses from seventeen red-spotted newts were surgically excised, measured in size and analyzed by the Transmission and Scattering Measurement System (TSMS) for optical clarity. Focal length and equivalent refractive index were also determined. Nine weeks following the first lentectomy, the regenerated lenses were removed and analyzed in the same fashion. After optical assessment, the lenses were prepared for histological sectioning.
Results: The nine-week time point was chosen for sampling regenerated lenses because the literature suggests that most of the histological and molecular aspects of regeneration are complete within 4-5 weeks. However, even at 9 weeks, there was a significant reduction in mean lens size between the original and 9-week regenerated lenses (the regenerated lenses were approximately 25% smaller). The regenerated lenses had improved optical transmission, but due to their smaller size, they had a lower focal length and a lower refractive index. Histology of the 9 week regenerated lenses showed less structural definition compared to the fully developed original lenses. Fully adult histological characteristics (including growth to the original size) were seen in 26-week regenerated lenses.
Conclusions:
TSMS analysis revealed that the 9-week old regenerated adult newt lens has improved light transmission in comparison to the native, older lens. However, the decreased size, focal length and refractive index of the regenerated lens suggest that the newt does not have functional vision after nine weeks of regeneration.

3-3. **A comparative study of collagen crosslinking for keratoconus in below-threshold versus above-threshold thickness corneas**

Salina Teja, W Bruce Jackson, George Mintsioulis, Pierre-Jerome Bergeron, Kashif Baig

Background: Keratoconus is a degenerative condition of the cornea, marked by progressive thinning and steeping of the cornea, which results in irregular astigmatism and visual loss. Corneal collagen cross-linking with riboflavin (CXL) is a procedure designed to increase the mechanical strength of the cornea, preventing disease progression in corneas > 400um. Many patients with severe keratoconus however, have thinned (<400um) corneas and advancements in procedure protocol to swell the cornea have been undertaken.

Purpose: To compare the 6-month anatomic and visual outcomes of CXL keratoconus corneas between below-threshold thickness (BTT: <400um after epithelium removal) and above-threshold thickness (ATT: >400um after epithelium removal) groups.

Methods: All patients received CXL entailing removal of the epithelium, 30 minutes of riboflavin (ATT received isoosmolar, BTT received hypoosmolar), and 30 minutes of UVA light. 50 eyes in the BTT group and 62 eyes in the ATT group matched inclusion criteria. These 112 charts were reviewed retrospectively. Baseline and post-op (1-, 3- and 6-month) measures of keratometry, uncorrected and corrected distance visual acuity (UDVA and CDVA), corneal thickness, and manifest refraction were recorded. The outcomes between the groups were compared in order to assess the procedure's relative effect on ectatic changes.

Results: Preoperatively, the BTT group had a mean maximum keratometry (Kmax) of 56D, mean thinnest corneal thickness (TCT) 403um (after epithelium removal mean 365um), astigmatism 4.4D, mean UDVA 20/630 and mean CDVA of 20/50. The ATT group showed less severe keratoconus at baseline with a mean Kmax of 49D, mean TCT of 469um (after epithelium removal mean 442um), astigmatism 3.3 D, mean UDVA 20/180 and mean CDVA of 20/30. At 6 month follow up, there were no significant changes in any variable in the BTT group. In the ATT group, significant reduction in mean Kmax to 47.5 D ($p<0.001$) and astigmatism to 2.3D ($p=0.01$), with an insignificant improvement in UDVA, CDVA and spherical refractive error.

Conclusions: Crosslinking has been shown to halt progression and cause regression of keratoconus in corneas that have a thickness greater than 400um. The procedure has been modified to treat patients with below-threshold thick corneas by using hypoosmolar riboflavin to swell the cornea. There are only 2 small retrospective case series reporting the outcomes of the modified protocol, however currently no studies comparing to the standard protocol. Our study shows that at 6 months, there is no significant improvement of keratoconus in corneas that are <400um however it does halt progression.

3-4. **Circulating Extracellular MicroRNAs as Potential Biomarkers in Patients with Pulmonary Arterial Hypertension**

Kenny Schlosser[1], R. James White[2], and *Duncan J. Stewart[1]

1 Ottawa Hospital Research Institute and University of Ottawa, Ottawa, Ontario, Canada

2 Division of Pulmonary & Critical Care Medicine, University of Rochester School of Medicine, Rochester, New York, USA

Rationale: Pulmonary arterial hypertension (PAH) is a progressive and fatal disease characterized by arteriolar remodelling and obliteration of the lung vasculature, ultimately causing right ventricular failure and death. Although the prognosis for PAH is poor, early diagnosis and treatment can increase patient quality of life. Unfortunately, prompt diagnosis remains a challenge as the early stages of PAH are asymptomatic, and there is no specific assay for this disease. Extracellular microRNAs (miRNAs) that circulate in the blood have been identified as promising biomarkers for a variety of cardiovascular diseases, but they have not yet been explored in patients with PAH.

Objective: To identify circulating miRNAs that may have utility as biomarkers of disease activity in PAH patients.

Methods and Results: RT-qPCR arrays were used to assay 1066 different miRNAs in plasma from 4 treatment-naive patients with idiopathic (I) PAH and 3 healthy controls. Twenty-five miRNAs differed between the IPAH and control groups within this discovery cohort (-2.8 to 3.4 fold change, $p<0.05$). Downregulation of four novel PAH-associated miRNAs was confirmed in a separate validation cohort of 14 PAH patients (6 IPAH and 8 associated PAH) and 13 healthy controls. Additionally, two miRNAs previously shown to be differentially expressed in PH lung tissue were decreased in plasma of PAH patients. The area under the receiver-operator characteristic curves was significant for five downregulated miRNAs, ranging from 0.77 to 0.85 ($p<0.05$). Several miRNAs also correlated with indices of PAH severity, including exercise capacity and pulmonary vascular resistance.

Conclusion: This global assessment of circulating miRNAs provides novel insight into the breadth of altered miRNA expression in human PAH, and supports their potential utility as biomarkers of disease activity.

Stem Cell Biology and Regenerative Pathways (3:00–3:50)

Moderator: Jennifer Collins

4-1. **The ovarian surface epithelium contains a population of LY6A (SCA-1) expressing progenitor cells that are regulated by ovulation-associated factors and BRCA1**

Lisa F Gamwell[1], Olga Collins[1], Barbara C Vanderhyden[1]

1 Centre for Cancer Therapeutics, Ottawa Hospital Research Institute.

The ovarian surface epithelium (OSE) is a single layer of poorly differentiated epithelial cells. This layer covers the surface of the ovary, is ruptured during ovulation and is one of the tissues of origin of ovarian cancer. Ovulation is the strongest non-genetic risk factor for ovarian cancer while deleterious mutations to the breast cancer type 1 susceptibility protein (BRCA1) represent the strongest genetic risk factor. Little is known about the changes that occur in this layer before or during ovulation, and even less is known about the regenerative processes that occur after the surface is ruptured to release a mature oocyte. Recently, a population of mouse OSE (MOSE) cells that exhibit progenitor/stem cell characteristics has been identified, though neither a genetic marker nor how these cells are regulated has been determined. We have identified a defined population of MOSE cells with progenitor cell characteristics that express the stem cell marker lymphocyte antigen 6 complex, locus A (LY6A; also known as stem cell antigen-1 [SCA-1]). By testing the effect of factors found in the follicular fluid at ovulation on proliferation, sphere formation, and LY6A expression, we have determined that the size of the LY6A-expressing (LY6A+) progenitor cell population is regulated by at least two ovulation-associated factors present in the follicular fluid: transforming growth factor beta 1 and leukemia-inhibitory factor. BRCA1 may also regulate LY6A+ progenitor cells, as inactivation of BRCA1 results in the expansion of this population. Our work has identified a population of LY6A+ MOSE progenitor cells on the surface of the ovary that is regulated by BRCA1 and may play a role in ovulatory wound healing.

4-2. **Efficient and expedited cellular reprogramming in stirred suspension bioreactors: a new tool for tissue replacement therapies**

Mehdi Shafa , Lavinia Ionescu, Derrick Rancourt, Bernard Thébaud

The clinical application of stem cells depends on the availability of pluripotent cells that are not restricted by ethical, immunological and technical considerations. The recent development of the derivation of induced pluripotent stem cells (iPSCs) from somatic cells is opening a new era in developmental biology. Having potential advantages in regenerative medicine, iPSCs are similar to their embryonic stem cell (ESCs) counterparts and possess all of the essential criteria such as pluripotency, self-renewal and potency. Despite major improvements in the methods of iPSC generation and expansion, the process still remains inefficient and poorly characterized. Based on our previous findings that stirred suspension bioreactors (SSBs) favor pluripotency over differentiation, we developed a novel method for the efficient and expedited derivation of iPSCs in SSBs. We found that suspension bioreactors increase both the kinetics and efficiency of iPSC derivation and can provide a selective advantage to enhance cellular reprogramming, presumably through application of shear stress. The resulting suspension-derived iPSCs (SiPSCs) resembled ESCs in their in vitro and in vivo characteristics, such as teratoma and chimera formation and displayed germ line transmission competency. These findings show that SSBs not only suppress differentiation but also provide a novel environment for the expansion and maintenance of iPSCs as well as their efficient derivation.

In order to demonstrate the potential therapeutic application and safety of iPSCs, we employed Bronchopulmonary dysplasia (BPD) as a disease model. BPD remains the most common complication of extreme premature birth. BPD has long term respiratory and neuro-developmental consequences that reach beyond childhood resulting in increased health care costs and decreased quality of life. We hypothesized that iPSCs can prevent/regenerate O2-induced alveolar damage in our BPD mice model. Our preliminary data suggest that we have identified specific growth factors and culture conditions that allow murine iPSC to differentiate into lung distal cells and to adopt a alveolar epithelial cells (AEC) phenotype. Differentiated iPSCs expressed specific markers of type I and type II AEC. The ability to differentiate iPSCs into AEC offers many other investigational (drug testing and understanding disease mechanisms) and therapeutic opportunities. In the next step, we will investigate the therapeutic potential of undifferentiated and iPSC-derived AEC in vivo to prevent/regenerate lung damage in our experimental BPD model. This research will provide proof-of-concept for the therapeutic potential of iPS cells for lung diseases.

4-3. **Alternative exon usage in Mef2D allows myocytes to evade the repressive effects of PKA signaling to complete the myogenic differentiation program**

Soji Sebastian [1] , Zizhen Yao[2], Patricia Rakopoulos [1], Yi Cao2, Herve Faralli [1], Kulwant Singh [1], Arif Azi [1], Carmen Pali [1], Marjorie Brand [1], Stephen J Tapscott[2] and Jeffrey Dilworth [1]

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2 Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

Background

The Mef2D protein is expressed in many cell types where it modulates gene expression to control proliferation, differentiation and cell survival. During skeletal muscle differentiation, a muscle-specific isoform Mef2D is generated through alternative exon usage. However the significance of this alternative splicing event to the function of Mef2D remains unknown.

Objective

Here we set out to characterize the functional differences between the muscle specific isoform Mef2D?′ and the more ubiquitously expressed isoform Mef2D?. Phenotypically, over expression of Mef2D? isoform behaved in a dominant negative fashion to block differentiation, while the expression of Mef2D?′ lead to precocious differentiation. To understand the mechanism through which the two isoforms of Mef2d mediated opposite outcomes during myogenesis we performed genomic and proteomic studies.

Methods and results

High-throughput analysis of immunoprecipitated chromatin demonstrated that both isoforms recognize a highly similar consensus sequence of CTAAAAATAG, and bind a largely over-lapping set of genes. Interestingly, proteomic studies demonstrated that while Mef2D? is efficiently phosphorylated by PKA, the Mef2D?′ is able to evade this repressive post-translational modification. By evading PKA phosphorylation, Mef2D?′ is not able to interact with transcriptional repressors, and instead preferentially interacts with the Ash2L methyltransferase complex to mediate transcriptional activation. Thus, the use of alternative exons allows Mef2D to behave as either an activator or repressor of transcription by modulating the ability of the transcription factor to be negatively regulated by PKA signaling.

Conclusion

Thus we have identified a novel mechanism by which a ubiquitously expressed transcription factor has evolved to undergo a tissue-specific alternative exon usage to permit the proper temporal activation of muscle genes during myogenesis.

4-4. Molecular Regulation of Muscle Stem Cell Function

Vahab D Soleimani, Michael A Rudnicki

In adult skeletal muscle, a rare population of muscle stem cells termed satellite cells is maintained in a quiescent state within the mature muscle tissue. Upon injury, these cells are activated and divide to produce progenies that will either self renew or differentiate into mature muscle cells. The self-renewal, specification, commitment, determination of their myogenic identity and their differentiation is regulated by the temporal expression of paired box homeotic proteins, Pax3/7 and the myogenic regulatory factors (MRFs) (Myf5, MyoD, Myogenin and Myf6). Together, these transcription factors initiate a hierarchical gene expression cascade that regulates self-renewal and differentiation of muscle cells. We have combined gene expression and genome-wide binding sites analysis of these factors in muscle stem cells-derived myoblasts and myotubes and mapped out their targets in growth and differentiation. Our studies have uncovered novel regulatory networks that play key role in muscle stem cell growth and differentiation. We have shown that MRFs function is subject to regulation by zinc finger repressors such as Snai1/2 proteins. In this talk a detailed presentation of MRFs function and their genetic network in myogenesis will be presented.

POSTER ABSTRACTS

Cancer Therapeutics Program

1. ### Role of PAX2 in the etiology of ovarian cancers

Ensaf Alhujaily,Yong Tang, Kenneth Garson, Barbara Vanderhyden

Background: Epithelial ovarian cancers appear to originate from multiple tissues of origin, including the ovarian surface epithelium (OSE), inclusion cysts that arise from this epithelium, and the distal fallopian tube epithelium (FTE). Growing evidence supports two distinct pathways (low-grade or high-grade) for ovarian carcinogenesis based on different molecular profiles, pathological features and clinical outcomes. The cancers appear to develop through a process that includes the formation of a transitional, preneoplastic structure that is characterized by increased proliferation and stratification of precursor cells. Recent characterization of these preneoplastic structures implicate altered expression of the transcription factor PAX2. The data suggest a rather complex role for PAX2 in ovarian cancer, where induction of its expression in OSE is an early event in the formation of low-grade cancers.We hypothesize that the early events of transformation leading to low-grade cancers are due to PAX2 expression in OSE with effects on proliferation, migration, survival, and p53-associated transformation of these cells.

Objectives: To identify the biological consequences of the presence of PAX2 in normal epithelium of the ovaries.

Methods: To define the contribution of PAX2 to early ovarian carcinogenesis, we are studying in vitro models of cancer progression: mouse OSE cells that are normal, p53-deficient, immortalized (non-tumorigenic) or K-Ras/c-Myc-transformed (tumorigenic). To determine the functional consequences of PAX2 in OSE cells, overexpression of Pax2 was induced using lentiviral infection (empty vector as control).

Results: PAX2 overexpression had no effect on the proliferation of OSE cells with and without p53 cultured under normal conditions. However, PAX2 expression reduces the ability of both cell lines to migrate. Moreover, the morphology of PAX2-expressing cells was notably different, resulting in smaller, less adherent, and more well-differentiated epithelial cells. While the growth rate of normal and p53-deficient OSE cells was not affected by PAX2, PAX2 overexpression increased proliferation of mouse ovarian cancer cells and increased both the number and size of colonies grown in soft agar, compared to controls. In terms of early transformation, our preliminary data indicates that immortalization of OSE cells using SV40 T antigen induced expression of Pax2, as determined by quantitative PCR.

Conclusion: These results indicate that the biological effects of PAX2 vary in OSE cells in association with their stage of progression from normal to cancer.

2. ### Characterization of Oncolytic MG1-mIL-12 in Tumor Bearing Mice

Almohanad Alkayyal [1,3,6], Lee-Hwa Tai[1], Jiqing Zhang[1,2], Christiano T de Souza[1], Charles Lefebvre[5], Andrew P. Makrigiannis[3], John C. Bell[1,3], David F. Stojdl[3,5], Rebecca C. Auer[1,4.]

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Background: Oncolytic viruses (OV) were originally designed to selectively infect and replicate in tumours, with the primary objective of directly lysing cancer cells. It is becoming increasingly clear, however, that OV infection results in a profound inflammatory reaction within the tumour, initiating innate and adaptive immune responses against it and that this is critical for its therapeutic benefit. This anti-tumour immunity appears to be mediated predominantly by natural killer (NK) cell and cytotoxic T cells. Interleukin 12 (IL-12) is a cytokine that induces proliferation and cytotoxicity of both NK cells and T cells. Direct intra-tumoural administration of IL-12 has been shown to stimulate a potent antitumor immunity.

Hypothesis: We have hypothesized that a replication competent OV expressing IL-12 would generate high intra-tumoural levels of IL-12, enhanced anti-tumoural immunity and result in improved cancer efficacy.

Methods and Results: We used a clinical candidate OV, an attenuated version of the Rhabdovirus Maraba (MG1) with mutations in M protein L123W and G protein Q242R. This bullet-shaped virus is a negative-sense single stranded RNA encoding five proteins; large protein (L), glycoprotein (G), nucleoprotein (N), phosphoprotein (P) and matrix protein (M). We created MluI restriction enzyme sites, upstream and downstream of the IL-12 gene using PCR and then, using a TA cloning technique, we inserted the IL-12 gene into an MG1 shuttle vector plasmid which was then sub-cloned into the MG1 plasmid. To rescue this virus, we transfected SNB19 cells with the MG1 plasmid in the presence of accessory plasmids encoding the N, P and L proteins in the presence of the T7 Vaccinia virus. The rescued virus was plaque purified and sent for sequencing to confirm the presence and orientation of IL-12. We demonstrated in-vitro that the virus

has an equivalent cytotoxicity to the parental MG1 virus on a panel of murine and human cell lines including B16, SNB75, OVCAR8, A549. We also have confirmed high levels of IL-12 expression in the supernatant 8 hours following infection of Vero cells with MG1-IL12. Future Directions: After the confirmation of IL-12 expression in vivo, we evaluate the effects of MG1-IL12 infection on the activation of NK cells and T cells. Following this, we will assess the efficacy of MG1-IL12 in two treatment models of disseminated malignancy: (1) A therapeutic infected cell vaccine in the B16 melanoma lung metastases model and (2) systemic administration resulting in an in situ tumour vaccine in the CT26 colon cancer lung metastases model. The effect of expressing IL-12 in MG1 on systemic and intra-tumoural NK cell and T cell activation and cytotoxicity will also be investigated.

3. ## Deletion of the ste-20 like kinase, SLK, is embryonic lethal

Khalid N. Al-Zahrani[12] **,**Marlene McKay[12], Kate Daniels[12], Luc A. Sabourin[12]
1 Centre of Cancer Therapeutics, Ottawa Hospital Research Institute
2 Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa.
The Ste-20 like protein kinase, SLK, has important roles in cell motility, downstream of the FAK/src complex, as well as invasion, downstream of ErbB2/HER2/Neu. In order to study the role of SLK in vivo we have created an SLK global knock-out mouse line using an SLK gene-trap vector. A LacZ insertion downstream of exon 10 creates a fusion protein that persists in the genotyped null animals until embryonic day 12.5. We believe that the SLK-LacZ fusion retains some kinase activity but lacks true regulation. SLK expression is then completely turned off between day 12.5 and 15.5 resulting in a global reduction of RNA and protein levels. Several morphological defects in the SLK-null embryos have been observed by haematoxilin and eosin staining. Defects in the structures of the brain, placenta, and muscle are observed and all lead to embryonic lethality. Work in characterizing these defects is ongoing and will be presented

4. ## Surgery-induced vaccine dysfunction in a murine model of melanoma

Abhirami Anu Ananth[1,2], Christiano Tanese de Souza[1], Lee-Hwa Tai[1], , Theresa Falls[1], Brian D Lichty[3], John C Bell[1,2], Rebecca AC Auer[1,4]
1 Center for Innovative Cancer Research, Ottawa Hospital Research Institute, Ottawa, ON
2 Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, ON.
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4 Department of Surgery, Division of General Surgery, The Ottawa Hospital, Ottawa, ON.
Background
Surgery is a necessary and crucial intervention to treat solid malignancies in cancer patients. However, previous works have shown that surgery can accelerate tumour progression and promote the metastatic potential of tumour cells. The study of this phenomenon has largely focused on impairment of innate immune cells such as natural killer (NK) cells; however, the impact of surgery on adaptive immunity is poorly understood. This remains an important question to clarify given that cancer treatment using genetic vaccination is an exciting therapeutic approach that is being actively pursued.
Objective
To the best of our knowledge, this is the first experimental study characterizing the impact of surgical stress on tumour antigen-specific adaptive immunity, with particular focus on surgery-induced impairment of cytotoxic T lymphocyte (CTL) function.
Methods
To illustrate the effect of surgery on acquired anti-tumour immunity, we adopted a C57Bl/6 mouse model of B16 melanoma prophylactically immunized with intramuscular AdhDCT, a replication-deficient adenovirus expressing human dopachrome tautomerase (hDCT), a melanoma-associated antigen. Surgical stress was induced by laparotomy and left nephrectomy. B16F10lacZ cells were inoculated intravenously (by tail vein) or subcutaneously (in the right hind flank) on the day of surgery. Perioperative DCT-specific CD8+ CTL function was assessed by flow cytometric analysis of IFN-gamma, TNF-alpha, and Granzyme B, IFN-gamma ELISPOT, and CD8+ T cell-mediated chromium release assay.
Results
In the IV B16 model, a dose of 1e6 pfu AdhDCT confers protection against lung metastases (3-fold decrease compared to PBS, $p<0.05$). However surgically stressed immunized mice are no longer protected (5-fold increase compared to AdDCT, $p<0.01$). In the SQ B16 model, surgery dramatically reduces both disease-free survival and overall survival in Ad-vaccinated mice, whereas no surgery control mice were cured of their tumours. Flow cytometry 18 hours post-surgery demonstrated that surgical stress decreases both the proportion ($p<0.001$) and absolute numbers of spleen ($p<0.05$) DCT-specific IFN-gamma+ CD8 T cells by over 2-fold. Also, the MFI of IFN-gamma gated on spleen CD8+ T cells is reduced by almost 3-fold ($p<0.001$) in surgically stressed Ad-vaccinated mice. Similar decreases were found in TNF-alpha and Granzyme B expression. IFN-gamma ELISPOT of isolated CD8+ T cells shows a 2-fold decrease in the number of spot-forming units 18 hours post-surgery.
Conclusions
We have shown that perioperative suppression of antigen-specific T cells leads to accelerated tumour progression and increased metastatic burden in a murine melanoma model. We hope to further characterize and recover this defect with perioperative immune-boosting therapeutics.

5. ## LMO4 regulates SLK-mediated cell migration through a direct interaction

Kyla D. Garbuio Baron[1,2], Chris J. Storbeck[1], Luc A. Sabourin[1,2.]
1 Centre for Cancer Therapeutics, Ottawa Hospital Research Institute
2 Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa

The Ste-20 like kinase, SLK, is activated downstream of ErbB2/HER2/Neu. SLK activity is required for cell migration and invasion. LIM binding domain proteins, Ldb1 and Ldb2, regulate SLK catalytic activity. Ldb1 is a known binding partner of the LIM-only domain 4 protein, LMO4 and due to sequence homology, Ldb2 is assumed to bind LMO4. LMO4 is over-expressed in 62% of ErbB2-positive breast tumors. Previously, LMO4 up-regulation induced mammary cell proliferation and tumour cell invasion. We have confirmed that LMO4 directly binds Ldb1, Ldb2 and SLK. Differential expression of LMO4 results in an increase in SLK activity and affects the ability of Ldb1 to form a complex with SLK. LMO4 is present in the cytosol in a pattern similar to that of SLK, localizing to the membrane ruffle. Loss of LMO4 expression inhibits cell migration. Work describing the contribution of the SLK-LMO4 complex to FAK/src-mediated cell migration will be presented.

6. ## Targeting Tumour Vasculature with Oncolytic Virotherapy

Naomi De Silva, Theresa Falls, Dominic Roy, Michelle Becker, Sara Cvancic, Caroline Breitbach, Manijeh Daneshmand, Aaron Fenster, Donald McDonald, Harold Atkins, David Kirn, John Bell

Background: Oncolytic viruses (OVs) are able to selectively target, infect, and kill cancer cells due to inherent differences between normal and cancer cells. OVs demonstrate remarkable clinical potential not only due to their ability to infect cancer cells, but also to induce secondary cell death through acute vascular disruption. In pre-clinical models, OV treatment causes widespread killing of uninfected tumor cells. This Bystander Effect, where uninfected cancer cells are rapidly killed, is due to the virus' ability to specifically target tumor vasculature. Oncolytic vaccinia virus is capable of targeting tumor vasculature by directly infecting tumor vessels and causing widespread vascular disruption, resulting in an acute loss of blood flow within tumours. This secondary activity causes extensive apoptosis and necrosis of the tumour core, yet leaves a viable rim that can be a potential source of outgrowth. Interestingly, these pre-clinical observations are mirrored in clinical studies with oncolytic vaccinia virus in advanced cancer patients.
Objective: To characterize acute changes in the tumour microenvironment in response to OVs and to determine how these changes may impact OV therapy.
Methods/Results: 3D modeling of a patient biopsy reveals that OV infection is widespread and specific to tumor tissue. The ability of oncolytic vaccinia virus to infect tumour vasculature in patients was confirmed through immunohistochemical analysis. Similar to pre-clinical models, there is an acute loss of tumour blood flow and necrosis five days following OV therapy. Despite the ability of OVs to mediate significant cell killing through direct viral oncolysis and acute vascular disruption, a viable rim often survives OV therapy. To investigate why the tumor rim remains viable and resistant to direct infection, live tumour slices were infected ex vivo. Tissue slice experiments revealed that in the absence of physical barriers to virus delivery, the majority of the tumour rim continues to be resistant to direct infection, indicating that intravenous delivery is not the sole barrier to OV therapy.
Conclusions: Oncolytic viruses are able to rapidly target tumour vasculature through direct infection of endothelial cells and acute vascular disruption.

7. ## Achieving Efficient Systemic Delivery of Oncolytic Vaccinia Virus

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Systemic delivery of oncolytic viruses is perhaps the ideal mode of treatment for metastatic disease because metastatic beds can be dispersed, inaccessible by direct injection, or their extent undiscovered. There are, however, many barriers to the systemic delivery of oncolytic Vaccinia virus presented by the innate and acquired immune systems. Most notably, complement and neutralizing antibody, specifically present in the demographic of patients vaccinated against Smallpox, act together to neutralize the virus, and induce phagocytosis in the blood and by the reticulo-endothelial system. In spite of these barriers, a recent Phase I dose escalation trial demonstrated the delivery of oncolytic Vaccinia virus following an intravenous infusion to patients with treatment-refractory solid tumours at a dose of 1.5 x 107 pfu/kg or greater. This high dose suggests that in order to achieve delivery, a given set of neutralization mechanisms must be saturated. We have assessed the neutralizing capacity of the blood of a panel of healthy vaccinated donors and determined that complement and antibody can neutralize up to 99% of the virus in vitro. However, the effect of anti-Vaccinia antibody is significantly abrogated when complement is inhibited. We therefore seek to modulate the activity of complement in order to achieve improved delivery. While many innate mechanisms limit delivery and spread, they also have important anti-tumoural roles. Our goal therefore is not to blunt the immune response, but to sculpt it to allow for optimal delivery and anti-tumoural effects.

8. **Inactivation of tumor suppressor lethal giant larvae via PTEN loss promotes the invasiveness of glioblastoma multiforme**

Alexander Gont , Ian Lorimer

Background: Glioblastoma multiforme (GBM) is the most aggressive and invasive form of brain tumor. From diagnosis the average survival time remains just past one year. While other forms of brain cancer can be successfully removed by surgical means, the invasive nature of GBM results in frequent relapses at secondary sites within the brain. PTEN loss is very common in GBM and leads to aberrant activation of the PI3K pathway. I have demonstrated that this event results in the phosphorylation, mislocalization and inactivation of a tumor suppressor called lethal giant larvae (LGL). Loss of LGL, one of the first tumor suppressors ever discovered, leads to over-proliferation of Drosophila and mouse neuronal tissue leading to a tumor like phenotype. LGL has also been previously implicated to interact with various cell trafficking and motility machinery. Its role, however, in proliferation and invasiveness in human brain cancer has not been investigated. Objective: To examine the role that LGL has in glioblastoma malignancy. Methods: U87MG cells stably transduced with a doxycycline inducible active LGL gene were used to study the effects of LGL on proliferation and invasiveness. Primary GBM cells were transduced with active LGL. Protein localization was studied by immunofluorescence. Cell growth in vitro was studied by trypan blue exclusion and in vivo as subcutaneous xenografts. In vitro invasion was assessed by migration through Transwell chambers coated with Matrigel. Results: I have demonstrated that in both cell culture and in vivo subcutaneous mouse models the introduction of a non-phosphorylatable, constitutively active LGL form (LGL-3SA) does not affect the proliferation of human glioblastoma cells (U87MG). U87MG cells expressing LGL-3SA demonstrate a marked decrease in the motility and invasiveness in vitro. LGL-3SA expression prevented the polarized delivery of matrix metalloproteinase 14, a key protein responsible in cleaving the extracellular matrix, to the leading edge of GBM cells and to the periphery of multiple primary GBM cell lines. Conclusions: The loss of PTEN in glioblastoma leads to the inactivation of LGL's tumor suppressive functions leading to increased motility and invasiveness.

9. **Identifying key genes mediating the tumour-promoting actions of 17-B estradiol in mouse models of ovarian cancer**

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4.University of Ottawa, Dept. of Obstetrics and Gynecology

Background:

Estrogen therapy increases the risk of developing ovarian cancer, and 17ß-estradiol (E2) promotes proliferation and decreases apoptosis in ovarian cancer cell lines. We have shown previously that E2 promotes ovarian cancer progression in mouse models.

Objective:

To determine whether this stimulation of tumour growth is caused by E2-altered transcription of one or more key genes.

Methods:

To investigate the mechanisms underlying the decreased survival caused by E2, we used a xenograft model in which SCID mice were injected with MASE cells. The MASE cell line was derived from the ascites of a transgenic tgCAG-LS-TAg mouse, an animal model of ovarian cancer.

Results:

Exogenous E2 decreased survival of both transgenic and xenograft mouse models. Microarray analysis of tumours from E2-treated mice showed upregulation of 196 genes and downregulation of 55 genes compared to tumours from placebo-treated mice. In addition to some known ER targets such as Pgr, several novel genes were identified which are involved in angiogenesis, proliferation or differentiation. Of particular interest is gene regulated by estrogen in breast cancer-1 (GREB1), which is necessary for E2-stimulated growth of MCF7 cells. We confirmed E2 regulation of GREB1 in ovarian cancer and investigated its function by lentiviral shRNA knockdown. Knockdown of GREB1 in MASE cells did not alter proliferation under high serum (10%) conditions, but preliminary results suggest that knockdown may decrease proliferation under lower serum conditions. GREB1 knockdown also altered morphology and decreased migration in a scratch wound assay.

Conclusions:

We are now investigating the potential role of GREB1 in invasion (in vitro) and tumour progression (in vivo). Characterization of the function of E2-targeted genes will aid the identification of mechanisms by which E2 increases the risk of ovarian cancer.

10. **Enhancement of Oncolytic Virus Efficacy by Novel Drug Candidates**

Ramya Krishnan[1,2], Mark Dornan[2], Vanessa Garcia[2], Rozanne Arulanandam[1], Christina Moi[2], Colin Davis[2], Andrew Chen[1], Karl Wasslen[3], Fabrice LeBoeuf[1], Jeff Smith[3], Christopher N. Boddy[2], Jean-Simon Diallo[1,2], John C. Bell[1,2]

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Background

Oncolytic rhabdoviruses such as vesicular stomatitis virus (VSV?51) have shown promise in pre-clinical studies. VSV?51 is genetically engineered to be safe and selective for cancer cells, but this also results in reduced spreading in some tumors. To overcome this problem, we have discovered drugs that sensitize cancer cells to

viral infection. VSe1 (Viral Sensitizer 1) enhances VSV?51 spread in resistant tumor cells up to a 1000-fold, resulting in synergistic cell killing and improved efficacy in vitro and in vivo. While we know VSe1 suppresses the ability of cancer cells to defend against viral infection, its biological targets are unknown.

Objectives

1 Characterize the structure-activity-relationship of VSe1

2 Identify the target of VSe1

3 Identify VSe1 analogues with improved pharmacological and pharmacokinetic properties.

Methods

VSe1 analogues were synthesized by members of Dr. Christopher Boddy's lab. Activity of VSe1 analogues was assessed by high through-put in vitro assays in a 96-well plate format. Potential targets were isolated through inhibitor-based affinity capture.

Results

In vitro assays and a rational approach in the design of VSe1 analogues allowed us to identify functional groups that can be modified without hampering activity. Interestingly, some analogues show improved properties such as potency, therapeutic index and stability. We used functional and non-functional analogues linked to amino PEGA resin to identify interacting proteins by affinity chromatography coupled with LC/MS/MS. This led to the identification of a 25 kDa protein that interacted specifically with the active resin-bound analogue.

Conclusions

We were able to modify VSe1to generate analogues with more attractive pharmacological and physicochemical properties. This also allowed us to identify interacting proteins. Future work will aim to identify these proteins and validate the results.

11. **Investigating the mutanome in conjunction with oncolytic virus therapy**

Monique Marguerie, Kelley Parato, Chantal Lemay , Madison Foster , David Conrad , Harold Atkins, John Bell

Background

Oncolytic Virus (OV) therapy has many advantages over other cancer therapeutics including that OVs can be delivered systemically to target distant tumours, are highly selected for tumor cells and are immune stimulatory. The immune stimulatory properties of OVs can be augmented by encoding specific tumour associated antigens (TAA) in the viral genome in order to augment the pre-existing anti-tumour immunity. TAAs derived from somatic mutations within the tumour, known as "the mutanome", are fundamentally different from the individual. As such these mutanome TAAs, while augmenting the immune response, should not induce an autoimmune response as is observed when vaccinations are with self-antigens, and thus represent a potentially novel source of potent tumour neoantigens.

Objectives

Currently we are investigating whether immunizing against mutanome tumour antigens from a murine melanoma in combination with an oncolytic virus therapeutic approach can enhance an already strong cancer therapy platform. Multiple different mutanome epitopes and administration approaches are being examined and compared for therapeutic efficacy. Ultimately we would like to determine whether using a more personalized cancer therapeutic approach utilizing an individuals mutanome TAAs would give any benefit to our oncolytic virus platform and if so how we can effectively incorporate it into a tumour-personalized vaccine.

Methods

To determine the ability of different mutanome peptides to elicit or enhance control over tumour growth we use both therapeutic and prophylactic studies and examine both survival curves and tumour growth over time. We conduct immune characterzation assays such as ELISAs and flow cytometry to examine the immune responses elicited by different vaccination methods.

Results/Conclusions

Significant results/conclusions yet to be attained/made as of the date of the abstract submission.

12. **Insect cell carriers for systemic delivery of oncolytic viruses**

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Background: Replication-competent oncolytic viruses are tremendously potent therapeutic agents when applied to tumour cells in vitro or by direct injection in vivo; however their capacity for systemic delivery is severely limited due to a host immune system that is well-trained to eliminate foreign pathogens from the circulation. In recent pre-clinical investigations, a novel approach using cells as carriers for oncolytic viruses has shown promise in overcoming such obstacles. A number of candidate cell types have been proposed ranging from immune cells to stem cells to transformed cell lines. However the clinical utility of carrier cell types investigated to date is limited by one or more of the following features: cumbersome isolation and/or manufacturing protocols, limited viral productivity (in the case of non-tumour cells), off-target tissue homing tropisms, and safety concerns (in the case of tumourigenic cell lines).
Objective: In efforts to improve the clinical feasibility of the carrier cell concept, we have investigated the potential of insect cells for systemic delivery of oncolytic viruses. We have identified insect cell lines that are efficiently infected with both rhabdo- and poxviral oncolytic agents. My objective is to investigate the ability of insect cell carriers to deliver oncolytic viruses to tumour cells both in vitro and in vivo.
Results/Conclusion: When systemically administered to tumour-bearing mice, virus-loaded insect cells were able to deliver both classes of oncolytic agent to distally-localized tumour beds in immune-competent animals. Importantly, systemically administered insect cell carriers were well tolerated and circulated extensively with little off-target tissue homing. Uniquely, the insect cell culture system offers the capacity for large-scale biotherapeutic production without the tumourigenicity that limits the safety of continuous mammalian cell lines. Therefore the insect cell system described here may help to fuel the launch of cell-borne oncolytic viruses into clinical trials.

13. **Ontario Tumour Bank Initiative at The Ottawa Hospital**

Arnaout, S.Gilbert, C.A. Jodouin. E. Pitre , **Marta Sienkiewicz** , J. Bartlett , S.Kodeeswaran

The Ontario Tumour Bank (OTB) is a province-wide biorepository and data bank focused on collection of tumour-related human biospecimens. It 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 Ontario Tumour Bank to provide research biospecimens of high quality, diversity, and integrity. Operating at state-of-the-art hospitals and cancer centres across Ontario, including The Ottawa Hospital since 2005. The Ontario Tumour Bank coordinates the collection, storage, analysis, annotation, and distribution of tumour and peripheral blood samples. Working in collaboration with local pathologists, medical oncologists, surgeons and other hospital personnel, specially trained staff obtain patient consent, collect tissues and assemble comprehensive clinical information about each donor and the corresponding samples. The Ottawa Hospital-OTB site has been acknowledged in the September 2012 issue of Nature for the contribution to the TCGA (The Cancer Genome Atlas) study. The researchers in this study identified promising therapeutic targets for lung squamous cell carcinoma, the second most common form of lung cancer.The Ontario Tumour Bank is a program of the Ontario Institute for Cancer Research (OICR). Funded by the Government of Ontario, OICR is a not-for-profit corporation that supports research on the prevention, early detection, diagnosis, treatment and control of cancer.

14. **Role of Periostin in ErbB2 induced mammary tumorigenesis**

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Periostin (postn) a glycoprotein of the extracellular matrix interacts with collagen, modulates its fibril formation and is implicated in cancers of breast, ovary and pancreas. Although postn is shown to act through Integrin-Akt-PI3K pathways to effect cancer cell proliferation and migration, the mechanisms by which postn interacts with oncogenic pathways remains unclear. Studies involving knock out of postn in MMTV-ErbB2 (neuNDL) mice show that lack of postn did not delay tumor initiation but significantly affected tumor progression. Immunohistochemical analysis using Ki67 show reduced proliferation rates in these tumors. Also, western blot analysis show that lack of postn in these tumors results in reduced levels of cyclinD1, pY397FAK, pErk, Egr1 and pErbB2 which are involved in proliferation and migration. The mechanisms through which postn interacts with the ErbB2 oncogenic pathway is further being characterized and will be presented.

15. **The effect of pre-existing leukemia on the efficacy of a leukemia cancer vaccine**

Jovian Tsang, Meaghan MacLean, David Conrad, John Bell , Harold Atkins

Background

A feature of some oncolytic virus therapies is the induction of an adaptive immune response. Dr. David Conrad has developed a vaccine to elicit this adaptive immune response toward L1210 leukemia in a murine model, which he has termed LOVV (leukemia-oncolytic virus vaccine). Our lab is currently studying the biological and
immunological phenomena associated with LOVV in order to better understand and eventually develop an individualized clinical equivalent.

Objective

We want to evaluate the efficacy of LOVV in a setting where the immune system has been previously exposed to leukemia cells. Specifically, we want to elucidate the reasons for the observed decrease in LOVV efficacy.

Methods

Separate cohorts (n=5) of DBA/2 mice were exposed to irradiated non-proliferative L1210 cells (irL1210) one week before leukemic challenge (with robust L1210 cells). Administration of gamma-irradiated MG1 virus-infected L1210 cells (LOVV) occurred the next day for a regimen of three weekly tail vein injections. Mice were observed for a subsequent 90 days for typical leukemic endpoints.

Results

Preliminary results by M. MacLean showed the cohort receiving the irL1210 prior to challenge and LOVV had 20% survival, while the challenge and LOVV cohort had 60% survival. Control cohorts receiving just leukemic challenge or irL1210 and challenge had no survival benefit as expected.

Conclusions

Preliminary results suggest that the vaccine might perform poorly in mice that have been previously exposed to non-proliferative leukemia cells. As this vaccine would be most beneficial to prevent relapse, this finding is significant because it implies that patients in remission may not benefit as initially hoped. We will explore potential immunological explanations for this observed loss in vaccine efficacy. While gaining insight into the strengths and weaknesses of LOVV, our conclusions may have implications on the biology of cancer vaccine.

16. **Mechanisms by which estrogen accelerates ovarian tumor initiation**

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Background: Ovarian cancers present as three different types, epithelial, germ cell, and stromal, with epithelial cancers consisting ~90% of cases. As a result, it is believed that the majority of cancers that develop in the ovary arise from the ovarian surface epithelium. Previous studies have shown that exposure of mice to exogenous estradiol (E2) causes preneoplastic changes in ovarian surface epithelial (OSE) cell morphology and accelerates ovarian tumor onset. Clinically, estrogenic compounds are used for treatments such as hormone replacement therapy for relief of menopausal symptoms or as a form of contraceptive such as birth control pills. However, women using estrogen-only hormone replacement are at higher risk of ovarian cancer. Objective: The aim of this project is to determine the mechanisms by which estrogen has detrimental effects that promote an increased risk of ovarian cancer, specifically by elucidating the actions of E2 on the OSE. The pathways controlling the premalignant transformation are unknown, but exogenous E2 promote stratification and apparent loss of cellular polarity in the OSE layer. We hypothesized that this change in phenotype may be due to decreases in disabled-2 (Dab2), as Dab2 is required for epithelial cells to maintain polarity and a genome-wide screen has identified estrogen response elements in regions proximal to the transcriptional start site of Dab2 in mice and humans. Dab2 is an adaptor protein that is found in a variety of tissues and is highly expressed in breast and ovarian tissue but its expression is lost in the majority of ovarian carcinomas. Methods: E2 effects were examined in two lines of normal mouse ovarian surface epithelial cells (mOSE-L1 and mOSE-L2), mOSE cells rendered immortal by infection with adenoviral vectors expressing Cre recombinase (AdCre) to activate SV40 large T-antigen (TAg) (CAG-TAg/AdCre mOSE), a mouse ovarian cancer cell line developed from ascites cells from E2-treated CAG-TAg mice (MASE), and tumors from CAG-TAg mice. CAG-TAg/AdCre mice were treated with E2 via a subcutaneous insertion of a 60 day timed-release 0.25mg E2 or placebo pellet. Changes in gene expression were measured by quantitative PCR (qPCR). Results/Conclusions: E2 suppresses expression of Dab2 in normal mOSE cells, mouse ovarian cancer cells and mouse ovarian tumors. Suppression is greatest in cells that express higher levels of ERa. This is the first demonstration that E2 regulates Dab2 expression.

17. **Productive infection is not required for innate immune stimulation and attenuation of surgery induced metastases by the oncolytic virus, Maraba MG1**

Jiqing Zhang[1,2], Lee-Hwa Tai[1], Christiano Tanese Souza[1], Carolina Ilkow[1], Almohanad A. Alkayyal[1,3], Abhirami A. Ananth[1,3], Lundi Ly[1], Shalini Sahi[1]Theresa J. Falls[1], Charles Lefebvre[5], David F. Stodjl[3,5], John C. Bell[1,3], Rebecca A. Auer[1,4]

Background: Surgical stress promotes the formation of metastatic disease secondary to profound suppression of innate natural killer (NK) cells. Our group has recently reported that NK cells are highly activated following infection with several Oncolytic Viruses (OV), resulting in NK cell cytotoxicity and IFN? secretion. We have further demonstrated that preoperative OV administration can reverse surgery-induced NK cell suppression and abrogate the prometastatic effect of surgery. Unfortunately there are several potential barriers to the administration of a live, replicating virus immediately before surgery, including the potential for an overwhelming systemic inflammatory response and the risk of infection of operating room staff.

Objective: In this study we sought to identify a safe, non-replicating OV that could stimulate NK cells and effectively prevent metastases when administered preoperative.

Methods and Results: The attenuated Maraba virus, MG1 was able to effectively infect and kill melanoma B16lacZ cells in-vitro and reduce metastases in B16lacZ lung metastases model. The efficacy, however, appeared to be independent of oncolytic activity, but dependent on intact NK cells. MG1 administration resulted in an immediate (24h) and intense activation of NK cells, as evidenced by significantly increased NK cell cytotoxicity and cytokines expression. In order to better understand the requirements for NK cell activation by MG1 we used three variations of MG1: (1) a G-less version (MG1-Gless) capable of a single cycle of viral replication, (2) UV inactivated MG1 for 2 minutes (MG1-UV2min) capable of cell entry and some viral transcription and (3) UV inactivated MG1 for 2 hours (MG1-UV2h) not capable of cell entry. This established that MG1-Gless, and MG1-UV2min had similar ability to stimulate NK cells (only slightly less than MG1) and to attenuate the development of postoperative metastases in the B16lacZ model of implanted lung metastases, as well as in the breast 4T1 model of spontaneous lung metastases. The MG1-UV2h was not able to activate NK cells and had limited efficacy in lung metastases models.

Conclusion: Viral infection, but not replication is required for NK cell activation and attenuation of postoperative metastases. Non-replicating forms of MG1, including MG1-UV2min, are novel cancer therapies that can be safely used in the immediate preoperative period to prevent the formation of metastatic disease.

Chronic Disease Program

18. **Understanding the Mechanisms by which IL-7 Down-regulates Expression of the IL-7 Receptor Alpha-chain (CD127) in Primary Human CD8 T-Cells**

Feras Al-Ghazawi, Paul MacPherson

Background: Interleukin (IL)-7 is an essential non-redundant cytokine influencing cell survival and proliferation throughout the life-span of a T-cell. We previously reported decreased expression of the IL-7 receptor alpha-chain (CD127) on CD8 T-cells in HIV+ patients and have since shown suppression of CD127 is mediated by both the HIV Tat protein and IL-7 both of which are elevated during HIV infection.
Objective: To elucidate the mechanisms by which IL-7 suppresses CD127 gene transcription and targets surface CD127 protein for degradation.
Methods: Primary human CD8 T-cells isolated from healthy HIV-negative volunteers were treated with IL-7 and c-Myb, SOCS1-7 and CIS transcripts and protein were examined by qPCR and Western. Nuclear run-on assays were used to measure rates of CD127 transcription. Candidate transcriptional repressors were identified using DNA Microarrays, PCR arrays, Western and siRNA-mediated gene knock-down assays. The region within the CD127 gene promoter required for IL-7 mediated transcriptional suppression was identified through progressive truncations using firefly luciferase as a reporter gene. The interaction of SOCS proteins with CD127 was examined by co-IP. Intracellular localization of SOCS and CD127 proteins was examined by confocal microscopy.
Results: Upon binding IL-7, surface CD127 is rapidly internalized while activation of the JAK/STAT5 pathway stimulates expression of CIS and SOCS2 transcripts and proteins. Following IL-7 stimulation, CIS binds directly to CD127 as demonstrated by co-IP and co-localizes with both CD127 and the early endosomal marker EEA1, and subsequent proteasomal degradation of CD127, CIS and SOCS2 is dependent on an E3 ligase. We also show that IL-7 suppresses CD127 transcription. Suppression of CD127 transcripts is dependent on JAK/STAT5 signaling which up regulates expression of c-Myb. The region within the CD127 gene promoter required for IL-7 mediated transcriptional suppression was identified and contains c-Myb binding sites. Using siRNA knock down and transient over-expression, we show c-Myb suppresses CD127 gene transcription in primary human CD8 T-cells.
Conclusions: IL-7 is currently being investigated as a potential therapy in HIV+ individuals with poor immunological response to antiretroviral therapy. In order to optimize the use of IL-7 in therapeutic settings, it is crucial to understand how expression of the IL-7 receptor is regulated. We show here that IL-7 suppresses CD127 expression by two mechanisms, transcriptional which is dependent on c-Myb and at the protein level by inducing expression of CIS and SOCS2 proteins which in turn bind to CD127 in early endosomes and shuttle the receptor complex to the proteasome for degradation.

19. **The oncogenic phosphatase PPM1D confers cisplatin resistance in ovarian carcinoma cells by attenuating checkpoint kinase 1 and p53 activation**

Ahmed Y. Ali , Mohammad R. Abedini , Benjamin K. Tsang

Cisplatin (CDDP: cis-diamminedichloroplatinum) resistance is a major hurdle in the treatment of human ovarian cancer (OVCA). A better understanding of the mechanisms of CDDP resistance can greatly improve therapeutic outcome for patients. p53, a determinant of CDDP sensitivity in ovarian cancer, is activated by Chk1 in response to DNA damage. Although the oncogenic phosphatase Protein Phosphatase Magnesium-dependent 1 (PPM1D) can deactivate both p53 and Chk1 through site-specific dephosphorylation, whether PPM1D plays a role in CDDP resistance is unknown. Here, using pair-matched wt-p53 CDDP-sensitive (OV2008) and –resistant (C13*) cells, and p53-compromised CDDP-resistant cells (A2780cp, OCC-1, OVCAR-3, and SKOV3), we have demonstrated (i) the existence of site-specific differences in phospho-Ser-Checkpoint kinase 1 (Chk1) content between sensitive and resistant cells in response to CDDP; (ii) PPM1D, but not phosphoinositide-3-kinase-related kinase (ATR), is important in the regulation of CDDP-induced Chk1 activation and ovarian cancer cell chemosensitivity; (iii) PPM1D down-regulation sensitizes resistant cells to CDDP primarily by activating Chk1 and p53. Our findings establish for the first time that PPM1D confers CDDP resistance in ovarian cancer cells through attenuating CDDP-induced, Chk1-mediated, p53-dependent apoptosis. These findings extend the current knowledge on the molecular and cellular basis of cisplatin resistance and offer the rationale for PPMID as a potential target for treatment of chemoresistant ovarian cancer.

20. **Influence of Depot Origin on the Susceptibility of Adipose Progenitor Cells to Apoptotic Stimuli**

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Obesity is a major risk factor for insulin resistance, which may lead to type 2 diabetes, hepatosteatosis and cardiovascular disease. These metabolic pathologies are collectively referred to as the metabolic syndrome. Hypernutrition and a sedentary lifestyle lead to an accumulation of adipose tissue through an increase in adipocyte size (hypertrophy) and number (hyperplasia). Differentiation of preadipocytes into mature adipocytes (adipogenesis) is crucial to maintain normal function of adipose tissue and to meet energy storage demands. Hypertrophic adipocytes lead to adipose tissue inflammation and insulin resistance. On the other hand, hyperplastic adipose

tissue expansion is associated with preserved insulin sensitivity in humans and animal models. It requires a sufficient number of functional preadipocytes to form new adipocytes. In addition, preadipocytes from different fat stores have been described to have different intrinsic rates of adipogenesis and apoptosis. The aim of our study is to determine if there are variations in preadipocyte apoptosis between subcutaneous (SC) and omental (OM) preadipocytes. To expand our understanding of preadipocyte cell death, we will investigate the influence of depot origin on the sensitivity of adipose progenitor cells to apoptotic stimuli. Preliminary results indicate that OM preadipocytes are more susceptible to cell death than SC preadipocytes. After 24 hour serum deprivation, cell enumeration revealed 19 ± 6 % vs 26 ± 2 % cell death in SC and OM preadipocytes, respectively (n=2). We will assess the difference in cell death rates between SC and OM preadipocytes in response to various apoptotic stimuli (serum deprivation, cycloheximide/TNFa treatment, CD95 ligand treatment). We will also evaluate the protective effect of macrophage-conditioned medium on the cell death rate of SC and OM preadipocytes. Finally, we will determine the pro- and anti-survival signalling gene expression in SC and OM preadipocytes by quantitative PCR and immunoblot analysis. Depot differences in the preadipocyte pool may be associated with proper metabolic function.

21. **Human Endothelial Colony Forming Cells Attenuate Ischemic Acute Kidney Injury in Mice**

Dylan Burger , Yuan Chung, Alex Gutsol , Anthony Carter, David Allan , Rhian M Touyz ,Kevin D Burns

Background: Acute kidney injury (AKI) occurs in approximately 5% of hospitalized patients with a 50% mortality. While the administration of certain progenitor cell populations has been shown to improve renal recovery following AKI, we recently reported that human cord blood-derived CD133+ progenitor cells unexpectedly exacerbated ischemic AKI in mice, associated with enhanced inflammatory responses and apoptosis (Burger D. et al. Nephrol Dial Transplant 2012). Accordingly, the choice and characterization of progenitor cell populations appears to be critical to cell-based therapy in AKI.

A hallmark of ischemic acute kidney injury (AKI) is microvascular injury that promotes pro-inflammatory and pro-coagulant responses, leading to persistent renal hypoperfusion beyond ischemic reperfusion. Accordingly, strategies targeting endothelial health may accelerate recovery of renal function following AKI.

Objective:

The purpose of this study was to examine the effects of human endothelial colony forming cells (ECFCs) in a mouse model of AKI, induced by bilateral ischemia-reperfusion (I/R).

Methods: ECFCs were isolated from human umbilical cord blood, expanded in culture, and identified as highly proliferative monolayers with a cobblestone morphology. ECFCs (106/mouse) were injected via the jugular vein into non-obese diabetic severe combined immunodeficient (NOD-SCID) mice (male, 8 weeks) at the time of reperfusion. Renal functional and structural outcomes were evaluated.

Results: Phenotypic analysis of human ECFCs by flow cytometry revealed expression of CD31 and VEGFR2 but not CD14, CD45, or CD133, consistent with an endothelial phenotype. Administration of ECFCs attenuated I/R-induced renal injury at 24 hours as revealed by reductions in plasma urea (untreated: 78±2 mM vs. ECFCs: 65±5 mM, $P<0.05$, n=5-6) and creatinine (untreated: 174±6 μM vs. ECFCs: 106±22 μM, $P<0.05$). At 24 hours post I/R, mice receiving ECFCs also displayed less tubular injury, as evidenced by a less pronounced loss of brush border, assessed by cortical megalin expression (untreated: 7.2±0.8% vs. ECFCs: 34.2±0.4 % of sham expression, $P<0.001$), and by reductions in the percentage of anuclear tubules (untreated: 64±1% vs. ECFCs: 57±2%, $P<0.001$). Furthermore, ECFC administration was associated with a significant reduction in I/R-induced renal fibrosis, assessed by a-smooth muscle actin staining, at 72 hours (untreated: 886.8±63.1% vs. ECFCs: 663.1±26.2% of sham expression, $P<0.001$).

Conclusions: Our data indicate that human cord-blood derived ECFCs attenuate renal injury in a mouse model of ischemic AKI. Such effects may be achieved by improvement in post-ischemic endothelial function, and imply a potential therapeutic role for ECFCs in human AKI.

22. **Regions of the CD127 cytoplasmic domain necessary for Tat-induced internalization**

Hafsa Cherid, Paul MacPherson

Background: Impaired cell mediated immunity is the clinical hallmark of HIV infection yet the manner in which CD8 T-cells are disabled is not yet fully understood. IL-7 signalling is essential for normal CD8 T-cell development and function, and our lab has previously shown decreased expression of the IL-7 receptor a-chain (CD127) on circulating CD8 T-cells in HIV+ patients. We also recently illustrated that the down regulation of CD127 is mediated by the HIV Tat protein which results in poor CD8 T-cell function. Soluble Tat protein is secreted by infected CD4 T-cells and taken up by neighbouring uninfected CD8 T-cells through endocytosis. Once in the cytoplasm, Tat translocates to the inner leaflet of the cell membrane where it binds directly to the cytoplasmic tail of CD127 inducing receptor aggregation, internalization, and degradation by the proteasome.

Objective: To demonstrate which region on the CD127 receptor is required for Tat binding.

Methods: To determine which of these regions of CD127 are required for Tat-induced internalization I will use the pCMV6-CD127 plasmid containing the CD127 cDNA cloned downstream of the CMV promoter. I will first construct a series of truncation or deletion mutants removing or replacing each of the four identified regions: a membrane-proximal basic region rich in lysine residues, an acidic region followed by a serine-rich region and a C-terminal tail containing three tyrosine residues, and transfect these into Jurkat cells which do not express the endogenous CD127 gene. Stable Jurkat clones each expressing a mutated version of CD127 will then be incubated with soluble Tat protein and CD127 expression will be monitored by flow cytometry, and physical interaction of the CD127 mutants with Tat

will be assessed by Co-Immunoprecipitation (CoIPs).
Results: We have successfully generated mutants of the CD127 cytoplasmic tail which have been transfected and expressed into Jurkat cells. We also successfully produced recombinant HIV-1 Tat protein to be used in the subsequent analyses.
Conclusions: This work will solve an important part of the puzzle in understanding how Tat down regulates the IL-7 receptor. This work also has important therapeutic implications. If the interaction between Tat and CD127 can be disrupted and IL-7 signalling restored in CD8 T-cells, we may improve cell mediated immunity in HIV+ patients and potentially establish immunologic control of HIV replication.

23. Oncolytic Viruses as a Potential Approach to Eliminate the HIV Reservoir

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Background:
Oncolytic viruses (OVs) are promising cancer therapies due to their ability to selectively replicate in, and kill, malignant cells. Like cancer cells, HIV-infected cells differ from uninfected cells due to alterations in interferon signaling pathways, the apparent reason for selectivity of certain OVs. Thus, use of an OV such as recombinant Maraba virus (MG1) may be a potential approach to eliminate latently infected cells which constitute the HIV reservoir.
Objectives:
1)To determine whether MG1 has a greater propensity to target and kill HIV-infected compared to non-HIV-infected cells
2)To determine whether MG1 decreases HIV proviral DNA in resting (CD4+CD25-HLADR-) T-cells from HIV-infected individuals with suppressed viral loads on HAART
Methods:
U1, ACH-2, OM-10 and J1.1 cells, harbouring 1-2 copies of integrated proviral DNA per cell, were infected with varying multiplicities of infection (MOI) of green fluorescent protein (GFP)-encoded MG1 with or without TNF-a stimulation. Controls included the respective parent HIV-uninfected U937, A301, HL-60 and Jurkat cell lines. Flow cytometry was used to quantify MG1 infection and MTT assay was used to assess cell viability 18 and 24 hours post-MG1 infection. CD4+CD25-HLADR- cells from virally suppressed HIV-infected individuals were infected with MG1 at MOIs of 0.01 and 0.001. Total HIV DNA was quantified 24 hours after MG1 exposure. Replication competent virus was measured by p24 Ag and HIV RNA in supernatants following MG1 exposure and a 2-week stimulation period.
Results:
MG1 infected and killed a greater proportion of U1 than U937 cells at MOIs 0.0001 to 0.01. No significant differences were noted between other HIV-infected lines and the respective parent lines with regards to infectivity or viability, except for J1.1 cells which appeared more resistant to MG1-induced death than Jurkat cells. MG1 did not infect or reduce viability of bulk CD4+CD25-HLADR- cells from HIV-uninfected or virally suppressed HIV-infected individuals. Preliminary results (n=9) suggest that MG1 did not significantly alter the quantity of total HIV DNA in cells nor levels of replication competent virus.
Conclusions:
MG1 infects and kills latently HIV-infected U1 cells to a greater degree than the HIV-uninfected parent U937 cells and may represent a promising model to facilitate further studies of OVs as a potential therapy for the eradication of latently HIV-infected cells. Preliminary results from primary cells suggest that MG1 alone does not appear to eliminate cells which comprise the major HIV reservoir.

24. The role of soluble IL-7 receptor a in murine CD8+ T-cell responses

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Background: The expression of membrane-bound IL-7 receptor a (CD127) is reduced in many viral infections. In HIV infection, this downregulation may be explained in part by increased plasma sCD127 levels. Our first study has shown that sCD127 decreases human IL-7 activity on CD8+ T-cells in vitro. Observed impairment of IL-7 activity in HIV infection may contribute to the decline of cytotoxic CD8+ T-cell (CTL) functions. The role of sCD127 in such immunopathogenesis is not known. Determining the role of sCD127-mediated immune modulation necessitates its study in vivo. To do so, a murine model of CD8+ T-cell activity will be used.
Objective: To establish in vitro conditions for sCD127 and IL-7 and to measure the effects of sCD127 on IL-7 activity on murine CD8+T cells in vitro.
Methods: The effect of sCD127 on IL-7-mediated activity of murine splenic CD8+ T-cells was evaluated using a recombinant CD127-Fc chimeric protein. CFSE-labeled bulk splenic CD8+ T-cells were incubated with medium, IL-7 or pre-complexed IL-7 + CD127-Fc and cell

proliferation (CFSE dilution) was assessed by flow cytometry. Cell viability was also determined using MTT assays. Plasma sCD127 concentrations were measured by ELISA.
Results and Conclusions: Unlike human T-cells, the survival of murine CD8+ T-cells in vitro is dependent on the addition of ?-chain receptor cytokines to the culture medium. The conditions under which CD127-Fc and IL-7 were pre-incubated altered on the activity of IL-7 on murine CD8+ T-cells in vitro. When pre-incubated in PBS or incomplete medium, sCD127 enhanced IL-7-induced proliferation of murine CD8+ T-cells. However, pre-incubation in either PBS-BSA(1mg/ml) or complete medium resulted in either no change or a slight decrease in IL-7-induced proliferation. Lastly, we detected 530 ± 135 ng/ml of sCD127 in murine plasma.

25. ## Cholesterol transbilayer distribution in mammalian cells

Kevin Courtney

Plasma membrane (PM) bilayer asymmetry is a ubiquitous feature of eukaryotic cells involved in maintaining cellular homeostasis. Both proteins and lipids are transversely asymmetric in the PM. Sterols are a major constituent in eukaryotic PM representing about 30% of total PM lipid. In model membranes, cholesterol transbilayer movement is rapid and spontaneous. This is in contrast to phospholipids, which require energy dependent flip-flop transporters. Curiously, recent studies with fluorescent cholesterol analogues suggest that sterols are enriched in the inner leaflet of the PM. It is however not known whether native cholesterol shares this transverse asymmetry. In addition, cholesterol is heterogeneously distributed laterally in the plane of the PM; it is enriched in various dynamic assemblies of lipids and proteins, known as lipid rafts, Lipid raft formation also requires sphingomyelin, a lipid almost exclusively residing in the outer leaflet of the PM. If cholesterol is enriched in the inner leaflet of the PM, similar to its fluorescent analogues, the lipid raft concept would need to be substantially updated.
Objective
The purpose of this study is to analyse mammalian PM cholesterol transbilayer distribution in order to investigate mechanisms and functions of cholesterol asymmetry.
Methods
We developed a protocol that can analyze cholesterol in a leaflet-specific manner by beta-cyclodextrin.
Results
We show that, at 37°C, cholesterol in large unilamellar vesicles (LUV) can be 100% extracted by methyl-ß-cyclodextrin (MCD), consistent with rapid cholesterol flip-flop between bilayers. However, at 0°C, MCD can only extract 50% of the cholesterol from LUVs. When cyclodextrin is used to extract cholesterol from erythrocytes, only 30% cholesterol can be removed from the outer leaflet.
Conclusions
The newly developed cyclodextrin protocol is effective at removing outer leaflet cholesterol at 37°C and 0°C. Cholesterol is evenly distributed in LUVs and 50% cholesterol can be removed at 0°C. However in erythrocytes, cholesterol extraction reaches a plateau at 30%, which suggests asymmetric transbilayer distribution in mammalian cells. We intend to characterize mechanisms and functions of this asymmetry.

26. ## Regulation of Cholesterol Trafficking and Its Effect on Cell Metabolism

Walaa,Eid , Xiaohui, Zha

Mammalian target of rapamycin complex 1 (mTORC1) plays a key role in lipogenesis through its activation of sterol regulatory element binding proteins (SREBPs). The molecular mechanism for this activation is yet not known. mTORC1 is also known to suppress a catabolic process, autophagy. In this study we propose that autophagy accelerates cholestryl ester cycle and increases cholesterol flux through the lysosomes. This leads to SREBP inhibition. Upon mTORC1 activation, autophagy is inhibited and thus activates SREBP. Our objective is to understand how mTORC1, autophagy, CE cycle and SREBP influence each other. Particularly, we propose to investigate whether suppressing autophagy is necessary for mTROC1 to activate SREBP. Our preliminary results show that autophagy accelerates CE cycle and suppresses SREBP. We plan to modulate autophagy by siRNAs and constitutive activations to establish its impact on mTORC1-mediated SREBP activation.

27. ## Phenotypic changes in naïve CD8 T cells induced by CD3/CD28 stimulation are enhanced by interleukin-7

Elliott Faller, Abdulkareem El Salfiti, Paul MacPherson

Background/Objective: IL-7 signaling is important for CD8 T-cell homeostasis, survival and function. In addition to ongoing homeostatic roles, IL-7 is an inducible factor upon viral infection and enhances CTL function. As a result, decreased IL-7 receptor (CD127) expression on CD8 T-cells in HIV+ individuals may not only reduce cell survival but may also disrupt naïve CD8 T-cell activation in response to antigens.
Methods: Naïve CD8 T cells (CD8+ CD45RA+CCR7+CD45RO-CD56-CD57-) were isolated from PBMC obtained from healthy donors. Cells were stimulated in vitro with anti-CD3/anti-CD28 beads +/- IL-7. Flow cytometry was used to follow surface expression of a number of

phenotypic markers over 96 hours and cell division was measured with CFSE..
Results: As expected, stimulation via the TCR resulted in phenotyoic changes in naïve CD8 T cells compared to cells maintained in media. Although IL-7 alone (5 ng/ml) had no effect on any surface markers measured with the exception of CD127, IL-7 significantly enhanced many TCR induced phenotypic changes (both as % expression and MFI). In particular, while TCR stimulation alone induced only a 6% increase in CD25 expression at 24h and an 8% decrease in CD45RA at 96h, the combination of IL-7 plus TCR stimulation resulted in a 56% increase in CD25 and a 60% decrease in CD45RA. Similar effects were seen in the down regulation of CD62L and the up regulation of CD27, CD28, CD8, CCR7, HLADR, CD69, CD38 and CD56. IL-7 also enhanced TCR induced proliferation as measured by CFSE dilution.
Conclusions: Our data suggest that IL-7 signaling plays an important role in CD8 T-cell responses following antigen stimulation. By enhancing expression of a number of activation markers including CD25, IL-7 may facilitate differentiation to the effector phenotype and increase CD8 T cell responsiveness to IL-2. Decreased CD127 expression and impaired IL-7 signaling in HIV+ individuals may then affect CD8 T-cell differentiation and function in response to antigens. Reduced CD127 expression could also play a role in foiling potential HIV vaccination strategies, and IL-7 based therapies.

28. **Extra-thyroidal action of TSH on adipocyte insulin signaling**

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Subclinical hypothyroidism is a condition resulting from mild thyroid failure and is diagnosed by high circulating thyroid-stimulating hormone (TSH), but with normal thyroid hormone levels. While asymptomatic, subclinical hypothyroidism has been independently linked to cardiovascular disease (CVD). Studies have also suggested a positive correlation between TSH levels and insulin resistance. The objective of this study is to evaluate the effect of TSH on insulin signaling in adipocytes, which has been shown to express the TSH receptor. The model systems used are the 3T3-L1 pre-adipocyte murine cell line and pre-adipocytes isolated from human adipose tissue samples. These cells are differentiated into adipocytes in vitro. Stimulation of human differentiated adipocytes with insulin caused a 5-fold increase in Akt phosphorylation (n=4) and a 3-fold increase in lipogenesis (n=5). However, TSH did not interfere with these insulin-dependent events. Further studies examining various time points and targets of insulin will be performed. Increased knowledge of TSH and insulin signalling interaction at the adipocyte level may lead to a greater understanding of the risks associated with elevated circulating TSH.

29. **In vitro HIV infection reduces IL-17 production by human Th17 cells**

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Background: T cell dysfunction persists in HIV+ individuals despite restoration of CD4+ T cell counts to near normal levels with HAART. Selective loss and deficiency of Th17 cells in the gut was recently described and may contribute to microbial translocation and persistent inflammation observed in HIV infection. It is unclear whether this deficiency results from HIV inhibiting Th17 cell differentiation or function. The known interaction of HIV with intracellular signaling pathways related to differentiation and cytokine production may explain the ongoing deficiency in Th17 cells in HIV patients even after HAART. We hypothesize that Th17 differentiation and function is impaired in HIV infection due to altered intracellular signaling.
Objective: The principle aim of this study is to identify the mechanisms by which reduced Th17 function is caused by HIV infection.
Methods: CD4+ T cells were isolated from peripheral blood mononuclear cells and treated with antibodies against CD3/CD28 in the presence of IL-1ß, IL-6, and IL-23 for 12 days to generate Th17 cells as described previously. The resulting Th17 cells were cultured with a dual tropic HIV strain (HIVCS204) for 24 hours. These cells were re-stimulated with PMA and ionomycin to stimulate cytokine production. Expression of Th17 cytokines and signaling pathways involved in Th17 differentiation were assessed by flow cytometry and RT-PCR.
Results: CD161 and CCR6 expression of cultured cells increased following activation in culture, consistent with literature describing in vitro Th17 differentiation. Re-stimulation of uninfected Th17 cells induced production of IL-17, a distinctive Th17 cytokine. IL-17 induction was inhibited when Th17 cells were cultured with HIVCS204 prior to re-stimulation with PMA and ionomycin.
Conclusions: In vitro HIV infection inhibits production of IL-17 by in vitro differentiated Th17 cells suggesting that HIV may play a role in altering Th17 responses. Preliminary analysis indicates that intracellular signaling in response to cytokines involved in Th17 differentiation is altered in the presence of HIV. Understanding the mechanisms of this inhibition may provide insight into therapies that will correct the gut-associated immunopathogenesis in HIV infection

30. **Increased Nox5 Activity Induces Podocyte Damage and Filtration Barrier Dysfunction**

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University of Glasgow, United Kingdom and [1] Background: Reactive oxygen species (ROS) play a key role in glomerular filtration barrier damage. As part of this barrier podocytes are particularly sensitive to ROS. NADPH oxidase (Nox) enzymes are a major source of ROS and their role in the kidney has been well studied. However a paucity of information regarding the role of Nox5 in the kidney remains partly due to its absence from mouse and rat genomes. Here we establish a previously unappreciated role for Nox5 in podocyte damage and diabetic kidney disease in both humans and transgenic mouse models.
Objectives: To investigate a potential role for Nox5 in inducing podocyte damage and filtration barrier dysfunction in diabetes in vitro and in transgenic animal models.
Methods: Human diabetic and control kidney biopsies were examined for Nox5 by immunofluorescence. Immortalized human podocytes (hPOD) were stimulated with TGFb, AngII, stretch, or high glucose and Nox5 expression and activity were examined. The effect of Nox5 knockdown via siRNA was also studied. Similar experiments were performed on mouse podocytes (mPOD) infected with Nox5 adenovirus. Transgenic mice (Nox5Pod+) expressing Nox5 specifically in podocytes were generated and characterized.
Results: Biopsies from diabetic individuals had higher immunodetectable Nox5 expression in glomerular structures. Stimulation of podocytes with TGFb or AngII induced Nox5 expression and activity. Inhibition of Nox5 via siRNA blunted ROS production in response to these stimuli. Increased Nox5 ROS production altered podocyte actin cytoskeleton, resulted in a more motile phenotype, and increased expression of markers of epithelial to mesenchymal transition. Nox5Pod+ mice displayed increased albumin to creatinine ratios as early as 4 weeks following birth. Kidney weight to body weight was increased in Nox5Pod+ compared to non-transgenic littermates indicative of kidney hypertrophy. QPCR on mRNA from kidney cortex demonstrated elevated Cox2 expression and decreased nephrin expression in Nox5Pod+ compared to non-transgenic littermates.
Conclusions: Upregulation of Nox5 in diabetic kidney occurs in response to classic diabetic stimuli and contributes to ROS–induced podocyte damage and filtration barrier dysfunction.

31. **(Abstract Withdrawn)**

32. **Dysregulated Ovarian Follicular Development in a Rat Model for Polycystic Ovarian Syndrome**
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Background: PCOS is a complex endocrine and metabolic syndrome, which is characterized by anovulatory infertility, hyperandrogenism and insulin resistance. Also, it is associated with suppressed granulosa cell proliferation, differentiation and ovarian follicular growth arrest at the early antral stage of development. We have recently established in our laboratory a rat model which recapitulates many of the phenotypes of human PCOS. Objective: To characterize the dysregulated ovarian follicular development in this experimental PCOS model. Methods: 21 day-old rats were implanted with a silastic capsule containing Dihydrotestosterone (DHT; daily release of 83 µg) to mimic the hyperandrogenic state in women with PCOS. Animals were sacrificed 12 weeks later and ovaries were fixed and paraffin- embedded for morphological (H & E and PAS staining) and immunohistochemical analyses. Cleaved casapse-3, aromatase, calpain, p-AKT and PTEN levels were analyzed by immunofluorescence. The influence of DHT on granulosa cells apoptosis was assessed in vitro. Results: DHT treatment resulted in ovary structural changes, which is associated with decreased follicle number in preantral to preovulatory stages and absence of corpus luteum, but increased number of condensed atypical follicles. Atypical follicles, constituting predominantly of theca cells, exhibited high expression of calpain and down-regulation of its cytoskeleton protein substrates vimentin, fodrin and ß-tubulin. Granulosa cell aromatase expression was down-regulated and apoptosis (active-caspase-3 and DNA fragmentation) increased. Whereas phospho-AKT (Ser473) content was decreased in granulosa cells in the PCOS rats, PTEN level was considerably higher than control animals, suggesting that down-regulation of phospho-AKT could have been induced by PTEN in vivo. DHT also increased granulosa cell cleaved caspase-3 level, decreased XAIP, PARP and phospho-AKT contents and induced apoptosis in a concentration-dependent manner in vitro. Forced expression of XIAP attenuated DHT-induced caspase-3 activation and PARP down-regulation in granulosa cells in vitro. Conclusion: The dysregulated follicular development in a PCOS rat model is characterized by marked changes in the ovarian structure, follicular growth dynamics, follicle cell fate and function, many of phenotypes resembling those of the human PCOS. These findings indicate that this chronically androgenizied rat PCOS model may be useful not only for studies on the long term effects of androgen on folliculogenesis, but also on the pathophysiology of this complex syndrome [Supported by grants from CIHR (BKT) and WCU Biomodulation Major. JYK is a recipient of a CIHR-QTNPR Postdoctoral Fellowship].

33. **Suppressed Opa1 Processing and Mitochondrial Fission in Chemoresistance in Ovarian Cancer Cells**
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Background: Mitochondria are highly dynamic organelles, constantly elongating and dividing (i.e. fusion and fission). Mitochondria undergo fragmentation when cells undergo apoptosis. Optic atrophy 1 (OPA1) is a dynamin-related GTPase required for mitochondrial fusion. It is also processed by proteases when mitochondria undergo fission. How mitochondrial dynamics and Opa1 processing are dysregulated and involved in the regulation of chemosensitivity is not known. Our overall objective is to better understand the molecular and cellular mechanisms of chemoresistance in human ovarian cancer (OVCA) by investigating a novel metallopeptidase Oma1and its substrate Opa1 in the control of mitochondrial dynamics. We hypothesize that chemosensitivity is determined by dysregulated mitochondrial dynamics and is dependent on the relative levels and activities of gene products regulating mitochondrial fission and fusion. Specifically, chemoresistance is associated with suppressed CDDP-induced Opa1 down-regulation and mitochondrial fission. Methods: chemosensitive cells (OV2008) and chemoresistant variant (C13*) were treated with cisplatin (CDDP; 0 – 10 μM). Mitochondria dynamics and protein contents were assessed by immunofluorescence and Western blot, respectively. Results: OV2008 cells exhibit much higher level of mitochondrial fission than C13* cells when challenged with CDDP. Whereas CDDP increases the content of the active protease Oma1 and decreases the levels of the long form of Opa1 in OV2008 cells in a concentration- and time-dependant manner, these changes were not evident in C13* cells. Contents of other proteins involved in mitochondrial fission (Fis1) and fusion (Mfn1 and Mfn2) were not affected by CDDP in both sensitive and resistant cells. Conclusion: dysregulated mitochondrial dynamics may be an important determinant of chemoresistance which may in part be mediated by suppressed processing of Opa1.

34. Calcium Signaling during Polar Body Emission in Xenopus laevis

Julie Leblanc, Rui Zhen Li; X. Johné Liu

Background

Meiosis is a process in which the ploidy number is halved in order to allow for the normal development of the embryo. Meiosis in the female is an extreme form of asymmetrical cell division, producing two polar bodies and one functional egg. Understanding the molecular mechanisms of this process will ultimately improve our knowledge of general cytokinesis, which is relevant to the field of cancer research; as well as oocyte health, a quality required for proper embryogenesis. Using confocal time-lapse 3D imaging, our lab has previously demonstrated that the GTPases, cdc42 and RhoA, are required for first polar body emission (PBE) in Xenopus laevis. Cdc42 regulates dynamic actin polymerization to create the membrane out pocketing required for PBE. RhoA, on the other hand, regulates the formation and constriction of the contractile ring required for cytokinesis. We have further demonstrated that cdc42 is required during second PBE. The question remains: "What is the universal cue that regulates PBE?". Calcium is an important candidate since it is a ubiquitous signal that is involved throughout the general cell cycle. The role of calcium signaling in meiosis is less well understood.

Objectives

Identify the calcium signals that occur during meiosis, as well as their biological importance.

Methods

Confocal 3D live cell imaging is used to follow Xenopus laevis meiosis is real time. Fluorescent probes identify the important factors involved. In addition, chemical inhibitors are applied to functionally assess the importance of these signals. An abscission assay is used to determine the "open" or "closed" state of the polar body.

Results

In order to specifically assess calcium signaling during PBE, we used a probe that specifically binds to phosphatidylserine, but only when it is bound to Ca2+. While calcium increases during anaphase, a most evident increase occurs as a "flash", later during anaphase and when the contractile ring is nearly constricted to its maximum. This "flash" localizes to the contractile ring, as confirmed by co-localization experiments. We developed an abscission assay that is able to determine the status of PBE. Preliminary evidence suggests that a weak calcium cheater (caged Diazo-2) is able to inhibit the physical closure of the polar body.

Conclusions

Calcium increases during meiosis at two distinct time points, as well as distinct structures during anaphase. In addition, preliminary experiments suggest that calcium regulates abscission in the Xenopus laevis oocyte. Being that the "flash" is spatially as well as temporally more relevant to abscission time, we believe that this novel "flash" regulates an essential regulatory step in the final stage of PBE – abscission.

35. Preovulatory rise of ovarian ornithine decarboxylase may exert functions in uterine decidualization and implantation

Dandan Liu

Background: It was discovered decades ago that ornithine decarboxylase (ODC), a key factor in the biosynthesis of the polyamines, markedly rises in the ovaries during late proestrus in marine. David V. Maudsley and Yutaka Kobayashi found that ODC can also be stimulated by luteinizing hormone (LH) or human chorionic gonadotrophin (hCG). A peak is observed between 3 to 5 hr after which the enzyme activity declines rapidly. It was reported that ODC-heterozygous mice are viable, normal and fertile, but homozygous null ODC mice are lethal after implantation. Carmen M. Bastida et al. reported that treatment of adult female mice with DFMO at proestrus result in

decreased blood vessels of corpus luteum and reduced plasma progesterone and estradiol at diestrus.
Objective: This suggests that ovarian preovulation ODC peak may have an impact on embryo implantation through progesterone and estradiol. Our goal is to investigate if ovarian preovulation ODC play any role in at early pregnancy.
Methods and results: Our recent research shows that blockade of the preovulation ODC surge with a-difluoromethylornithine (DFMO), a spicific inhibitor of ODC, in mice ovary produce normal number of oocytes, unremarkable change of implantation sites and litter size. This may be because, as reported by Milligan and Finn, that as low as 25% of physiological level of progesterone is sufficient to allow implantation . So we are going to find out if levels of progesterone in pregnant mice decreased when preovulatary ODC surge has been inhibited. And combination of DFMO and RU486, a progesterone receptor antagonist, will be used to investigate the function of ODC on mouse implantation and decidualization.
Conclusions: According to literatures and our primary data, we hypothesize that ovarian preovulation ODC surge may exert functions in early pregnancy.

36. ## A Novel Cell Patterning Method Using a Photo Labile Caged-Peptide for Applications in Spinal Cord Injuries

Chunyu Lu

Background:
Currently, the way to cure the injury of spinal-nerve has been hindered by the learned method to grow nerve cell especially in a particular manner. Recently, the discovery of photo-sensitive caging groups, which could shelter the function of molecule and the invention of two-photon laser instrument has made this goal more achievable.
Objective:
1. To prepare a novel RGD containing photo-caged peptide.
2. To apply the above peptide in various cell lines of interest in spinal cord injuries for examination of their growth in a directed pattern.
3. To immobilize the above peptide in biocompatible resin like hydrogel for future applications in model animals.
Methods:
The following circular peptide has been designed that contains the RGD sequence labeled with ANP group (3-Na-Fmoc-Amino-3-(2-nitrophenyl)propionic acid) at the amino terminus. This peptide also contains a Carboxyl terminal Lys group which allows it to cyclize with the free amino terminus.
Cyclic-(HN-ANP-Arg-Gly-Asp-Ser-Adoa-Lys-CO)
The linked Adoa(Fmoc-8-Amino-3,6-Dioxaoctanoic Acid) is incorporated before the Lys for the purpose of easy cyclization. The purpose of having an extra Lys is to allow the peptide when needed to attach to hydrogel.
Product is confirmed by HPLC, MS and Circular Dichroism Spectroscopy and its bioactivity is tested by photo-controllable directing cell culture.
Results:
This Peptide has already been successfully synthesized by using Solid Phase Peptide Chemistry and fully characterize by HPLC, Proton NMR and MS spectroscopies. The secondary structure analysis of this peptide has been conducted in aqueous solution of neutral PH using Circular Dichroism Spectroscopy. Our result indicated a predominant turn structure in the molecule.
Conclusions:
In future, our product following immobilization could be useful for implantation in the damaged area of spinal cord injury. This may form a novel technic for treatment of such injuries.

37. ## Pre-diabetic Signature in Prospective Pancreas Biopsies and Microbiome of the Diabetes-prone Rat

J. Ariana Noel, Dr. Gen-Sheng Wang , Christopher Patrick, Turki Abujamel, Dr. Alain Stintzi, Dr. Fraser W. Scott

Introduction: Type 1 diabetes (T1D) is a polygenic autoimmune disorder targeting ß-cells of the pancreas that develops as a result of a complex interplay between genetic and environmental factors. The T1D disease fingerprint remains poorly understood. In this study, the gene expression signature of pre-diabetic and T1D-resistant BioBreeding diabetes-prone rat (BBdp) pancreata and fecal microbiome was studied early in the disease program to identify novel candidate biomarkers and microbial populations in T1D development.
Materials and Methods: Young, pre-insulitic BBdp rats (n=26) maintained in specific pathogen free conditions and fed a cereal-based diet underwent partial pancreatectomy (30% PPx) or sham operation (n=23) at age 30 d. Rats were monitored for T1D until 150 d at which time the pancreas biopsies were designated as pre-diabetic or T1D-resistant. RNA from biopsies was isolated for analysis using Affymetrix Rat Gene 1.0 ST microarrays. Semi-quantitative qRT-PCR was used to validate microarray candidates in individual animals (n=6-7). Tissues were fixed in Bouin's fixative and stained for candidate markers TRIM26, REG3ß, HDAC1 and REG3? followed by morphometric analysis. Prospective fecal samples obtained at 30 d were analyzed by Illumina Hiseq2000 sequencing and categorized by phylogeny.
Results: Diabetes-incidence was similar in PPx and sham-operated rats. Pancreas genes involved in regeneration and chromatin remodeling were upregulated in pre-diabetic rats: Trim26 (9.8 fold change), Hdac1 (3.9), Reg3a (3.8), Reg3ß (3.1), Reg3? (1.8). qRT-PCR validation showed upregulation of Trim26 (p=0.05), Reg3a (p=0.03), and Reg3ß (p=0.02) in the pre-diabetic cohort. Trim26 was 2-fold

higher in pre-diabetic rats. Reg3a and Reg3ß, factors with regenerative and antimicrobial functions, were 7-fold and 2-fold higher by qRT-PCR in pre-diabetic rats. Staining for TRIM26, REG3ß, HDAC1 and REG3? was observed mainly in the islet core, suggesting a role in ß-cell biology. Morphometric analysis of HDAC1, REG3? and REG3ß revealed a similar trend as observed by qRT-PCR, but this did not reach statistical significance. Staining with the M1 macrophage marker, CD68, revealed vasculitis as an early sign of inflammation in pre-diabetic rats (p=0.04). Compared with rats that remained diabetes-free, the 30 d gut microbiome from pre-diabetic rats displayed more abundant sequences from Acinetobacter sp. and other selected bacteria.
Discussion: Trim26 is a member of the tripartite motif containing protein family with several pleiotropic functions related to cell growth, differentiation and antiviral defense. However, the function of Trim26 itself is unknown and it has not previously been associated with T1D. The upregulation of Reg3a and Reg3ß suggests that antimicrobial innate defenses and/or regenerative processes are invoked in the pancreas of very young animals in an unsuccessful attempt to counter diabetogenic processes. These changes occur soon after weaning and before insulitis. Around the same time, there is inflammation in the pancreas vasculature which includes a population of M1 macrophages, suggesting vascular inflammation is among the earliest features of T1D pathogenesis. The upregulation of several islet Reg genes and Trim26 could also suggest a novel role for antimicrobial responses early in the pre-diabetic program. This would be consistent with the unique pre-diabetic microbiome and defects in the gut immune system.

38. **Cathelicidin Anti-Microbial Peptide (CAMP) - a potential islet trophic factor and biomarker of gut CD163 regulatory macrophages**

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Background: Intestinal inflammation elicited by environmental factors such as dietary proteins and microbes is implicated in type 1 diabetes (T1D) pathogenesis. T1D incidence is low in diabetes-prone BioBreeding (BBdp) rats fed a low-antigen hydrolyzed casein (HC) diet compared with a standard cereal diet. We found that Cd163 gene expression and the number of CD163+ tolerogenic M2 macrophages in the BBdp jejunum was increased in diet-protected HC-fed rats compared with cereal-fed rats.
Objective: We sought to identify additional diet-modifiable immune factors in the small intestine of 130 d BBdp rats fed diabetes-modifying diets by screening a panel of innate and adaptive pro- and anti-inflammatory immune factors.
Methods: BBdp rats were fed either a cereal- or HC diet and asymptomatic rats were killed at 130 d. RNA from jejunum was isolated and cDNA was synthesized. The long-term effect of diet on gene expression of intestinal immune factors in BBdp rats was surveyed using real time PCR Arrays for Innate and Adaptive Immune Responses (PARN-052A, Qiagen/SABiosciences); n=4 arrays/diet). Immunohistochemistry, morphometry, and confocal imaging were used for additional confirmation.
Results: There was a significant 2.4-fold up-regulation of the Camp gene in rats fed a T1D-protective HC diet compared with a standard cereal-based diet (p=0.03). Camp encodes the cathelicidin anti-microbial peptide (CAMP), a major multifunctional anti-microbial protein and immunomodulatory host defence factor. In BBdp rats, intestinal CAMP+ cells were distributed throughout the epithelium and lamina propria; CAMP co-localized with CD163 and CD14, revealing it is a possible novel biomarker of tolerogenic M2 macrophages in gut. Serendipitously, CAMP+ cells were identified in pancreatic lymph nodes and islets. CAMP expression was detected independently of infection or microbial exposure in adult germ-free and embryonic (E14) BBdp rats, suggesting a role in islet development. Consistent with this, CAMP+ cells were also detected in pancreatic tubular complexes which are regenerative structures implicated in ß-cell neoformation. In ~150 d asymptomatic cereal-fed BBdp rats, there was a positive correlation between the frequency of CAMP+ cells per islet area and total islet number. CAMP was also detected in islet remnants of all pancreata from recent-onset T1D subjects (n=15, 6-27 years of age). CAMP+ cells were also observed in peri-ductular areas and in pancreatic lymph nodes.
Discussion and conclusion: The Camp gene was up-regulated in the jejunum of HC-fed, diet-protected BBdp rats. Intestinal co-localization of CAMP with CD163 and CD14 suggests it could be a marker of regulatory M2 macrophages. In addition, expression of an anti-microbial peptide in rat islets, even under sterile conditions and in tubular complexes further suggests CAMP could represent a naturally-occurring pancreatic islet trophic factor. The identification of CAMP in human pancreas further suggests a role for this protein in T1D patients. Thus, CAMP could have several pleiotropic functions in regulating intestinal immune homeostasis, gut microbiota and islet homeostasis.

39. **Glucose Modulates the Ability of Macrophages to Suppress Adipogenesis and Attenuate Triacylglycerol Synthesis**

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Obesity is an excess accumulation of adipose tissue that results from a chronic positive energy balance, via the recruitment and differentiation of preadipocytes into adipocytes (adipogenesis) and/or hypertrophy of existing adipocytes. Insufficient adipogenic capacity leads to dysfunctional adipocyte hypertrophy that raises the risk of insulin resistance and type 2 diabetes. Obesity is also

associated with increased inflammatory (M1 state) macrophage infiltration within adipose tissue. Our laboratory and others have shown that macrophage-secreted factors prevent adipogenesis. Glucose is a dietary factor that activates macrophages. Therefore we hypothesize that exposure of macrophages to increased glucose levels modulate their secretion of anti-adipogenic factors. Monocyte derived macrophages (MDMs) and THP-1 human macrophages were exposed to either high (25 mM) or normal (5 mM) glucose for 24-48 hours. The conditioned medium was collected and its effect on differentiation of human subcutaneous abdominal preadipocytes was evaluated. THP-1 conditioned medium (THP-1 CM) generated in the presence of 25mM glucose inhibited human preadipocyte differentiation as assessed by the expression of adipogenic markers, PPAR? and aP2, by 66% and 11%, respectively; however this inhibition was only minimally observed in THP-1 CM generated in the presence of 5mM glucose (N=1). Furthermore, using CM generated from MDMs (MDM CM) in high glucose attenuated triacylglycerol accumulation in mature adipocytes by 49%, compared to only 23% inhibition with MDM CM prepared in normal glucose (N=2). These preliminary results suggest that dietary factors such as glucose may influence adipose tissue function by modulating anti-adipogenic factors produced by macrophages.

40. **The Role of Ubiquitin C-Terminal Hydrolase L1 (UCH-L1) in ACTN4-Associated Focal Segmental Glomerulosclerosis (FSGS)**

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Background: Podocyte UCH-L1 is upregulated in ACTN4-associated FSGS and may promote ubiquitin proteasome system (UPS) and macroautophagy (MA) impairment.
Objective: Loss of UCH-L1 should improve podocyte injury in ACTN4-associated FSGS.
Methods: UCH-L1+/- and K256E-ACTN4pod+ mice were crossed to generate K256E-ACTN4pod+/UCH-L1-/- mice. Glomeruli were scored for sclerosis using PAS stained kidney sections. Albuminuria (albumin/creatinine) was determined by ELISA. Conditionally immortalized podocytes were infected with adenovirus containing GFP, WT or K256E a-actinin-4 (aA4) for 3 days and treated with LDN57444 (UCH-L1 inhibitor; 50μM; 24h), or mechanically stretched (24h). Protein expression and localization were assessed by western blot and IF.
Results: K256E-ACTN4pod+ mice show segmental and global (35.5% and 31.3% of glomeruli) glomerulosclerosis, with 2519μg/mg albuminuria by 10 weeks of age. K256E-ACTN4pod+/UCH-L1-/- mice exhibit reduced segmental (14.2%) and global (16.6%) glomerulosclerosis at 10 weeks with 569μg/mg albuminuria. Renal ubiquitin (Ub) is elevated in K256E-ACTN4pod+ mice while levels are normalized in K256E-ACTN4pod+/UCH-L1-/- mice. K256E aA4 levels were not reduced by UCH-L1 inhibition nor was peripheral localization ameliorated. Similar to WT aA4 expressing cells, UCH-L1 inhibition increased poly-Ub proteins and cleaved caspase-3 in K256E aA4 expressing cells. LC3II levels were also elevated, suggesting MA activation. Interestingly, UCH-L1 inhibition led to loss of UCH-L1 expression in podocytes expressing GFP or WT aA4 but not K256E aA4. Preserved UCH-L1 expression may be due to an impaired UPS or MA pathway. Similar reductions of mono-Ub correlated with UCH-L1 expression. Finally, mechanical stretch decreased LC3II levels in K256E aA4 expressing podocytes, suggesting MA impairment.
Conclusions: Our data suggest that K256E aA4 is associated with UPS/MA dysfunction and that loss of UCH-L1 may relieve these pathways in a mouse model of FSGS, reducing poly-ubiquitinated K256E aA4 aggregates.

41. **Clusterin re-localization during mouse sperm capacitation: a bioindex for sperm capacitation?**

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Background and Objectives: Clusterin (CLU) is a ubiquitously expressed sulfated glycoprotein involved in biological processes such as lipid transport, cell interaction, and as a chaperone preventing certain proteins from precipitation. CLU is present abundantly in the epididymal fluid, presumably the source of CLU on the sperm surface. As a chaperone, CLU may prevent some egg-binding proteins in the epididymal fluid from precipitation and transport them onto the sperm surface. To date, information on the amounts and functions of epididymal CLU is not available. To gain a better understanding of its functions, we therefore characterized CLU distribution profile in the epididymal fluid and sperm. CLU has affinity for cholesterol, its proposed binding partner on the sperm surface. Since cholesterol efflux occurs during capacitation, we then determined whether sperm CLU localization changed during this event.
Methods: Caput epdidymal sperm were centrifuged through a 45% Percoll solution to remove loosely bound components. Motile populations of caudal epididymal sperm were prepared by Percoll gradient centrifugation. Epididymal fluid was centrifuged (14,000g) to remove subcellular particles. Caudal sperm were capacitated in medium containing 0.3% BSA or 0.75 mM methyl-ß-cyclodextrin. Antibody against CLUa was used in immunofluorescence/flow cytometry, and immunoblotting from which CLU from epididymal fluid and sperm was quantified by densitometric analyses using recombinant CLU as a standard.
Results and Conclusions: Caput epididymal fluid contained 9x higher CLU than caudal fluid (10.48 vs 1.22 μg in one animal). In contrast, CLU was present only on caudal sperm. Possibly, the sperm migration through the caput region is too fast for CLU to deposit onto the

sperm surface. Significantly, total CLU amounts in fluid (0.61µg) and sperm (0.55 µg) in each epididymis side were almost the same, suggesting that CLU adsorption onto the sperm surface occurred with ease. Immunofluorescence revealed CLU staining patterns only in the caudal sperm head. Interestingly, sperm CLU re-localized during capacitation. On non-capacitated caudal sperm, the CLU staining pattern was punctate over the entire ventral head region; however, following capacitation, CLU staining became more intense on capacitated sperm and it was only at the hook region of the head. This movement and more exposure of CLU on the capacitated sperm surface suggested that CLU may be involved in capacitation. Possibly, its relocalization to the hook region reflects its dissociation from the egg-binding proteins, allowing them to bind to the zona pellucida. These CLU associated events may thus serve as a capacitation bioindex.

42. Acquired immunity in patients on different hemodialysis modalities

Andreea Slatculescu, Todd Fairhead

Background:

Patients with end-stage renal disease (ESRD) exhibit various deficiencies of acquired immunity. Clinical analyses indicate that renal failure is associated with a state of chronic inflammation, marked by elevated levels of serum inflammatory markers (IL-6, TNF-?, CRP, IL-1, IL-12); and, with suboptimal immune responses against infections and vaccines (HBV, DTP, influenza). Further research studies suggest that this immune impairment in ESRD is partially caused by deficiencies in T-lymphocytes and antigen presenting cells (APCs). Monocyte-derived dendritic cells (moDCs), in particular, exhibit reduced endocytotic abilities, delayed maturation, and diminished antigen presentation. Concomitantly, T-lymphocytes also show decreased activation and proliferation after stimulation.

Renal replacement therapy, which includes hemodialysis (HD), is life sustaining for ESRD. However, HD is still accompanied by various complications and a high rate of mortality compared to the general population. Extended home hemodialysis (EHHD) is an alternative method in which patients receive longer and more frequent hemodialysis. These patients show many health improvements compared to conventional HD patients; however, it is currently unknown if these ameliorations are correlated with enhanced immunity.

Objectives:

We hypothesize that patients receiving EHHD have decreased inflammation and exhibit improved immunity compared to conventional HD patients. More specifically, we aim to measure the baseline serum markers of inflammation, to test the T-lymphocyte response to stimulation, and to assess the functional improvements in moDCs by determining antigen uptake, expression of cell surface maturation markers, and cytokine production. Furthermore, we intend to explore the possible mechanisms by which ESRD and chronic inflammation affect the functionality of both moDCs and T-lymphocytes in our study population.

Methods:

We have designed a prospective matching-cohort research study to compare immune responses in HD patients vs. EHHD patients vs. healthy controls. HD patients will be matched based on co-morbidities with our EHHD patients. We plan to recruit at least 20 individuals in each study group and to date we have enrolled several healthy volunteers and EHHD patients.

Results/Conclusion:

We have completed troubleshooting experiments and finalized our experimental designs for the analysis of moDCs maturation by flow cytometry and T-lymphocyte proliferation by carboxyfluorescein diacetate succinimidyl ester (CFSE) labeling. Our preliminary studies show that moDCs from healthy volunteers and EHHD patients exhibit similar maturation phenotypes and phagocytic function suggesting that EHHD may help improve immunity in patients with renal failure.

43. Antimicrobial Peptide, LL-37, as a Potential Vaginal Contraceptive with Anti-Sexually Transmitted Infection (STI) Property

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The continuous increase in world population calls for new contraceptive development. Significantly, the highest population growth occurs in the areas (e.g., Subsaharan) where sexually transmitted infections (STIs) are prevalent. It is therefore desirable to find a compound that can act dually as a contraceptive and anti-STI agent. LL-37 is a cationic antimicrobial peptide produced in the vagina (at 2- 10 μM) hours after the intercourse by which time motile sperm have entered the uterine cavity. LL-37 has microbicidal/virucidal activities against STI-causing pathogens including HIV-1. Should LL-37 disable sperm functionality, it would be an ideal vaginal contraceptive that can protect women from microbial/viral attacks. We hypothesize the dysfunctional activities of LL-37 on sperm based on the fact that LL-37 interacts with microbial surface anionic molecules (e.g., lipopolysaccharide), leading to membrane damage and microbial necrosis. Sperm uniquely possess anionic sulfogalactosylglycerolipid (SGG) at 10 mole% of total lipids. Therefore, LL-37 may cause sperm damage following its interaction with SGG. We indeed demonstrated direct binding of LL-37 to SGG monolayers (Kd=455.7±59.1 nM). We further showed that LL-37 (0.36 and 3.6 μM) bound to mouse and human sperm heads and tails, and pretreatment of capacitated mouse sperm with LL-37 inhibited their fertilizing ability in vitro in a dose-dependent manner, with zero fertilization rate at 3.6 μM. The decreased fertilizing ability of sperm was attributed to their reduced motility and binding to the zona pellucida, as well as the premature acrosome reaction. At 3.6 μM LL-37, all sperm became immotile and 80% acrosome reacted. As observed in microbes, LL-37 exerted its primary action on the sperm surface membranes. LL-37-treated mouse and human sperm became positively stained with Sytox Green, a membrane impermeant DNA dye, a result indicating membrane permeabilization. Electron microscopy further supported these results; specific damages on the mouse sperm head plasma membrane and the outer acrosomal membrane were observed on LL-37 treated sperm prior to the premature acrosome reaction. Finally, the in vivo contraceptive effects (with 70% efficacy) of LL-37 were revealed in female mice inseminated with sperm suspended in 3.6 or 5.4 μM LL-37-containing medium. However, histology of the reproductive tract of these inseminated females was normal. In addition, LL-37 at the contraceptive doses did not significantly affect the membrane integrity of the human vaginal and ecto-/endo-cervical cell lines, as assessed by Sytox Green exclusion. Therefore, LL-37 with microbicidal/virucidal properties is a promising candidate to be developed into a vaginal contraceptive.

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44. Deficiency of ovarian ornithine decarboxylase contributes to aging-related egg aneuploidy in mice

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It has been known for more than four decades that during mammalian estrous cycles, luteinizing hormone stimulates a transitory rise in the ovaries of ornithine decarboxylase (ODC) activity and its enzymatic product putrescine, concurrent with oocyte maturation in vivo. Inhibition of this transitory ODC/putrescine rise, however, does not appear to affect oocyte maturation or ovulation. Using several mouse models and combining in vitro and in vivo approaches, we demonstrated that deficiency of ODC during oocyte maturation is correlated with increased levels of egg aneuploidies. These results suggest that the transitory ovarian ODC rise in late proestrus is important for ensuring proper chromosome segregation during oocyte maturation. Older mice (8 months of age) exhibited about 1/3 that of young mice in LH-stimulated ovarian ODC activity and a corresponding increase of egg aneuploidies. Moreover, a combination of putrescine supplementation in mouse drinking water leading up to oocyte retrieval and in oocyte maturation medium reduced egg aneuploidies of the old mice from 12.7% to 5.3%. Therefore, ovarian ODC deficiency might be an important etiology of maternal aging-related aneuploidies, and peri-ovulatory putrescine supplementation might reduce the risk of aneuploid conceptions in older women.

45. The Prostaglandin E2 EP1 Receptor Promotes Glomerular and Tubular Dysfunction in OVE26 Diabetic Mice

Jean-Francois Thibodeau, Anthony Carter, Chris Kennedy

Background:

Cyclooxygenase (COX)-derived prostanglandin E2 (PGE2) synthesis and downstream EP receptor activation contributes to diabetic nephropathy (DN). COX inhibition lowers albuminuria and renal damage in human DN and experimental models. Given that pharmacological EP1 antagonism is beneficial in diabetic rats, we hypothesized that the Gq-coupled EP1 receptor promotes glomerular and/or tubular damage in DN.

Methods:

Our prior studies revealed that gene-targeted EP1 knockout mice (EP1-/-), subjected to the low-dose streptozotocin (STZ) model of type 1 diabetes (T1DM), were significantly less albuminuric than wild-type (WT) mice at 16 weeks. Despite comparable glomerular damage, EP1-/- mice were protected from megalin downregulation, a marker of proximal tubule (PT) injury. We backcrossed EP1-/- mice onto the OVE26 transgenic model of T1DM (OVE26EP1-/-) and measured various functional and structural parameters.

Results:

At 8 and 26 weeks, albuminuria was exacerbated in the OVE26 cohort compared to the OVE26EP1-/- mice (8 weeks: OVE26, 865±119 vs. OVE26EP1-/-, 508±66 μg/24 hrs, $p<0.001$; 26 weeks: OVE26, 2762±1067 vs. OVE26EP1-/-, 1022±395 μg/24 hrs, $p<0.05$) mirroring our STZ-study findings (WTstz, 1546±282 vs. WT, 525.8±110 μg/24 hrs, $p<0.001$). However blood pressure and glomerular filtration rates were elevated in both diabetic groups. EP1 receptor deletion reduced the extent of glomerular hypertrophy (OVE26, 9447±396vs.

OVE26EP1-/-, 7397±315 µm2, p<0.001) as well as mesangial expansion (OVE26, 35.07±1.19vs. OVE26EP1-/-, 26.34±1.01% of glomerular area, p<0.001) while dramatically increasing survival rates. Semi-quantitative PCR revealed that OVE26EP1-/- mice were protected from DN-induced megalin downregulation, while nephrin mRNA decreased in both diabetic groups.
Conclusions:
These results implicate the EP1 receptor in DN, downstream of the classic COX2/ PGE2 pathway. Our data are consistent with the idea that EP1 activation may exacerbate filtration barrier damage in DN by increasing podocyte and/or mesangial cell damage while promoting dysfunctional post-glomerular albumin processing via the PT's megalin/ cubilin complex.

46. **Role of chemerin and prohibitin on FSH-induced Steroidogenesis during follicular development**

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Background: Follicular growth and differentiation are tightly regulated by endocrine, paracrine and autocrine factors. The coordinated biosynthesis of ovarian steroids (estradiol and progesterone) in response to gonadotropins and intraovarian factors is critical for reproductive cycle, successful ovulation and eventual pregnancy. However, the action of gonadotropin during follicular development is complex. Chemerin, a novel adipokine, has recently been reported to be expressed in the ovary and its serum and ovarian levels are elevated in women with polycystic ovarian syndrome (PCOS) and a rat PCOS model, known to be associated with dysregulated steroids production. Prohibitin is a multifunctional protein important for cell cycle regulation, apoptosis and cellular differentiation. However, whether and how chemerin and prohibitin are involved in the regulation of ovarian steroidogenesis is unknown.
Our objective is to better understand the function and regulation of chemerin and prohibitin during follicular development, especially during FSH-induced steroidogenesis. We hypothesize that chemerin and prohibitin are two novel factors which negatively regulate FSH-induced steroidogenesis.
Methods: Granulosa cells from immature rats were cultured with FSH (100 ng/ml), or chemerin (100 ng/ml) or combinations and protein expression and mRNA abundance of steroidogenic enzymes (cyp19 and cyp11a1) were determined (Western blot and real-time PCR). Estradiol and progesterone secretion in spent medium were assessed by EIA. Granulosa cells were infected with adenoviral-shProhibitin (shNeg as negative control) for 48h to knockdown prohibitin in this study.
Results: The expression of chemerin and prohibitin in granulose cells was down-regulated by gonadotropin in vivo and in vitro. Both recombinant chemerin and exogenous prohibitin significantly reduced FSH-stimulated steroids production. They also suppressed the expression of steroidogenic enzymes at the transcriptional and translational levels. Prohibitin expression was up-regulated by chemerin and knockdown of prohibitin attenuated the suppression of aromatase expression induced by chemerin.
Conclusion: This study demonstrates that chemerin and prohibitin are novel negative regulators of follicular steroidogenesis. Our findings raise the possibility that the dysregulation of chemerin and prohibitin expression and function may be linked to the infertility associated with compromised follicular growth and differentiation (e.g. PCOS). Supported by CIHR (BKT) and CIHR-QTNPR scholarship (QW)

47. **Angiotensin-Converting Enzyme 2 (ACE2) is Shed from Proximal Tubular Cells Via ADAM-17**

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BACKGROUND: Angiotensin-converting enzyme 2 (ACE2) is highly expressed in the proximal tubule (PT) where it converts (damaging) angiotensin (Ang) II to (protective) Ang-(1-7). We recently reported that urinary ACE2 activity increases in renal transplant patients with diabetes (PLoS ONE 2012), suggesting that ACE2 may be shed from PT cells.
OBJECTIVE: The purpose of this study was to determine pathways for proximal tubular ACE2 shedding, and to characterize shed fragments.
METHODS: Studies involved primary cultures of mouse PT cells, as well as urine samples from mice with streptozotocin (STZ)-induced diabetes and renal transplant patients. ACE2 activity was measured using a fluorogenic substrate, and ACE2 fragments were analyzed by immunoblots and mass spectrometry.
RESULTS: In cultures of mouse PT cells, a time-dependent increase in ACE2 activity was observed in the media. ACE2 activity was also increased in the media from PT cells from ACE2 knockout mice that were transfected with a human ACE2 vector (p<0.001). Incubation of PT cells in high D-glucose (25 mM) for 48-72 h significantly increased ACE2 activity in the media (p<0.001), an effect blocked by a disintegrin and metalloproteinase (ADAM)-17 antagonist, TAPI-1 (p=0.004). Similarly, incubation of cells for 72 h with Ang II (10-7 M) enhanced ACE2 activity in the media (p<0.05), an effect blocked by TAPI-1. High D-glucose significantly increased ADAM-17 activity in cell lysates (p<0.05). In culture media, mouse ACE2 was detected as two bands at ~90 and ~70 kDa on immunoblots. Deglycosylation reduced fragment sizes to ~75 and ~60 kDa. Mass spectrometry of immunoprecipitated fragments identified peptides with the mouse ACE2 sequence at positions 18-706 in the 75 kDa form, and 18-577 in the 60 kDa form. In STZ-diabetic mice, a time-dependent increase in urinary ACE2 activity occurred, compared to baseline levels. In urine samples from diabetic mice, ACE2 was initially detected as two

bands of ~100 and ~75 kDa. After deglycosylation, three ACE2 fragments were observed, with sizes of ~85, ~78 and ~65 kDa. Similarly, in urine samples from renal transplant patients, three ACE2 fragments were detected after deglycosylation, and mass spectrometry confirmed their origin from ACE2.
CONCLUSIONS: These data suggest that ACE2 fragments are shed from proximal tubular cells into the urinary space. ACE2 shedding is stimulated by high glucose or Ang II, at least partly via an ADAM-17-mediated pathway. In diabetes, loss of membrane-bound ACE2 might alter the peritubular levels of Ang II and Ang-(1-7) and thereby affect kidney disease progression.

48. **Insulin-like 3 through growth differentiation factor 9 stimulates rat preantral follicular growth**

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Insulin like 3 (INSL3), primarily expressed in the theca cells, is a 6 kDa peptide consisting of two chains (A and B) and belongs to the insulin superfamily. Androgens and oocyte-derived growth factors growth differentiation factor 9 (GDF9) are essential for proper follicular development. GDF9 signaling is mediated by the activin receptor-like kinase (ALK) 5/SMAD3. Clinical studies have shown that serum INSL3 concentrations are positively correlated with androgen levels. Using a rat preantral follicle culture model, we examined the action and interactions of theca INSL3 and androgen and oocyte-derived GDF9 in the regulation of preantral follicular growth. In the present study, the receptor of INSL3, LGR8 was exclusively expressed in oocyte. INSL3 stimulated preantral follicular growth, testosterone production and GDF9 expression in vitro. Blocking cAMP/PKA signal pathway (with PKA inhibitor, RP-8-Br-cAMPS) attenuated INSL3-induced GDF9 expression. Inhibiting the action of androgen (with androgen receptor antagonist flutamide) or GDF9 signaling (with ALK5 inhibitor, SB431542 or SMAD3 inhibitor, SIS3) reduced INSL3-induced preantral follicular growth. Furthermore, INSL3 is responsive to LH stimulation, which is mediated by nuclear receptors NR4A1 and NR5A1and its expression is down-regulated by dihydrotestosterone (DHT). In conclusion, INSL3 is a key theca cell-derived growth factor in preantral follicular growth.

49. **Angiotensin 1-7 Attenuates Endothelin-1-Induced Endothelial Cell Inflammation and Growth Through Nitric Oxide Production and Activation of Mas and EndothelinB Receptors**

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In pulmonary hypertension, where the endothelin system plays a major role, the vasoprotective axis of the rennin angiotensin system (ACE2-Ang (1-7)-Mas) seems to be protective. However, the exact mechanisms are still elusive and whether Ang 1-7 counterbalancing effects are beyond interactions with Ang II system is unknown. In this study, we assessed whether Ang 1-7 influences/interacts with the ET-1 system in endothelial cells. Cultured human microvascular endothelial cells (HMEC) were studied. HMEC were stimulated with ET-1 (10-7 mol/L) in the absence and presence of Ang 1-7 (10-7 mol/L), BQ788 (an ETBR antagonist), BQ 123 (an ETAR antagonist) and A779 (Mas receptor inhibitor) (10-6 mol/L). Expression of pro-inflammatory mediator (VCAM-1), cell growth marker (PCNA), Mas, ETBR expression and eNOS activation was determined by immunoblotting. ET-1 significantly increased expression of VCAM-1 (138.90% vs control, $p<0.05$) and PCNA (125% vs control, $p<0.05$). Ang 1-7 alone did not modulate pro-inflammatory and growth mediators, but significantly inhibited the effects of ET-1 on VCAM-1 (95.55%) and PCNA (103.83%) expression, an effect mediated by Mas receptor activation (after A779: VCAM-1: 226.15%; PCNA: 120% vs control, $p<0.05$). Ang 1-7 increased NO production (Ctl: 7.5 vs Ang 1-7: 20 RFU/ug of protein, by microfluorescence). Inhibition of Ang 1-7-induced NO production by L-NAME, inhibited Ang 1-7-mediated effects on ET-1-induced VCAM-1 (160%) and PCNA (125%), $p<0.05$. Ang 1-7 significantly increased expression of ETB receptors (175.63% vs control, $p<0.05$), an effect attenuated by A779. Ang 1-7 (166.94% vs control, $p<0.05$) and ET-1 (146.04% vs control, $p<0.05$) increased eNOS phosphorylation in HMEC. Blockade of Mas and ETB receptor inhibited Ang 1-7 and ET-1 effects on eNOS activation. BQ123, but not BQ788, blocked ET-1-stimulated inflammation/growth in HMEC (VCAM-1: 75%, PCNA: 100%, $p<0.05$). In conclusion, Ang 1-7 negatively modulates proinflammatory and mitogenic actions of ET-1, through crosstalk between Mas and ETB receptors, and increase in NO production. These data highlight some molecular mechanisms whereby Ang 1-7 may exert beneficial effects in pulmonary hypertension and suggests a novel mechanism for Ang 1-7 signalling in HMEC.

Clinical Epidemiology Program

50. **Early Versus Late Treatment for Smoldering (Asymptomatic) Multiple Myeloma: A Systematic Review**

Mubarak AlGhamdi

Background: Expectant management remains the current standard of care for patients with smoldering (also known as asymptomatic) myeloma. Recent appreciation of "high risk" smoldering myeloma and the advent of novel therapeutic agents may allow one to better tailor the timing of therapeutic interventions.
Objectives: To summarize available evidence comparing an early treatment strategy for smoldering (asymptomatic) multiple myeloma (SMM) with observation.
Methods: Our systematic search strategy includes MEDLINE, EMBASE, Cochrane Database, and relevant bibliographies where the following concepts were used: Randomized controlled trials (RCT), smoldering myeloma, and treatment. RCT addressing clinical outcomes of early treatment for smoldering (asymptomatic) multiple myeloma as per IMWG Criteria were eligible for inclusion.
Main Results: Six RCT (2 articles and 4 abstracts) representing 801 patients compared early versus deferred treatment for smoldering multiple myeloma were included. The median age is 66 (range 61-72). Three studies received a Jadad score of 3 while other three studies received a score of 2. Allocation concealment was described in 5 studies. Two studies compared early Melphalan plus Prednisone (MP) versus deferred MP did demonstrate differences in overall response rate (ORR), time to progression (TTP), and overall survival (OS). Three studies assessed bisphosphonates versus abstention revealed a lower incidence of skeletal-related events with bisphosphonate use, but did not translate into benefits in terms of ORR, TTP, and OS. One study compared lenalidomide plus dexamethasone in high risk patients with therapeutic abstention revealed significant clinical benefits in ORR, TTP, and OS.
Conclusion

51. **The Contribution of Child Behaviour Problems to the Health of Caregivers**

Mathieu Chalifoux, Jamie C. Brehaut , Dafna Kohen, Kelly Carroll, Brian Hutton, Dean Fergusson

Background: Parenting is a daunting and demanding task, especially for those who must care for a child with health problems. Considering that caregivers of children with health problems have been demonstrated to show generally poorer physical and psychological health, efforts have been made to understand the factors that influence caregiver health. It has been suggested that child behavioural problems are key and account for a large proportion of the variance in caregiver health. The mechanisms by which child behaviour problems affect caregiver health remain unclear, but it has been proposed that stress may be part of that explanation. Behavioural problems have been categorised into two groups: Externalizing behavioural problems which are overt in nature and internalizing behavioural problems which are covert in nature. We expect externalizing behavioural problems (e.g. acting out) to have a greater effect on caregiver health since they would require more active disciplining of the child and create more stress for the caregiver than internalizing behavioural problems. Currently, the literature linking child behaviour problems and caregiver health is varied, and the relation between the two kinds of behaviour problems in terms of their effect on caregiver health is unclear. The current study proposes to perform a systematic review in order to describe and compare the effect of internalizing and externalizing behaviour problems on caregiver health.
Methods: Studies were included if: 1) They measured a psychological or physical outcome on the caregiver, 2) The caregivers were caring for a child (2 to 18 years old) with a behaviour problem, and 3) These caregivers were compared with caregivers of healthy children or caregivers of children with a different health problem not related to behaviour problems. Studies were screened and inclusion was determined by two independent investigators. Comparisons will be made between children with internalizing behavioural problems and healthy children, externalizing behavioural problems and healthy children, and between both types of behavioural problems. The second screening will select studies based on if there is a healthy comparison group, and if the goal of the study is to look at caregivers. The databases used during this project are going to be Medline, Embase, PsycInfo, CINAHL, and Sociological Abstracts.
Results: To date, first screening is under way. Currently, 2551 articles have been retrieved for screening.
Discussion: This study is expected to shed light on the mechanisms by which child behaviour problems lead to poorer physical and psychological health in their caregivers.

52. ## Estimating the Incidence of Intussusception in Ontario Using Health Administrative Data

Robin Ducharme

Background

The importance of post-marketing vaccine safety surveillance was highlighted by the withdrawal of the RotaShieldTM vaccine from the US market in 1999 due to evidence of an increased risk of intussusception. In view of the recent addition of RotarixTM vaccine to Ontario's immunization program, planning for post-marketing safety surveillance in Ontario is essential. The implementation of a surveillance system for rotavirus vaccine using health administrative data requires a) an accurate method of identifying cases of intussusception in administrative data; and b) an estimate of the baseline incidence of intussusception.

Objective

In this study, we aimed to develop and validate an algorithm as an accurate method of identifying cases of intussusception using Ontario's health administrative data housed at ICES. We then aimed to apply the validated algorithm to ICES data to ascertain the baseline incidence of intussusception, prior to the introduction of the RotarixTM vaccination program.

Methods

We calculated measures of diagnostic accuracy for various combinations of diagnostic and procedural and billing codes. The reference standard for our study was the true disease status of patients seen at CHEO, as determined by chart abstraction and application of a validated case definition for acute intussusception. We selected the algorithm with the highest positive predictive value (PPV) while maintaining a high sensitivity.

Results

The selected algorithm (ICD-9 or ICD-10 code for intussusception in the CIHI DAD) was sensitive (89.33%) and highly specific (99.99%) in ascertaining both true cases and non-cases of intussusception. The positive predictive value of the algorithm was 72.43%, and the negative predictive value was 100.00%.

Using the algorithm, we estimated the crude annual incidence of intussusception in Ontario children (<18 years of age) to range from 2.93 to 5.30 per 100,000 person-years, between the fiscal years 1995/96 and 2010/11. After standardizing the rates to the Canadian population by age group and sex, the annual incidence rates ranged from 3.33 and 4.96 per 100,000 person-years. In infants (<1 year of age), the crude annual incidence rates ranged from 18.64 to 49.28 per 100,000 person-years between fiscal years 1995/96 and 2010/11.

Conclusions

Our results demonstrate that cases of intussusception can be accurately identified within health administrative data using a validated algorithm or ICD diagnostic code. We have also demonstrated that a validated algorithm can be used to establish disease incidence at a population level. Our study establishes the foundation required for safety monitoring related to rotavirus vaccine in Ontario.

53. ## Protocol Management of Severe Traumatic Brain Injury in Intensive Care Units: A Systematic Review

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BACKGROUND Severe traumatic brain injury is a a complex and life-threatening event that is managed using a multitude of treatment interventions. Management protocols (MPs) have been proposed to guide patient care and incorporate best evidence but their effect on patient outcome is unclear.

OBJECTIVE We aimed to examine the effect of MPs for adult patients in ICUs with acute severe TBI as compared to usual care on outcome. Our a priori primary outcome was 6 month GOS and secondary outcomes were mortality, LOS, ICP/CPP control and adverse events.

METHODS Searches of major electronic databases were completed for all citations available from 1950, to April 18, 2011. Published abstracts from major international meetings and a hand search of the references of all included studies were also reviewed. From selected studies, data on patient characteristics, management protocol characteristics, outcomes and methodological quality was extracted independently and in duplicate.

RESULTS 488 potential articles (from 6151 identified) were reviewed independently and in duplicate leading to the inclusion of 12 observational studies, one abstract publication and no randomized controlled trials in the analyses (n = 3293 patients). A random effects model showed use of MPs was associated with a favorable outcome (GOS 4 or 5) at 6 months (OR and 95% CI 3.84 (2.47, 5.96)) but not at 12 months (OR, 95% CI 0.87 (0.56-1.36)). Use of MPs were associated with reduced mortality at hospital discharge and at 6 months (OR and 95% confidence interval 0.72 (0.45-1.14) and 0.33

(0.13, 0.82) respectively), but not at 12 months (OR, 95% CI 0.79 (0.5-1.24)). Sources of heterogeneity included variation in study design (prospective or retrospective MP group) and methodological quality, MP design (algorithm based vs. descriptive, and the use of ancillary MP), MP neurophysiologic endpoints and type of ICU.
CONCLUSIONS MPs for severe TBI were associated with reductions in death and improved neurological outcome. Although no definitive conclusions about MPs for severe TBI can be drawn from our study, these data illustrate the need for randomized controlled trials to examine the efficacy of MPs for severe TBI.

54. **Vitamin K Antagonists or Antiplatelet Agents After Infrainguinal Venous - A Systematic Review & Meta-analysis**

Danielle Hammond, Esteban Gandara , Phil Wells

Background: Prevention of revascularization failure and the consequent need for re-intervention is of major clinical and economic importance in the surgical management of lower extremity peripheral arterial disease (PAD).
Objectives: To determine whether vitamin K antagonist (VKAs), either alone or in combination with antiplatelet therapy, are superior to antiplalet agents alone in improving graft patency and relevant outcomes in patients undergoing infrainguinal venous bypass.
Methods: The Cochrane Central Register of Controlled Trials, MEDLINE and EMBASE were searched for reports from randomized clinical trials (RCTs). RCTs comparing VKAs, either alone or in combination with antiplatelet agents, versus antiplalet agents in adult patients undergoing infrainguinal venous bypass were selected. Two reviewers extracted data on study design, methods, clinical characteristics, antithrombotic therapies, outcomes, and major bleeding events and rated study quality using a pilot version of the Systematic Review Data Repository (SRDR). The main outcome of the study was primary patency of the venous bypass
Results: Of 10,723 titles screened, 57 were retrieved in full text. Four RCTs evaluating 2158 participants were satisfied our inclusion criteria. Compared to antiplatelet agents VKAs appear to increase graft survival at two years of follow up OR 1.55 (95% CI = 1.2 to 1.9; i2 0%). There was no evidence of publication bias. Information on other relevant outcomes could not be retrieved.
Conclusions: Based on limited evidence, VKAs appear to improve the patency of infrainguinal venous bypass. Our findings call for well-designed RCTs to be conducted.

55. **Capturing the knowledge translation footprint from step one: A new approach to KT integration**

Kelly Weegar, Linda Li, Yara Kadulina, **Lynn MacLeay**, James MacDougall, Shawn Marshall

Background: KT, or knowledge translation, is the fundamental process that ensures new knowledge reaches and is used by the intended users. KT is deemed an essential component of research by health care, research and grant-funding communities worldwide. Despite this widespread recognition, knowledge implementation strategies and evidence-based practice and policy are lacking. Discussion: Several issues remain in current KT practices, such as the after-thought that is often given to KT, which impact the transition of new evidence into today's health care systems. As an alternative, we propose a prospective and comprehensive approach, rather than consideration in the final stages of a research project, when planning of KT activities is daunting and overdue. Further, we propose an interactive and prospective KT process that we believe is a valuable investment for any research program – the Integrated Team-oriented Knowledge Translation Tool (IT-KiTT). The IT-KiTT is a web-based tool that enables researches to accurately report KT processes to funders and supporters, to develop an organizational/team memory of KT activities and to learn from the KT experience. Summary: There is an internationally recognized lack of guidance for researchers on how to design KT plans, record processes and evaluate outcomes. Without the record, researchers lose an opportunity to examine and learn from their KT efforts, as well as achieve recognition by their respective academic institutions for investments in non-traditional KT activities: for instance, media interviews and policy development. This impedes improvement in the transfer of evidence from research to practice. Hence, a comprehensive, user friendly and publicly accessible KT reporting tool is needed to allow researchers, funders and health care providers the opportunity to observe and subsequently enhance their KT planning.

56. **Patient Decision Aids Web Site: A Resource to Support Patient Involvement in Healthcare Decisions**

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Successful engagement of patients in shared decision making (SDM) about healthcare requires interventions such as patient decision aids (PtDAs), training of healthcare professionals (HCP), and performance feedback. The Patient Decision

Aids web site (http://decisionaid.ohri.ca) provides access to these resources and more to facilitate implementation of PtDAs and enhance patient involvement in healthcare decisions. In the past year (May/2011-Apr/2012) the site had approximately 82,000 unique visitors, 647,000 page views and 189,000 downloads.
One can search for publicly available PtDAs using the A to Z Inventory, find decision aids in development using the Complete Inventory, or register PtDAs in the Decision Aid Library Inventory (DALI).
The Ottawa Personal Decision Guide (English, French, Spanish, Japanese) helps people assess their decision making needs and plan the next steps for any health-related or social decision. It can be used by HCPs when supporting patients making treatment decisions.
The Ottawa Decision Support Tutorial (English, French) is an online training program based on the Ottawa Decision Support Framework and supported with evidence from client and HCPs needs assessments, and trials evaluating PTDAs. Since May 2007, it has been completed by over 1500 people in French or English.
The Decision Support Analysis Tool (English, French) is a valid and reliable instrument for appraising the quality of the patient-HCP interactions. Findings can be used to audit the quality of decision support and provide feedback to HCPs for enhancing their skills.
The web site also includes many other resources for PtDA development, implementation and evaluation.

57. ## Associations between cognitive performance, self-reported driving comfort, abilities and behaviour in a large cohort of healthy older drivers

Kelly Weegar,Mark J. Rapoport, Duncan Cameron, Anita Myers, Holly Tuokko, Nicol Korner-Bitensky, Shawn Marshall, Malcolm Man-Son-Hing, Gary Naglie, Candrive Investigators

Background: Driving is a complex and demanding task that requires a high level of visual, motor/somatosensory and cognitive skills. Accordingly, there is concern surrounding how cognitive functioning may affect driving patterns among older drivers, in whom both car crashes and dementia are seen in increasing numbers. Objective: The main focus of this study is to examine the association between selected cognitive measures and self-reported driving comfort, abilities and behaviour, while controlling for a variety of other factors known to impact driving. Methods: This study reports on a cross-sectional analysis of data from the first year of a five-year longitudinal study of healthy older drivers, the Candrive II prospective cohort study. A total of 928 participants aged 70-94 from seven Canadian cities were investigated. Cognitive assessment tools included: the Montreal Cognitive Assessment (MoCA) as well as Trail Making Tests A and B. Driving behaviour was assessed using three psychosocial measures and two measures of self-reported driving practices, which altogether assess several underlying aspects of driving: perceived abilities, situational frequency and avoidance, day and night driving comfort and current driving restrictions. Results: Using univariate regression analysis, it was observed that the times to complete Trails A and B were modestly associated with self-reported driving avoidance ($p = .001$), as well as with day and night driving comfort, current driving restrictions and perceived driving abilities ($p < .05$). Both MoCA total score and errors on Trails A and B were not associated with any of the self-reported driving variables ($p > .05$). The association persisted after adjusting for age and sex, as well as variables pertaining to health, vision, mood, and physical functioning. Conclusions: Psychomotor speed and executive functioning, as measured by time to complete Trails A and B, respectively, were statistically significant, but only modest predictors of self-reported driving comfort, abilities and behaviour in this cross-sectional analysis. Stronger associations between cognitive performance, driving behaviour and other important driving outcomes (e.g., actual driving exposure and crashes) are anticipated in the longitudinal study currently in progress, as cognitive and functional decline are likely to become more prevalent in a sub-group of this current cohort of healthy older drivers.

58. ## Temporal Variability in Urinary Concentrations of Triclosan among Pregnant Women in Canada

Lorelle Weiss, Tye Arbuckle, Tim Ramsay, Mandy Fisher, Ranjeeta Mallick

BACKGROUND
Triclosan is an anti-microbial agent added to personal-care and household products, which can be absorbed into the body upon exposure. Following absorption, triclosan levels can vary, making it difficult to reliably identify the source and timing of potential exposure. Due to its potential endocrine disrupting properties, human exposure to triclosan must be monitored, particularly among pregnant women. Biomonitoring surveys largely rely on a single urine void to quantify exposure. For chemicals with a short half-life (hours to days), such as triclosan, information on the reliability of these indicators of exposure is lacking. To date, no data exist on temporal variability in exposure to triclosan.
OBJECTIVE
Using proposed urinalysis methods by Hauser et al., Teitelbaum et al., Mahalingaiah et al., and Braun et al., this study will measure the variation of triclosan measurements over the course of 24 hours, within a week-end and weekday, and at

various stages throughout pregnancy. This study will also determine within- and between-individual variability, and calculate the validity of a spot urine sample to predict average exposure to triclosan in pregnant women.
METHODS: Biomonitoring data for triclosan levels were obtained through those enrolled in the Health Canada P4 Study. Specifically, through maternal urine samples of 80 pregnant women collected in each trimester of their pregnancy as well as post-partum, with each woman contributing up to 23 samples. Urinary triclosan levels were determined with the GC-MS-MS BPA method (gas chromatography coupled to tandem mass spectrometry following solvent extraction and derivatization).
RESULTS
The statistical analysis is in progress; descriptive statistics on urinary triclosan levels overall, by day and throughout pregnancy will be presented. Mixed effect models will be fit to determine the association of urinary triclosan concentrations with predictors; intraclass correlation coefficients will be calculated to assess intra- and inter-individual variability. A surrogate category analysis will summarize the predictive ability of single spot samples to predict average exposure to triclosan. Trimester-specific results will report both unadjusted levels and those adjusted for specific gravity.
CONCLUSIONS
Measurements of temporal variation of urinary triclosan concentrations in pregnant women will provide a complementary data set to the ongoing research aiming to assess the daily exposure of Canadians to this chemical. Results will provide useful information towards determining population-specific exposure assessment strategies for this population.

59. ## Improving practice: Rx for Change - an intervention research database for healthcare decision-makers and researchers

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Background:
Improving health care often requires changing the behaviour of healthcare professionals and consumers. The Rx for Change (www.rxforchange.ca) database attempts to close the gap between research discovery and program implementation by gathering and translating the abundant evidence from systematic reviews into a single accessible package to inform healthcare decision makers.
Objectives:
To describe Rx for Change and disseminate evidence gathered on the effectiveness of interventions designed to change professional practice and medicines use by consumers.
Methods:
We identify, analyse, summarise and report our findings from systematic reviews using standardised methods. We organise and present this data on the Rx for Change web-site using a multi-layer format that includes: an expandable list of intervention categories; summaries of evidence found for each intervention; a list of all systematic reviews that address the intervention topic with corresponding quality scores; and a description and summary of results and conclusions from each individual review.
Results:
The database contains: summaries of key findings for 275 systematic reviews, and summaries and statements of effectiveness for 39 intervention categories the reviews addressed. Examples of effective interventions include: distribution of educational materials and use of educational meetings to improve professional practice (including prescribing) and the use of decision aids to minimise risks or harms to consumers medicines use. Research gaps are evident in 11 intervention categories.
Implications:
Rx for Change is an internationally recognised interventions research database with multiple applications for a range of users. The evidence will help guide clinicians, healthcare administrators and policy-makers towards finding solution to improve evidence based practice behaviour and consumers use of medicines and identify areas where further research is needed. Guidelines and other policy and research initiatives should be informed by this.

60. **Behavioral Characterization of Transgenic Mice with Neuronal Overexpression of Human Cathepsin D**

Qiubo Jiang[1], Dina Elleithy[1], Tohru Kitada[1,2], Piotr Kolodziej[1,2], Paul Manninger[1], Merila Hasu[2], Jason MacLaurin[2], Yves De Repentigny[1], Ruth Slack[2], Rashmi Kothary[1], Diane Lagace[2], Julianna J Tomlinson[1], Michael G Schlossmacher[1,2]
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Background: Parkinson's disease and dementia with Lewy bodies are characterized by Lewy inclusion formation and cell loss in the brainstem and forebrain. Alpha-Synuclein (SNCA) is the main constituent of Lewy bodies, and its prefibrillar aggregation is thought to promote neurotoxicity. The molecular mechanisms leading to synucleinopathy are still unknown, but select models showed that preventing SNCA accumulation reduces neurodegeneration. Several laboratories including ours recently demonstrated that over-expression of human cathepsin D (CTSD) cDNA lowered SNCA concentration in neural cells. In addition, our analysis of CTSD mutant mice and CTSD-deficient humans revealed evidence of SNCA dysregulation in the forebrain (Cullen et al., Mol Brain 2009), thereby identifying CTSD as a potential drug target.

Objectives and Method: To further examine the potential value of CTSD in preclinical studies, we successfully generated hCTSD-transgenic (tg) mice in which CTSD expression is driven by the CamKIIa promoter. We have recently completed the biochemical, histological and behavioral characterization of these mice (see also accompanying poster by Kitada et al.). To assess any potential motor and cognitive effects of overexpressed CTSD, we conducted a series of behavioral tests .including Pole Test, Rotarod and Morris Water Maze (MWM) at 4 different ages from 8 weeks to 1.5 years of age.

Results: We successfully generated the first transgenic line with neuronal overexpression of hCTSD. Careful biochemical and histological characterization confirmed increased enzyme expression and activity in heterozygous transgenic animals compared to wt littermate controls with strong expression in the forebrain and hippocampus (see poster by Kitada et al.). The mice develop and age normally and appear healthy. Importantly, behavioral analysis revealed no impact of increased CTSD on motor and cognitive function between the tg mice and their wt littermate controls as assessed using the Pole Test, Rotarod and MWM respectively. The results of our behavioral testing will be discussed herein.

Conclusions: Our hCTSD transgenic model is the first mouse to be reported that features overexpression of human, wild-type CTSD in forebrain neurons. By all measures assessed, these mice appear normal and show no behavioral deficits. Together, this suggests that increasing the lysosomal activity of CTSD in CNS neurons appears to be well-tolerated, thereby adding an important safety parameter to this potential target. Assessing its further usefulness as a protease for the degradation of alpha-synuclein (and possibly, of other misfolding prone proteins) in preclinical models is ongoing in our laboratory.

Neurosciences Program

61. **IRF2BP2, A Novel Regulator of Macrophage Polarization and Neuronal Ischemia**

Kianoosh Keyhanian , Xun Zhou, Nihar R. Pandey, Alexandre Stewart, Hsiao-Huei Chen

Background

Inflammation at the site of a stroke is responsible in part for further damaging the tissue but also for the regeneration process. Macrophages are key players in the immune response. Two classes of macrophages are assigned based on their functional characteristics. M1 macrophages are characterized with high IL12, low IL10 production and enhanced inducible nitric oxide synthase (iNOS) levels, which contribute to tissue damage and neuronal death after injury. M2 macrophages diminish the inflammatory response and induce tissue repair with low IL12 and high IL10 production as well as increased Arginase 1 expression.

Interferon regulatory factor 2 binding protein 2 (IRF2BP2) is a nuclear protein that was first discovered by its binding to interferon regulatory factor 2 (IRF2). Recent studies proposed an antiapoptotic role for IRF2BP2 in different cancer cell lines. IRF2BP2 was also reported to bind to NFAT and to repress the expression of NFAT regulated proteins. A recent study from our lab showed that IRF2BP2 is upregulated in ischemic muscle. However, the effect of this protein had yet to be investigated in neural ischemia. Based on its binding to two important regulators of macrophage polarization and apoptosis, IRF2 and NFAT, we hypothesized that IRF2BP2 is important in determination of macrophage and neuron fate after ischemia.

Methods

Primary cortical neurons and F11 cell line were subject of Oxygen Glucose Deprivation (OGD) for 6 hours and reperfusion for 12-14 hours. Then the cells were harvested for apoptosis analysis or western blotting. IRF2BP2 protein and mRNA levels were measured after stimulation of the mouse macrophage RAW264.7 cell line with LPS or IL4 inducing M1 or M2, respectively. Primary Bone Marrow Derived Macrophages (BMDM) were isolated from wild type or IRF2BP2 flox/flox mice and infected with adeno-Cre viral vector. Cells were then stimulated with LPS or IL4 and harvested for mRNA and protein analysis.

Results

IRF2BP2 protein levels were increased after OGD in primary cortical neurons and the F11 cell line. Moreover, IRF2BP2 knockdown lead to increased apoptosis in F11 cells. In macrophages, IRF2BP2 protein and mRNA levels were increased in IL4 stimulated cells (M2) compared to control or LPS induced macrophages (M1). In addition, IRF2BP2 knockdown in primary BMDMs resulted in decreased Arginase1 mRNA levels when stimulated with IL4, demonstrating a defect in macrophage polarization toward M2 phenotype. IRF2BP2 knockdown in BMDMs stimulated with LPS resulted in increased TNFa mRNA showing a shift to macrophage M1 polarization.

Conclusion

IRF2BP2 is an important regulator of macrophage polarization as well as neuronal apoptosis after ischemia.

62. **Exploration of a putative synucleinase through Characterization of Human Cathepsin D Transgenic Mice**

Tohru Kitada[1,2], Qiubo Jiang[1], Piotr Kolodziej[1,2], Adel Farah[1], Julianna J. Tomlinson[1], Juliana Ng[1], Jason MacLaurin[2], Yves De Repentigny[1], Ruth Slack[2], Rashmi Kothary[1], Michael G. Schlossmacher[1,2]

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[Background] Parkinson's disease (PD) and dementia with Lewy bodies are characterized by Lewy inclusion formation and cell loss. Alpha-Synuclein (SNCA) is the main constituent of Lewy bodies, and its prefibrillar aggregation is thought to promote neurotoxicity. The molecular mechanisms leading to synucleinopathy are still unknown, but select models show that preventing SNCA accumulation reduces neurodegeneration. Thus, future cause-directed therapies for PD (and DLB) may include SNCA reduction. Three laboratories including ours recently demonstrated that over-expression of human cathepsin D (CTSD) cDNA in dopaminergic cells promotes the degradation of excess SNCA. In addition, our analysis of Ctsd mutant mice and sheep as well as of CTSD-deficient humans revealed evidence of SNCA dysregulation in the brain (Cullen et al., Mol Brain 2009). These results identified in CTSD a potential target to lower SNCA. [Purpose and Method] To determine whether elevated CTSD levels promote lysosomal 'synucleinase' activity in vivo, we generated hCTSD-transgenic (tg) mice under the CamKIIa promoter that is utilized in the forebrain. We furthermore generated hCTSD/SNCAA53T double-transgenic mice to observe whether hCTSD over-expression reduces synucleinopathy in SNCAA53Tmice, which were provided by Dr. RL Nussbaum. [Result] We confirmed generation of hCTSD over-expression mice by Southern, Northern and Western blotting and immunohistochemistry. CTSD activity assay resulted in 27% and

31% increase in single Tg mice at the age of 9 and 18 months, respectively. However, Western blots and ELISA analysis for SNCA levels in brain lysates did not show any difference between single Tg mice and controls at the age of 9 and 18 months. We also performed the quantification of SNCA by ELISA and Western blotting using lysosomal fractions of whole brains from double transgenic mice at the age of 6 months. However, we did not detect any significant difference versus lysosomal fractions from control mice. Behavioral analyses are discussed in an accompanying poster (Jiang Q et al). In summary, we were unable to detect any decrease of mouse or human SNCA in the brain despite an approximate 30% CTSD activity increase in vivo.

63. **Diffusion Tensor Imaging in Multiple Sclerosis: A method of differentiating symptomatic and asymptomatic cervical cord lesions**

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Background and Objective
Diffusion tensor imaging (DTI) is a novel magnetic resonance imaging technique that can image specific fiber tracts in-vivo and has been used to examine the spinal cord in patients with multiple sclerosis (MS). Studies have shown that DTI can identify cervical cord damage in MS however, correlation with clinical symptoms is limited. Our objective was to see if DTI could differentiate symptomatic from asymptomatic cervical cord lesions in MS.
Methods
Fourteen MS patients with cervical cord lesions (10 female, 4 male; mean age: 46, SD= 13.26) and eight healthy controls (6 female, 2 male; mean age: 30, SD= 5.09) were evaluated in this study. Sagittal T2-weighted images and DTI of the cervical cord were obtained using a 3T Siemens Magnetom Trio MR imager.
Within 24 hours of their imaging, all patients were neurologically assessed using the American Spinal Injury Association (ASIA) classification of spinal cord injury and the Expanded Disability Status Scale (EDSS). Patients were classified as having symptomatic or asymptomatic cervical spinal cord lesions based on their clinical evaluation. DTI measures of mean radial diffusivity, (RD) and mean diffusivity (MD) were assessed at the MS lesion, peri-lesion and normal appearing white matter (NAWM) levels as identified by the T2 weighted images.
Results
Patients with symptomatic lesions had a significantly higher mean radial diffusivity (RD) in lesion sites ($p=0.047$), as well as a higher mean diffusivity (MD) ($p=0.041$) and mean RD ($p=0.034$) in peri-lesional sites compared to asymptomatic patients. No significant differences in DTI measures were found in NAWM between these two groups.
Conclusion
Our study demonstrated that DTI measures could differentiate symptomatic from asymptomatic spinal cord lesions in patients with multiple sclerosis. Further studies will evaluate the use of DTI in monitoring disease progression and disability in MS patients with cord lesions.

64. **Increase in adverse effects without significant improvement in pain management in rats administered 3-day versus 1-day buprenorphine regimen**

Nischal Ranganath, Kystal Walker, Stahs Pripotnev, Matthew J. Coyle, Karunanithi Rajamanickam, Dr. Steffany Bennett, Eve C. Tsai

Background: Alleviation of pain and optimization of analgesic management are critical tenets to the humane and ethical treatment of laboratory animals. The challenge in spinal cord injury (SCI) research is that the analgesic regimen selected must provide effective pain-control associated with severe vertebral manipulations, without altering the underlying regenerative cellular and molecular processes.
Objective: The objective of the study was to evaluate the effectiveness of 1-day versus 3-day buprenorphine hydrochloride regimen in a post-operative pain model in rats. In tandem, the effect of the buprenorphine regimen on cellular proliferation and neural repair at the SCI site was profiled.
Methods: Female, Sprague-Dawley rats received a complete T8/T9 spinal cord transection surgery and were randomized to receive the current standard of practice of one injection of 0.03 mg/kg buprenorphine (n = 6; 1-day cohort) or three daily injections for three days of 0.03 mg/kg of buprenorphine (n=6; 3-day cohort) post-operatively. Behavioural control rats (n=3) were used to compare spontaneous emitted pain behavioural responses. The effectiveness of analgesia on post-operative weight change, pain behaviour, and adverse effect patterns were assessed daily throughout the 3-day and 14-day endpoints. Rats were sacrificed by transcardial perfusion. Brain and spinal cord was dissected en bloc, fixed, and cryopreserved. Spinal cord cryosection was performed 5 mm caudal and rostral from transection site and staining was

performed using Bromodeoxyuridine (BrdU).
Results: Post-operative analysis of mean weight between the 1-day and 3-day buprenorphine regimen was not significant at both the 3-day endpoint ($p = 0.08$) and the 14-day endpoint ($p = 0.05$). While, the 3-day endpoint results are consistent with the expected 10% weight loss post-SCI, the 14-day endpoint results suggest minimal benefit in post-operative weight gain with the 3-day buprenorphine regimen. The behavioural pain score measured between the 1-day and 3-day regimens was not significant ($p = 0.65$). However, the adverse effect score between the two cohorts was significant ($p = 0.04$), with greater incidence of adverse events in the 3-day buprenorphine regimen. Sub-group analysis of adverse events attributed this significance to increased gastrointestinal side effects ($p = 0.02$) in the 3-day buprenorphine group. Staining and analysis of spinal cord cryosections from 1-day, 3-day, and control rats are currently in progress.
Conclusions: The preliminary results from comparing the 1-day, 3-day, and control buprenorphine regimen suggest a lack of significant improvement in both objective weight-gain and behavioural pain-management, with an increased adverse effect profile.

65. **Circulating microparticles are elevated in the plasma of rats exposed to chronic cerebral hypoperfusion and initiate apoptotic cell death in normal rat kidney cells**

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Background: Microparticles (MPs) are small (100 nm – 2 micron diameter) membrane bound vesicles released from most cell types and found in virtually all bodily fluids. They have been shown to be involved in many physiological processes including inflammation, coagulation, angiogenesis and vascular dysfunction. In the present study MPs isolated from the plasma were found to induce apoptosis when applied to cultures of normal rat kidney (NRK) cells.
Methods: At various times following permanent occlusion of both common carotid arteries rats were sacrificed and MPs were isolated from the plasma by ultra centrifugation, labeled with annexin V and anti-VE-cadherin and quantified by flow cytometry. Cell death in cultured NRK cells was estimated with a lactate dehydrogenase assay.
Results: Total MPs and VE-cadherin positive MPs were elevated for up to 6 months following surgery. MPs induce caspase 3 dependent cell death in cultured NRK cells which is both time and dose dependent. Pharmacological inhibition of NADPH oxidase, xanthine oxidase or NOS did not protect NRK cells and there was no increase in glutathione peroxidase activity following exposure to MPs. Inhibition of the fas signaling pathway was not protective while inhibition of caspase 3, the TNF alpha inhibitor SPD-304 and TRAIL blocking peptide provided some degree of protection.
Conclusions: Plasma levels of total MPs and VE-cadherin positive MPs are elevated following the onset of chronic cerebral hypoperfusion. MPs initiate caspase 3-dependent cell death in cultured NRK cells that is not associated with an increase in oxidative stress but is induced by activation of the TNF-alpha and TRAIL signaling pathways.

66. **Ketamine reduces glutamate induced astrocytic migration**

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Background: Post spinal cord injury (SCI), endogenous neural progenitor cells (eNPCs) proliferate and differentiate predominantly into glial cells. These cells then migrate and contribute to the formation of the glial scar, which can impede spinal cord repair. We examined whether ketamine, a widely used anesthetic, altered glial cell migration and affected proliferation of differentiating eNPCs present in the spinal cord using an in vitro model.
Methods: Rat eNPCs derived neurospheres were exposed to glutamate (50 uM) and ketamine (dose ranging from 0-100ug/mL) for two days, and then visualized using immunocytochemistry. Cells were exposed to 5-bromo-2-deoxyuridine 24 hours prior to fixation. Proliferation of differentiating cells was assessed using cell counts. Migration was assessed measuring the distance from the edge of the neurosphere to the centre of the respective cell body.
Results: There was a significant decrease in eNPCs derived GFAP(+) and O4(+) cell migration following exposure to 100 ug/mL of ketamine (Anova, $p<0.05$). There was no significant difference in the proliferative ability of differentiated eNPCs following glutamate and ketamine exposure.
Conclusions: Ketamine, may be useful in decreasing the migratory potential of GFAP(+) and O4(+) glial cells, which may be beneficial in delaying their contribution to the glial scar formation. Therefore, ketamine may be an useful therapeutic component for SCI repair.

67. **A role for Prickle and Van Gogh in specifying neuronal positioning in C. elegans**

Raymond Tanner, Theodore Perkins , Antonio Colavita

C. elegans is a microscopic nematode with a simple nervous system of 302 neurons, and is an ideal model for dissecting the genetic pathways underlying neuronal development and positioning. We have recently shown that the worm orthologues of Van Gogh (vang-1) and Prickle (prkl-1), core components of planar cell polarity (PCP) signaling, are required for the proper positioning of the DD-class motor neurons. The six DD neurons (DD1-6) are normally positioned equidistant from each other in the ventral nerve cord, with DD1 most anterior and DD6 most posterior. In prkl-1 and, to a lesser extent, vang-1 mutant worms, the DD neurons are shifted anteriorly. This positioning defect can be rescued by expressing a prkl-1 cDNA under pan-neuronal and cell-specific promoters, suggesting a cell-autonomous role for PRKL-1 in the DD neurons. Conversely, overexpression of PRKL-1 in the DD neurons results in posteriorly shifted DD neurons. Interestingly, the more penetrant anterior shift of the DD neurons in prkl-1 mutants can be suppressed by simultaneous loss of vang-1. This is evidence of a genetic interaction between vang-1 and prkl-1. These results suggest that PRKL-1 and VANG-1 are required for proper DD neuron spacing along the AP axis. We further hypothesize that these defects may represent impaired neuronal migration.

68. **The role of ALS8-linked VAMP-associated protein B (VAPB) in Caenorhabditis elegans motor neurons**

Wendy Zhang, Jiravat Visanuvimol, David Carr, Antonio Colavita, Johnny Ngsee

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects both upper and lower motor neurons, resulting in a loss of motor neurons of the spinal cord, brainstem and motor cortex. A familial form, ALS8, is linked to a single missense mutation in the VAPB gene, resulting in a substitution of Proline at residue 56 by Serine (P56S). Although the formation of dilated ER membranes induced by VAPB-P56S is suggested to compromise various intracellular processes, ALS is a motor neuron disease that specifically causes the death of motor neurons.

Objective: Currently, neither the Drosophila nor the mouse model shows evidence of motor neuronal death. Therefore, this study aims to generate a C. elegans animal model for mutant VAPB characterizing the role of VAPB in motor neurons, and to determine the underlying mechanism of selective vulnerability of motor neurons.

Method: Expression of VAPB-WT and VAPB-P56S was placed under the control of the unc-4 promoter, which is active in "dorsal A" (DA) motor neurons that control backward locomotion. These transgenic worms were further crossed to a GFP reporter line to highlight the cell bodies and axons. Locomotor defect, axonal guidance phenotype and age-dependent death in these worms were measured.

Results: The VAPB-WT and VAPB-P56S transgenic worms showed a backward locomotory defect. Crossing this to a GFP reporter line to highlight the cell bodies and axons, it was found that axons of DA motor neurons were misguided in the transgenic worms. Moreover, there was a significant increase in DA motor neuron loss from Day 3 to Day 7 in the transgenic worms.

Conclusion: The phenotype is consistent with the specific expression of the transgene in the DA neurons. The age-dependent neuronal loss recapitulates the age-dependent onset of disease symptoms in human ALS8.

Regenerative Medicine Program

69. **The role of MLL1 in expression of Myf5**

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Background: Following muscle injury, and in degenerative diseases such as muscular dystrophy, satellite cells residing in the muscle proliferate and fuse regenerating the muscle. During development and in the adult, the transcription factor Pax7 specifies cells to the myogenic program, and in the absence of Pax7, adult muscle cannot regenerate. To activate genes, Pax7 recruits a H3K4 histone methyltransferase leading to an epigenetically active status at and transcription of target genes. Objective: This study aims to identify and further define the role of the specific methyltransferase required for Pax7 mediated transcriptional activation of target genes during muscle regeneration. Methods: Conditional knockout myoblasts were isolated from mice expressing a tamoxifen activated Cre recombinase. Myoblasts were cultured and tamoxifen was used to initiate knockouts. Retroviruses were used to exogenously express factors to rescue the effects of gene knockout. Gene expression was determined with RT qPCR, western blot, and microarray. Results: Expression of the myogenic transcription factor Myf5, which is directly regulated by Pax7, is lost after knockout of MLL1 in cultured myoblasts. After MLL1 knockout, expression of Pax7 is reduced by 40% but Myf5 expression is reduced by more than 90%. H3K4 methylation at Myf5 is also lost suggesting that MLL1 is required for expression of Myf5. While the reduction in Pax7 expression does not seem to account for the loss of Myf5 expression, over-expression of Pax7 overcomes the MLL1 knockout defect and Myf5 expression and H3K4 methylation at Myf5 are rescued. Microarray identified a short list of genes also having reduced expression after MLL1 knockout but re-expression of these genes had no rescue effect on Myf5 expression. Double knockout of MLL1 and the homologous MLL2 also had no effect on exogenous Pax7 mediated rescue of Myf5 expression. Conclusions: Since MLL1 knockout only weakly effects Pax7 expression but Myf5 expression is lost, it seems that MLL1 is necessary for Pax7 to activate target genes, however over-expression of Pax7 in MLL1 knockout cells is able to rescue Myf5 expression indicating that MLL1 is not necessary for Pax7 to activate target genes. In rescue experiments, Pax7 was over-expressed at inordinate levels prior to MLL1 knockout, because MLL1 regulates genes epigenetically, it is possible that over-expression of Pax7 results in a strict epigenetic program at Myf5 that requires an equally inordinate intervention for down-regulation of Myf5 expression. Alternatively, MLL1 may be required for appropriate expression of Pax7 at levels leading to normal Myf5 expression.

70. **Caspase Mediated Chromatin Remodeling during early Myoblast Differentiation**

Mohammad Al-Khalaf, Lynn A. Megeney

Effector caspases have been implicated in pathways governing stem cell differentiation to various types of mature cell types. Recent studies have shown the role of caspase 3 in propagating differentiation of satellite cells into mature myotubes. Caspase-dependent cleavage of proteins like MST1, ICAD and PAX7 lead to initiation of differentiation in primary myoblasts. In this s tudy, we show for the first time the involvemnent of effector caspase 7 in this early myoblast differentiation model. We identify a new target, Special A-T rich Binding porotein 2 (SATB2) that is cleaved specifically for caspase 7 during early myoblast differentiation. This work sheds light onto the role of effector caspases in chromatin remodeling of myoblasts during differentiation and myotubes formation.

71. **The Smarca1 and Smarca5 chromatin remodeling proteins drive cerebellar development by transcriptional regulation of key patterning genes**

Matias Alvarez-Saavedra[1,3], Yves De Repentigny[1], Michael S. Huh[1], Keqin Yan[1], Pamela Lagali[1], Emile Hashem[1], Alan Mears[2], Rashmi Kothary[1,4], Tomas Stopka[5], Arthur I. Skoultchi[6], David J. Picketts[1,4.]
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The role of ATP-dependent chromatin remodeling complexes during mammalian brain development remains poorly understood. Smarca5 and Smarca1, the mammalian ISWI homologues, are the catalytic cores for multiprotein chromatin remodeling complexes highly expressed in the brain. In this work, we show that Smarca5 is essential for GNP proliferation

and Purkinje neuron development, while Snf2l promotes GNP differentiation. Molecularly, Smarca5 and Smarca1 antagonistically modulate the expression of the master homeobox transcription factor Engrailed-1. ISWI-dependent deregulation of Engrailed leads to severe deficits in the development of topography maps that establish functional cerebellar circuitry domains, resulting in altered sensorimotor control, cognitive malfunction, and ultimately young mortality. Importantly, we show that mammalian ISWI are essential for higher order chromatin structure and Smarca5 orchestrates gene expression programs via the transcriptional regulation of core histone and patterning genes. As a whole, this study provides novel cellular, molecular, physiological and ultrastructural insight into the role of ISWI-containing chromatin remodeling complexes in the mammalian brain, revealing a hierarchical epigenetic landscape driving evolutionary conserved developmental gene expression programs.

72. ## MicroRNA expression profiles reflect endothelial progenitor cell differentiation and outgrowth

John Behbahani

Background: Generation of endothelial cells (ECs) is paramount for cardiovascular health, and uncovering modes to facilitate this generation is pivotal for improving cardiovascular disease (CVD). Endothelial progenitor cells (EPCs) are an important model for pursuit of this discovery, and exist in two varieties, early (E-EPC) and late (L-EPC) outgrowth. E-EPCs emerge after 3 days of culture, but lack robust EC phenotype and therapeutic potential of L-EPCs (which emerge after two weeks). Uncovering the sub-cellular mechanisms that drive the strong EC phenotype in L-EPCs holds great therapeutic potential.

MicroRNAs (miRs) are short non-coding RNAs that can regulate entire networks of genes, di-rect numerous cellular pathways, control cell differentiation and fate. Most miR generation is the result of transcription and mRNA processing, and thus can be generated as EPCs differentiate. As well, some miRs can be directly transcribed, or function as immediate or dormant modulators of transcription fac-tor activity. Numerous studies have implicated miRs in angiogenic roles, impacting both EPC and EC function. Moreover, it has been shown that miR levels correlate with E-EPC function; and at least in hypoxic conditions, the differentiation of L-EPCs.

Hypothesis: Changes in expression of specific miRs contribute to the emergence of L-EPCs from cultures of E-EPCs.

Methods and Results: Leukapheresis product was collected from 8 healthy participants and processed through ficoll density gradient centrifugation to isolate the monocyte-enriched buffy coat. The apheresis cell product was plated on fibronectin-coated flasks and cultured for 1, 3, 5, 7 and 9 days. L-EPCs were also cultured from 5 of 8 participants. RNA was collected and analyzed using qRT-PCR for each time point including day 0. All miRs were normalized to RNU6B and RNU48. Based on a pilot study consisting of thirteen miRs, we selected five miRs to pursue.

Research Approach: MicroRNA mimics and antagomirs will be used to drive L-EPC differentiation. Typical EPC characterization will be performed by assessing cell morphology, AcLDL and Ulex (UEA-1) lectin uptake; flow cytometry will be performed to define the presence or absence of relevant surface markers of mononuclear cells (CD14, CD45), EPCs (CD34, CD133) and ECs (KDR, CD31). The functional characteristics of resulting cell populations and a matrigel assay to assess capillary-like tube formation will be performed.

Conclusions: Uncovering a regulatory role of miRs in driving EC phenotype in L-EPCs has potential for therapeutic approaches targeting these miRs; thereby improving therapeutic interventions and patient outcomes.

73. ## Delayed skeletal muscle development and intrinsic muscle pathophysiology in mouse models of spinal muscular atrophy

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Background

The disruption of the survival motor neuron gene (SMN1), either by deletion, rearrangement, or mutation leads to the neurodegenerative disease called Spinal Muscular Atrophy (SMA). Although SMA has traditionally been considered a motor neuron disease, the muscle specific requirement for SMN has never been ruled out.

Objective

Histological analyses of skeletal muscles from SMA mice revealed intact sarcomere organization albeit with an increased number of immature myofibers. This observation led us to investigate the expression of the myogenic regulatory factors (MRFs) in SMA muscle.

Methods

We have taken advantage of two different SMA mouse models, the severe Smn-/-;SMN2 mouse and the less severe

Smn2B/- mouse to study the possible contributions that muscle may have on the SMA phenotype.
Results
The expression of myogenic proteins Pax7, MyoD, myogenin and MRF4 was delayed in muscles from severe Smn-/-;SMN2 and less severe Smn2B/-SMA mice. At the functional level, we report that tibialis anterior muscles of Smn-/-;SMN2 and Smn2B/- SMA mice generate over 60% less maximal force than control muscles independent of motor neuron loss and denervation. Immunoblot analyses from hindlimb skeletal muscle samples revealed aberrant levels of protein that are developmentally regulated and important for proper contraction. These include the sarcoplasmic reticulum Ca2+ ATPase and the skeletal muscle sodium channel protein Nav1.4.
Conclusions
In summary, we demonstrate delayed expression of the myogenic program and pronounced muscle weakness independent of motor neuron loss and motor neuron denervation. Our results suggest that reduced Smn levels retard muscle development which leads to intrinsic muscle weakness in SMA mice.

74. ## Development of a Protein-Based Therapy for the Treatment of Spinal Muscular Atrophy

Joseph Burns, Rashmi Kothary, Robin Parks

Background:
One of the leading genetic causes of death among infants is spinal muscular atrophy (SMA), a neurodegenerative disease in which the motor neurons of the spinal cord are progressively lost, resulting in paralysis and often death. Deficiency in full-length survival motor neuron (SMN) protein causes the disease, the severity of which decreases in cases of lesser deficiency. Studies in which exogenous full-length SMN protein was introduced to alpha motor neurons yielded promising results. In order to be effectively delivered to motor neurons, SMN must be fused to a protein transduction domain (PTD), such as the PTD from the trans-acting activator of transcription (TAT) from HIV, which allows it to be taken across the cell membrane. A combination gene therapy/protein therapy approach in which an adenoviral vector delivers a transgene encoding a secreted TAT-SMN to the liver would allow sustained production, secretion, and systemic delivery of the therapeutic SMN to motor neurons and other important cells in an SMA patient.
Objectives:
To develop an effective therapeutic protein for spinal muscular atrophy by fusing an HIV TAT domain and a secretory peptide (SP) to SMN. We will first evaluate the capabilities of the transduction and secretion domains using green fluorescent protein (GFP) as an indicator before continuing into development of the fusion SMNs. We will proceed with in vivo experiments using SMA mice once adenoviruses delivering secreted TAT-SMN have been developed.
Methods:
We generated plasmids encoding three TAT PTD domain variants fused to GFP. We assessed expression and transduction of the fusion GFPs using fluorescence microscopy and verified the results with Western blotting. We precipitated the secreted fusion GFPs from media using trichloroacetic acid (TCA) and analyzed the precipitates with Western blotting. We generated fusion SMNs and characterized them in the same manner.
Results:
We observed expression of GFP via fluorescence microscopy in cells transfected with DNA encoding the TAT-GFP constructs and also in cells treated with purified TAT-GFP proteins. We have recovered TAT-GFPs and TAT-SMNs in the media of cells previously transfected with plasmids or infected by adenoviruses encoding these secreted proteins.
Conclusions:
Characterization of the TAT-GFPs suggests that all TAT variants are capable of transduction and that the secretory peptide is functional. Secretion of the fusion SMNs has been verified and preliminary evidence suggests that they are capable of transduction. We have generated adenoviruses capable of infecting cells and causing them to secrete TAT-SMNs.

75. ## Bone Marrow Microenvironment in Acute Myeloid Leukemia

Priya Chandran, Michael Rosu-Myles, David Allan

Background: Acute myeloid leukemia (AML) often remains refractory to current chemotherapy and transplantation approaches despite many advances in our understanding of mechanisms in leukemogenesis. The bone marrow “niche” or microenvironment, however, may be permissive to leukemia development and studying interactions between the microenvironment and leukemia cells may provide new insight for therapeutic advances. Mesenchymal stem cells (MSCs) are central to the development and maintenance of the bone marrow niche and have been shown to have important functional alterations derived from patients with different hematological disorders. The extent to which MSCs derived from AML patients are altered remains unclear. Objective: The aim of this study is to detect changes occurring in MSC’s obtained from human bone marrow in patients with AML by comparing their function and gene expression profile in comparison with normal age-matched controls.

Results: To date, MSCs have been expanded from 7 patient samples out of 9 diagnosed with acute leukemia and 5 healthy controls. Adherent cells obtained from AML patients were observed to have heterogeneous morphological characteristics compared to the normal samples. Immunohistochemistry and flow data confirm the typical cell surface immunophenotype: CD90+CD34-CD105+ although MSCs from 2 patients with AML revealed reduced surface expression of CD105 and CD90 respectively. Differentiation assays demonstrate the ability of MSCs to differentiate into bone, fat and cartilage for n=5 normal donors and n=1 AML sample. The ability of MSCs to support hematopoietic function has been quantified in co-culture experiments with CD34+ hematopoietic precursors isolated from human umbilical cord blood for n=5 normal donors and n=1 patient sample.
Conclusion: These results indicate that there exist differences in the biologic profile of MSCs from AML patients compared to MSCs derived from healthy donors. Differential gene expression profiling of MSCs from patients with leukemia will be performed to gain additional insight regarding the extent to which MSC function may be altered in patients with leukemia and throughout anti-leukemia treatment.

76. Regulation of Carm1 in myogenic commitment of satellite stem cells

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Background
The paired-box transcription factor Pax7 is required for direct transcriptional activation of the myogenic regulatory factor Myf5 and myogenic commitment of satellite stem cells. Pax7 recruits the MLL2/Ash2L/Wdr5 histone methyltransferase (HMT) complex to the Myf5 gene resulting in tri-methylation of histone H3 lysine 4 of the surrounding chromatin and activation of Myf5 expression. Moreover, recent findings from our lab indicate Carm1, an arginine methyltransferase, as a direct regulator of Pax7. Carm1 methylates Pax7 in vivo and methylation is required for Pax7 recruitment of the HMT complex. Importantly, de novo activation of Myf5 expression following asymmetric satellite stem cell division requires Carm1.
Objective
As a key regulator of Pax7, Carm1 plays an essential role in regulating asymmetric cell division and myogenic commitment of satellite cells. I therefore hypothesize that the activation of Carm1 represents a key regulated step during asymmetric satellite stem cell division.
Methods
In an effort to elucidate the molecular mechanisms that regulate Carm1 activity in satellite stem cells, a candidate kinase approach was employed to identify Carm1 regulatory kinases. In parallel, tandem affinity purification (TAP) of Carm1 protein complexes coupled with mass spectrometry will be performed to identify Carm1 interacting partners. Following the identification and validation of Carm1 kinases and Carm1 interacting proteins, the functional and biological contributions of these proteins to asymmetric stem cell division and satellite cell function will be determined.
Results
Kinases with established roles in asymmetric cell division and myogenesis were investigated for their ability to phosphorylate Carm1 in vitro. Interestingly, I found that Carm1 is a substrate for p38-gamma MAP kinase. p38-gamma is highly expressed in skeletal muscle and has been shown to phosphorylate MyoD to prevent premature myogenin expression in satellite cells. The interaction between Carm1 and p38-gamma proteins was confirmed by co-immunoprecipitation analysis. Moreover, proximity ligation assays performed on cultured myofibers revealed that Carm1 and p38-gamma interactions occur at the cell membrane in activated satellite cells.
Conclusions
p38-gamma phosphorylates Carm1 and is a candidate kinase for the regulation of Carm1 in asymmetric satellite stem cell division and myogenic commitment. The biological relevance of Carm1 phosphorylation by p38-gamma in satellite cell function is actively being pursued.
Acknowledgements
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77. Modelling Hutchinson-Gilford Progeria Syndrome Using Induced Pluripotent Stem Cells

Zhaoyi Chen, Wing Y. Chang, William L. Stanford
(Background) Hutchinson-Gilford Progeria Syndrome (HGPS) patients exhibit traits of premature aging. The leading cause of death in HGPS individuals is due to deterioration in their vascular tissues. This disease is characterized as a laminopathy-based disease, where a heterozygous mutation in the LMNA gene cause the accumulation of mutant lamin A protein termed Progerin, leading to downstream effects such as alterations in gene expression and heterochromatin

formation. The molecular mechanism of altered gene expression is unknown.
(Aim) HGPS-induced pluripotent stem cells (iPSCs) were used to model the disease to dissect the molecular mechanism underlying this genetic disorder. By characterizing HGPS iPSCs, we aim to examine whether reprogrammed cells have the capacity to differentiate normally, whether reprogramming can reset altered gene expression in HGPS cells, and how mutation in LMNA gene in HGPS cells can affect chromatin formation upon differentiation into vascular smooth muscle cells (VSMCs).
(Method) Reprogramming: Cells derived from Progeria patients were reprogrammed into iPSCs through retroviral infection using four transcription factors: Oct4, Klf4, Sox-2, and c-Myc. Cell potency of each reprogrammed cell line is characterized using alkaline phosphatase staining and immunofluorescence staining of cell surface pluripotent markers. iPSC colonies were induced to form embryonic bodies (EBs), which are then plated to allow growth and differentiation into the three germ layers. Their differentiation capabilities were characterized through immunofluorescence staining using differentiation marker antibodies. Differentiation of iPSCs to VSMCs: EBs were plated on 0.1% gelatin coated plates for 3-5days, EB outgrowth were trypsinized and cells were replated on Matrigel in Medium 231 with growth supplement. Immunostaining on VSMC markers was performed on these cultures. Phospho-H2A.X localization was characterized using western blot as well as Duo-link assay.
(Results / Conclusions) iPSCs derived from patients diagnosed with HGPS exhibited traits of pluripotency, and could differentiate into cells characteristic of the three germ layers. Reprogramming can reset perturbed gene expression in iPSCs caused by mutation in the LMNA gene, reverse HP1 binding distribution back to the normal state. However, the localization pattern of HP1 changes upon differentiation upon heterochromatin formation. VSMCs differentiated from HGPS iPSCs exhibited similar marker expression as coronary artery VSMCs. However, an up-regulation in phospho-H2A.X marker was observed at the DNA replication fork in HGPS VSMCs, suggesting that there is an increase in DNA damage in HGPS VSMCs. In general, a disease model to investigate the progression of HGPS was developed from the study.

78. **Short, Long Term and Paracrine Effect of Human Umbilical Cord-derived Stem Cells in Lung Injury Prevention and Repair in Experimental BPD**

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Background: Bronchopulmonary dysplasia (BPD) remains a main complication of extreme prematurity and currently lacks efficient treatments. Rat bone marrow derived-mesenchymal stem cells (MSC) prevent lung injury in an O2-induced model of BPD. Human cord is an advantageous source of stem cells especially appealing for the treatment of neonatal diseases. The therapeutic benefit after established lung injury and long-term safety of cord-derived stem cells is unknown.
Objective: To determine whether cord-derived cell based therapy is efficient and safe for the prevention and/or treatment of chronic lung disease of prematurity.
Methods: Human cord-derived perivascular cells (PCs) or cord blood-derived MSCs, were delivered prophylactically or after established alveolar injury into the airways of newborn rats exposed to hyperoxia, a well-established BPD model.
Results: Rat pups exposed to hyperoxia showed the characteristic arrest in alveolar growth with air space enlargement and loss of lung capillaries. PCs and MSCs partially prevented and rescued lung function and structure. Despite therapeutic benefit, cell engraftment was low, suggesting that PCs and MSCs act via a paracrine effect. Accordingly, cell free-derived conditioned media from PCs and MSCs also exerted therapeutic benefit when used either prophylactically or therapeutically. Finally, long-term (6 months) assessment of stem cell or conditioned media therapy showed no adverse lung effects of both strategies with persistent improvement in exercise capacity and lung structure.

Conclusions: Human umbilical cord-derived PCs and MSCs exert short- and long-term therapeutic benefit without adverse lung effects in this experimental model and offer new therapeutic options for lung diseases characterized by alveolar damage.

79. **Caspase 3 Promotes Skeletal Muscle Regeneration by Limiting Satellite Cell Self-Renewal**

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Background: Satellite stem cells are indispensable for the growth and regeneration of skeletal muscle. They are a heterogeneous population consisting of stem cells and committed progenitors. The transcription factors which regulate the control of satellite cells lineage progression have been well characterized. Nevertheless, the mechanisms that determine whether activated satellite cells self-renew or differentiate into committed muscle progenitors remains unknown. Caspase proteases are traditionally viewed as conserved cell death proteins, yet recent observations have demonstrated that caspase activity is required for muscle stem cell differentiation.
Objective: Here, we sought to identify whether caspase 3 influenced cell fate by altering the balance between self-renewal and differentiation.
Methods/Results: Using the isolated single fiber model, we have observed that caspase 3 activity is elevated during early stages of satellite cell derived myogenesis. Specifically, we have noted that caspase activity is restricted to Pax7(+) satellite cells and not the mature myonuclei. Peptide inhibition of caspase 3 activity resulted in increased numbers and clusters of the self-renewing Pax7(+)/MyoD(-) satellite cells with a corresponding decrease in the number of differentiating Pax7(-)/MyoD(+) cells. We have shown caspase 3 cleaves full length Pax7 in an in vitro cleavage assay and have begun identifying and examining the functional role of this cleavage event. The targeted destruction of Pax7 may be a critical step in the decision to self-renew or differentiate, as Pax7 expression must be down-regulated to allow the differentiation program to proceed. Caspase 3 cleavage events are often modulated by phosphorylation of the target protein. Phosphorylation via casein kinase 2 (CK2) is a known inhibitor of caspase 3 mediated cleavage. Initial observations suggest CK2 may be playing a Pax7 protective role in self-renewing satellite cells.
Conclusion: These results suggest that caspase 3 is a key determinant in cell fate decisions and promotes differentiation in part by limiting satellite cell self-renewal.

80. **Elucidating the role of dystonin in autophagy**

Andrew Ferrier, Daniel Kuo, Dr. Rashmi Kothary

Dystonin/Bpag1 is a giant cytoskeletal linker protein whose loss of function in dystonia musculorum mice (dt mice) results in a hereditary sensory neuropathy with a progressive loss of limb coordination. Autophagy is a degradative pathway implicated in the recycling of portions of the cytosol and in the removal of superfluous or damaged organelles. Moreover, the autophagic mechanism is highly reliant upon cytoskeletal filaments, and plays a protective role in several human neurodegenerative diseases, including Huntington's, Parkinson's, and Alzheimer's diseases. We therefore wanted to assess the influence loss of function of dystonin has on autophagy in the murine nervous system. By using dtTg4 mice (devoid of both major neuronal dystonin isoforms, dystonin-a1 and dystonin-a2) we assessed autophagic flux in both primary cortical and sensory neurons. While no difference in autophagic flux was found between dtTg4 and WT primary cortical neurons, a difference in autophagic flux was observed in primary sensory neurons. Specifically, we found an accumulation of the autophagasome marker MAP1-LC3-II, an increase in the autophagic substrate p62, and an increase in poly-ubiquitinated proteins. Moreover, we find a decreased co-localization between autophagasomes and lysosomes, suggesting impaired fusion and degradation. To assess which neuronal dystonin isoform was responsible for these aforementioned autophagic defects we developed dystonin-a2 rescue mice. More specifically, exogenous dystonin-a2 expression was driven by the Prion Protein Promoter (PrP-dystonin-a2) on the dtTg4 background. Interestingly, PrP-dystonin-a2 expression on the dtTg4 genetic background reduced the protein levels of MAP1-LC3-II, p62, and poly-ubiquitinated proteins compared to dtTg4 sensory neurons. Taken together, dystonin-a2 plays a role in autophagy in sensory neurons, but not cortical neurons. Further investigation will explore the biological mechanism by which dystonin-a2 contributes to autuphagy and will aid our understanding of dt pathogenesis and other human neurodegenerative diseases.

81. **Adenovirus chromatin: nucleoprotein complex of the viral genome in the infected cell**

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Human Adenovirus (Ad) is a widely studied DNA virus. However, the nucleoprotein structure of the viral genome in the infected cell nucleus is still poorly characterized. Our objective is to establish whether wild type Ad DNA (wtAd) undergoes chromatinization and how this process affects the viral life cycle. Association of DNA binding proteins (protein VII, histones) with wtAd DNA was characterized by ChIP. Micrococcal nuclease (MNase) accessibility assays and Southern blots analysis of infected cell were used to investigate the assembly of viral DNA into nucleosomes. Most of the virus-encoded DNA condensing protein, protein VII, is lost within a few hours of infection and this loss is independent of transcription. Cellular histones associate with the viral DNA soon after removal of protein VII, with a preferential deposition of H3.3. Knockdown of the H3.3 chaperone HIRA reduced early viral gene expression. MNase accessibility assays on DNA isolated 6hrs post infection showed laddering of the viral DNA, suggesting the genome is wrapped in physiologically spaced nucleosomes. Although viral DNA continues to associate with H3.3 at late times of infection, the overall level of association with histones is greatly reduced, which is consistent with the lack of laddering at 18hrs. Our work suggests that wtAd undergoes decondensation (removal of protein VII) and associates with cellular histones within the first hours of infection, with a preferential deposition of H3.3, and that the viral DNA is wrapped in physiologically spaced nucleosomes, at least in early times of infection. This chromatinization process is essential for optimal early gene expression and timely progression of the viral life cycle.

82. **Investigating the role of Pax7 DNA binding domains**

Andrew Jones, Yoichi Kawabe, Jeff Ishibashi, Michael Rudnicki

The postnatal development of skeletal muscle is dependent on a population of myogenic stem cells known as satellite cells. The development and maintenance of the satellite cell lineage is dependant on the activity of the paired-box transcription factor Pax7. Alternative splicing events of the highly conserved paired-box domain (PD) of Pax7 generates four functional isoforms due to the inclusion or exclusion of a glutamine (Q+/-) residue and/or a glycine-leucine (GL+/-) dipeptide. While these various isoforms are believed to exhibit distinct tertiary structures and possess differential DNA binding affinities and specificities, a specific function for the Pax7 alternative splicing events has not been described. Our findings suggest that while all isoforms can induce the proliferative myogenic regulatory factor Myf5 in myogenic cells, only two Pax7 isoforms (Q- GL-and Q+GL-) can initiate Myf5 expression in non-myogenic cell types, suggesting a functional importance for this GL+/- splicing event. In addition, mutational analyses of the closely related Pax3 indicate a functional interaction between the two Pax DNA binding domains; the PD and the homeodomain (HD). To date, the functional specificities of the Pax7 PD and HD remain to be elucidated. In an attempt to determine how Pax7's ability to bind DNA alters target gene expression, we employed Pax7 mutant constructs containing functionally relevant point mutations in the paired domain (G48S) or the homeodomain (N265A). In addition, the HD has been suggested to mediate potential dimer formation and early studies suggest the HD of Pax7 binds cooperatively to specific DNA sequences. Elucidation of the molecular mechanisms regulating Pax7 expression and DNA binding, and further understanding of the isoform-specific target gene regulation, will provide a greater understanding of the molecular regulation present during myogenesis.

83. **MicroRNA misregulation in Multiple Sclerosis - impacts on oligodendrocytes and their ability to differentiate, mature and myelinate axons in the central nervous system**

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Multiple Sclerosis (MS) is a progressive, demyelinating disease of the central nervous system (CSN). MicroRNAs (miRNAs) have been identified as factors that are consistently misregulated in brain lesions from patients with MS. However, the impacts of these misregulated miRNAs in MS have yet to be deduced. In the course of MS, oligodendrocytes (OLs) - the cells responsible for the myelination of axons in the CNS - appear to have a lessened ability to repair damaged myelin as well as a reduction in maturation from precursor cells. Since miRNAs have been shown to have strong control over the differentiation and myelination processes in OLs, it is likely that misregulation of miRNAs during MS has negative effects on OL maturation and myelination. A subset of these misregulated microRNAs is predicted to target cytoskeletal factors that may be important to OLs' capacity for membrane extension and myelin deposition around axons. Our preliminary research has revealed that all of these miRNAs are expressed in Oli-neu cells, an immortalized mouse OL cell line. To

further characterize their role in OL maturation, we have investigated their expression patterns by RT-PCR in Oli-neu cells across three time points. Future work will include manipulation of the expression levels of these miRNAs using lentiviral vectors initially in Oli-neu cells and primary mouse cultures, and finally in a mouse model of MS. Target validation for these microRNAs will be also be explored by PCR array and luciferase assay. Ultimately, rescue of myelination by OLs through manipulation of miRNA levels along with miRNA target validation may reveal valuable therapeutic targets in the treatment of MS.

84. The role of Wnt7a in skeletal muscle

Melanie Lacaria, Julia von Maltzahn, Michael Rudnicki

Previously, our lab performed a gene expression analysis of satellite stems cells and identified a central role for Wnt7a/Fzd7 in regulating the pool size of the satellite stem cell compartment in skeletal muscle. We also determined that Wnt7a is markedly upregulated in newly formed myofibers during regenerative myogenesis, and that the exogenous application of recombinant Wnt7a protein dramatically stimulated the symmetric expansion of satellite stem cells through a process involving components of the non-canonical planar cell polarity (PCP) signaling pathway. Based on these studies, we hypothesize that Wnt7a-stimulated satellite stem cells will exhibit markedly enhanced muscle cell proliferation and engraftment capabilities for the treatment of DMD and other disorders marked by muscle wasting and degeneration. To this end, we propose to investigate whether whole body transgenic over-expression of Wnt7a in skeletal muscle can attenuate the dystrophic phenotype of mdx mice, which are a well-established model for DMD. We will generate transgenic mice that overexpress Wnt7a in an inducible and tissue-specific manner, and cross them to mdx mice; several muscle groups will be isolated, weighed and subjected to immunohistological and morphometric analysis to ascertain muscle mass/volume, fiber number and caliber, and satellite cell number. We will also assess force generation of these muscles ex vivo and perform behavioral tests for motor function to evaluate the effect of Wnt7a overexpression on outward muscle phenotypes in vivo. Successful stimulation of satellite stem cell proliferation and prevention of muscle atrophy in the mdx mouse model may also provide compelling rationale for future studies of other neuromuscular diseases, including other forms of muscular dystrophy, motor neuropaties, such as Dejerine-Sottas syndrome (HSMN Type III), and cachexia.

85. Investigating the Cellular and Molecular Basis of Atrx-Mediated Retinal Interneuron Survival

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Background: Retinal degenerative diseases involve the progressive loss of photoreceptor cells of the retina and are the leading cause of blindness in the developed world. Therapeutic strategies that aim to replace or bypass the lost photoreceptors require the integrity and proper connectivity of the remaining retinal neurons. The survival and functional circuitry of retinal interneurons downstream of the photoreceptors is essential for visual signal processing and transmission leading to effective visual perception. We have generated a mouse model in which retinal amacrine and horizontal cells, the inhibitory interneurons critical for modulation and integration of synaptic activity in the retina, are selectively lost. We are using this model system to delineate the mechanisms that govern retinal interneuron homeostasis and communication in normal and disease states.

Objective: To determine the neuronal circuitry and genetic regulation underlying the loss of retinal amacrine and horizontal cells in mice lacking the chromatin remodeling protein Atrx

Methods: We use conditional knockout approaches to selectively remove Atrx from different retinal cell populations in vivo, including the production of transgenic mice and the surgical delivery of genetic excision tools targeted to specific cell types. Phenotypic analysis of the Atrx-deleted tissues is performed using immunohistochemistry and fluorescence microscopy. Retinal function is examined by electroretinography. Gene expression changes are assessed with DNA microarrays and quantitative RT-PCR.

Results: Amacrine and horizontal cell disorganization and loss occurs when Atrx is deleted in multipotent progenitor cells early in retinal development, but not when the gene is inactivated in lineage-restricted, post-mitotic amacrine and horizontal precursor cells. Electroretinograms show functional deficits in interneuron communication within the inner retina of conditional knockout animals, suggesting a role for bipolar neurons in the observed phenotype. Deletion of Atrx postnatally recapitulates some features of the early knockout phenotype, further suggestive of a causative role played by later born neurons such as bipolar cells. In addition, genetic profiling of the mutant mice reveals dysregulation of bipolar

cell marker genes as well as genes that function in retinal synaptic communication.
Conclusions: The loss of amacrine and horizontal cells from Atrx-deleted retinas appears to occur through a cell non-autonomous mechanism. Electrophysiological analysis, cell type-selective gene inactivation in the retina and gene expression profiling implicate a role for bipolar cells in mediating Atrx-dependent retinal inhibitory interneuron survival and function. Atrx may be involved in the regulation of specific genes that play a role in retinal neuron homeostasis, synaptic activity, and connectivity.

86. ## Proteomic Analysis Identifies Translationally Controlled Tumor Protein (TCTP) as a Potential Novel Mediator of Occlusive Vascular Remodeling in Pulmonary Arterial Hypertension

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Background: Pulmonary arterial hypertension (PAH) is a lethal disease, characterized by excessive proliferation of pulmonary vascular cells. Hereditary (H) PAH is mainly caused by “loss-of-function” mutations in the bone morphogenetic protein type II receptor (Bmpr2). However, the mechanisms by which these mutations cause PAH remain unclear.
Objective: To identify dysregulated proteins in blood-outgrowth endothelial cells (BOECs) of HPAH patients compared with healthy controls that may contribute to the pathogenesis of this disease.
Methods: BOECs were expanded ex vivo from peripheral blood mononuclear cells from 4 patients with HPAH and 4 healthy subjects. Protein isolates were subjected to 2D gel electrophoresis and stained for total proteins and subjected to quantitative computer-assisted analysis (PDQuest software). Differentially regulated proteins were identified by mass spectrometry (LC-MS/MS).
Results: Of the 416 proteins detected, 11 were significantly downregulated in HPAH cells and 11 proteins were upregulated, notably translationally controlled tumor protein (TCTP). TCTP has previously been shown to be enriched in EC apoptotic nanovesicles and to mediate pro-survival signaling in adjacent cells. Therefore, the potential role of TCTP in PAH was studied in vivo in the SU5416 (SU) rat model of severe, angioproliferative PAH. Immunofluorescence staining revealed high expression of TCTP in arteriolar ECs of PAH lungs tightly localized to proliferating cells within occlusive intimal lesions; whereas, only minimal TCTP expression was seen in vascular ECs of normal lungs. Similarly, abundant TCTP immunostaining was also seen in human PAH lung sections, again associated with complex vascular lesions. In BOECs, TCTP has been found to participate in cell growth and survival as its knockdown with siRNA lead to a decreased cell growth, measured by BrdU incorporation, together with decreased cell survival, measured by annexinV and PI staining by flow cytometry.
Conclusions: TCTP could play an important role in PAH by mediating pro-survival and growth signaling in vascular cells, contributing to occlusive pulmonary vascular remodeling triggered by EC apoptosis.

87. ## Role of PCL2 in Acute Myeloid Leukemia

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Background: We have shown that PCL2/MTF2 is a component of the Polycomb Repressive Complex 2 (PRC2), is a critical regulator of mouse embryonic stem cell (mESC) fate, and have drafted the PCL2-PRC2 Gene Regulatory Network (GRN) in mESCs. As Pcl2 represses the expression of a number of oncogenes, we generated a Pcl2 gene signature from the PCL2-PRC2 GRN which was used to screen various cancer databases. The Pcl2 gene signature resembled Acute Myeloid Leukemia (AML) transcriptome data. We postulated that PCL2-PRC2 complex may be involved in hematopoietic stem cell growth and differentiation, promoting leukemogenesis upon misregulation.
Hypothesis: Aberrant expression of PCL2 changes the behaviour of human hematopoietic stem and progenitor cells (HSPCs) such that they are highly susceptible to leukemogenesis.
Results: We screened bone marrow aspirates from 57 AML patients and found 11 patients with loss of PCL2 expression

and 31 patients with over-expression. We then drafted a PCL2-PRC2 GRN specific to the hematopoietic system and tested it within the 57 AML patients. The 11 patients that showed loss of PCL2 expression also showed reduced expression of the PRC2 complex members, while the 31 patients that had over-expression of PCL2 also showed increased expression of the PRC2 complex members.
Cell sorting by FACS and qPCR analysis of Umbilical Cord Blood samples showed the expression of PCL2 to be high within the hematopoietic stem and progenitor cells (HSPCs). The PCL2 expression levels decreased as cells differentiated. We identified efficient shRNA clones for PCL2 KD and successfully cloned them into a lentiviral backbone and used them to KD PCL2 in HSPCs. The PCL2 KD HSPCs were then used to perform in-vitro clonogenic assays (CFU-C and LTC-IC). HSPCs with low levels of PCL2 did not form any Erythroid progenitors; however, they formed highly proliferative Granulocyte and Macrophage colonies. We are currently cloning our PCL2 overexpression vector to study the effect of PCL2 over expression in HSPCs.
Furthermore, we are sequencing the entire genomic sequence of PCL2 and the PRC complexes and the associated demethylase UTX-MLL3 complex in 11 AML patient samples with very low (less than 10 fold) PCL2 expression, 2 AML patient samples with intermediate (3-10 fold PCL2 over-expression) and 11 AML patient samples with very high (over 10 fold) PCL2 expression. This will help us determine the contribution of mutations to abnormal PCL2 expression. We are also performing bi-sulphite sequencing to determine whether the 42 AML patients have methylation alterations.

88. The in vivo role of Polycomb-like 2 (Pcl2)

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Background: Polycomb genes are epigenetic repressors critical in cell fate decisions. We identified Polycomb-like 2 (PCL2) as a novel regulator of embryonic stem cell (ESC) self-renewal and drafted gene regulatory networks demonstrating that PCL2 serves as a lynch-pin in the pluripotency feed-forward network (Walker, 2010). Knockdown of Pcl2 in ESCs causes defects in differentiation and increased self-renewal characteristics. PCL2 exhibits its effects through targeting of the Polycomb Repressive Complex 2 (PRC2) to specific targets and modulating chromatin remodelling via PRC2-mediated histone modification.
Objective and Methods: Pcl2 is expressed at its highest level during development as well as in some adult tissues, such as spleen, thymus, lymph nodes and hematopoietic stem cells. As a follow up to our ESC analysis, we aim to study Pcl2 in vivo through generation of a Pcl2 knockout mouse model using targeted ES cells.
Results: Mutant mice that lack Pcl2 die at e15.5 and exhibit growth defects, hemorrhage and anemia. Pcl2-/- mice have fewer total fetal liver cells compared to their wild-type littermates, but a comparable proportion of CD45+ fetal liver cells. Using a colony forming assay to assess progenitor cell function, Pcl2-/- mice formed more CFU-GEMM colonies, indicating that cells lacking in Pcl2 are in a more primitive/progenitor state. Based on flow cytometric analysis and peripheral blood smears, Pcl2-/- mice have significantly fewer enucleated erythrocytes, suggesting Pcl2 is necessary for definitive erythropoiesis.
Conclusion: We propose that Pcl2 is critical in normal development of the hematopoietic system and plays an essential role in definitive erythropoiesis.
Funding provided by CIHR and CCSRI.

89. Addition of large polypeptides to adenovirus capsid protein IX (pIX) adversely affects viral growth

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Human adenovirus (Ad) has been used in numerous preclinical studies as a platform for vaccine development to treat cancer and other diseases. Ad vectors typically provide very high levels of expression of a foreign transgene protein, which is accompanied by generation of very strong humoral and cellular immunity to the foreign protein. In addition, several studies have shown that foreign antigens can be presented on the surface of the Ad capsid itself resulting in induction of strong, long-lasting immunity in the absence de novo expression of the antigen. The Ad capsid protein IX (pIX) has proven particularly amenable to the addition of large polypeptides to the Ad capsid, such as autofluorescent proteins for virus tracking, single-domain antibodies to redirect virus infection to specific cell types and tissues, or presentation of foreign proteins for vaccination. Ad pIX's normal function is to stabilize the capsid, permitting packaging of full-length Ad genome (36 kb); virions lacking pIX are heat labile and can only accommodate sub-genomic DNA molecules (<35kb). Whether addition of large polypeptides to pIX compromises its ability to stabilize the Ad virion has

not been determined. We show that addition of GFP to pIX does not affect growth or stability of Ad vectors containing small genomes (~32 kb). In contrast, vectors encoding pIX-GFP with a genome size of 36 kb or greater were not viable, suggesting that addition of large polypeptides to pIX compromises pIX's ability to stabilize the Ad capsid. Our data indicates that although pIX can acts as a platform for presentation of foreign antigens on the surface of the Ad capsid, the genome of the Ad must be kept below ~35 kb in order to ensure efficient growth and recovery of the vector.

90. **Defects in Neuromuscular Junction Remodelling in the Smn2B/- Mouse Model of Spinal Muscular Atrophy**

Lyndsay M. Murray, Ariane Beauvais, Kunal Bhanot, Rashmi Kothary

Spinal Muscular Atrophy (SMA) is a devastating childhood motor neuron disease caused by mutations and deletions within the survival motor neuron 1 (SMN1) gene. Although other tissues may be involved, motor neurons remain primary pathological targets, with loss of neuromuscular junctions (NMJs) representing an early and significant event in pathogenesis. Although defects in axonal outgrowth and pathfinding have been observed in cell culture and in lower organisms upon Smn depletion, developmental defects in mouse models have been less obvious. Here, we have employed the Smn2B/- mouse model to investigate NMJ remodelling during SMA pathology, induced reinnervation, and paralysis. We show that whilst NMJs are capable of remodelling during pathogenesis, there is a marked reduction in paralysis-induced remodelling and in the nerve-directed re-organisation of acetylcholine receptors. This reduction in remodelling potential could not be attributed to a decreased rate of axonal growth. Finally, we have identified a loss of terminal Schwann cells which could contribute to the defects in remodelling/maintenance observed. Our work demonstrates that there are specific defects in NMJ remodelling in an intermediate SMA mouse model, which could contribute to or underlie pathogenesis in SMA. The development of strategies that can promote the remodelling potential of NMJs may therefore be of significant benefit to SMA patients.

91. **The MoFlo Flow Cytometry and Fluorescence Activated Cell Sorting (FACS) Facility**

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Flow cytometry is the science of examining physical and chemical properties of live cells or other biological particles as they pass in a fluid stream through a measuring apparatus. This technique uses laser light scattering and/or fluorophore detection to measure cell populations. Some flow cytometers are equipped to separate and collect cells of interest in a technique called Fluorescence Activated Cell Sorting (FACS).

Our Flow Cytometry and Cell Sorting Facility is jointly operated by the Ottawa Health Research Institue (OHRI) and StemCore Laboratories. We provide academic and corporate clients with access to flow cytometry analysis as well as high-speed cell sorting services. We also offer comprehensive training and education as well as expert consultation services to enable our users to utilize this technology to enhance the scope and quality of their research.Our Beckman Coulter MoFlo instrument can be used for high speed cell analysis and sorting (up to 20,000 cells/second). This recently upgraded system contains five excitation lasers, two light scatter detectors, and ten fluorescence detectors, allowing for the analysis of up to nine fluorescence colours at once. Specifically, we are equipped with a UV laser, as well as a 405 nm (violet), 488nm (blue), 561nm (green), and 640nm (red) laser. The two light scatter detectors are forward scatter (cell size) and side scatter (cell granularity). Additionally, the ten fluorescence detectors are completely customizable to the experiment at hand.

The following services are available with the current configuration of the MoFlo:

- Immunophenotyping/immunofluorescence by monoclonal antibodies.
- Total/Absolute population counts.
- Simultaneous analysis of surface and intracellular fluorescent labelling.
- Monitoring gene transfer and expression using fluorescent proteins.
- Simultaneous detection and sorting of GFP and various red fluorescent proteins.
- FRET measurements using GFP and RFP or DSRed.
- DNA content, cell viability, and cell cycle analysis using PI, Hoechst, BrDU, etc.
- Hoechst 33342 side population (SP).
- Cell viability with exposure to drugs, toxins, etc.
- Apoptosis.
- Telomere length measurement.
- Neutrophil functions (phagocytosis and oxidative burst).
- NK cytolytic activity.

• MLR / CTL cytotoxicity.
• Caspase activity.
• Mitochondrial function (DiO, JC-1)
• Intracellular Calcium Flux.
• Q-Prep whole blood lysis.
• Customizable fluorescence assays.

In addition, up to four subpopulations can be sorted at high-speed into bulk collection tubes or multi-well plates for single cell clonal expansion. We will be presenting results from a few of the above-mentioned techniques that have been performed in our facility. Specifically, FACS sorting of satellite cells from mice, SP sorting, and cell cycle analysis results will be presented.

92. **The role of integrin-linked kinase in oligodendrocyte development**

Ryan O'Meara, John-Paul Michalski, Rashmi Kothary

Background: Interactions between the extracellular matrix (ECM) and the ß1 integrin signaling pathway in oligodendrocytes (OLs) are key for the production of myelin. A major downstream effector of ß1 integrin signaling is integrin-linked kinase (ILK), which is involved in stabilizing focal adhesions and transducing ECM signals.

Objective: The current study is aimed at determining the importance of ILK in the development of primary OLs.

Methods: In order to address this aim, we have devised a cell culture system in which we can conditionally ablate ILK from OLs using the cell-permeable TAT-Cre recombinase. Addition of TAT-Cre to cultured ILKfl/fl OLs results in the excision of the Ilk kinase domain, in conjunction with EGFP expression

Results: Upon ILK knockout, there is a delay in the expression of developmental stage-specific markers MAG and MBP. In addition, MAG+ ILK-null OLs are deficient in their ability to extend processes, and produce myelin-like membrane. While some ILK-null OLs are able to produce such membranes, the overall area of these structures is reduced as compared to controls. When co-cultured with dorsal root ganglion neurons (DRGNs), the morphological impact of losing ILK is seemingly less severe. At DIV3 of co-culture, there is no effect of ILK loss on the capacity of OLs to contact and overlap with DRGN neurites. However, at DIV6, ILK-null OLs have a reduced capacity to contact neighbouring DRGN neurites and wrap them with MAG+ membrane. In addition, ILK-null OLs are deficient in their ability to produce myelin-like leaflets when cultured with DRGNs. The reduced production of these structures appears to be the driving force underlying the diminished wrapping of DRGNs.

Conclusions: In summary, ILK is important for various aspects of OL development. Its loss results in delayed development, both in terms of morphology and expression of differentiation markers. When cultured with DRGNs, the morphological phenotype persists but is less severe as compared to when cultured alone. Future work is aimed at investigating signaling alterations in ILK-null OLs that may explain said defects.

93. **Charis Putinski (Abstract not being published)**

94. **New Insights and Contributions for Short Read Sequencing Data Analysis**

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Background & Objective: Reliably estimating the mean fragment length and the genome-wide read density profiles are important problems to be solved in NGS analysis pipelines. Although many analysis tools arrive at these estimates in various ways, the problems are far from being adequately solved as demonstrated by the variability of the results returned by these algorithms. Here we describe some of the recent results of our ongoing investigation aimed at developing improved techniques for obtaining these estimates.

Methods & Results: We first employ strand cross-correlation to estimate mean fragment length and show that the traditional cross-correlation is an unreliable estimator. Based on our insight that the mappability of different parts of the genome can introduce an artificial bias into the cross-correlation function, resulting in incorrect fragment-length estimates, we propose a new approach, called Mappability-Sensitive Cross-Correlation (MaSC), to remove this bias and allow for more accurate fragment-length estimation. We evaluate the performance of MaSC on a test suite of NGS datasets, demonstrating its superiority to traditional cross-correlation analysis.

We then investigate the application of kernel density estimation (KDE) for identifying enriched regions in high-throughput sequencing data. KDE builds a probability density function (PDF) representing the distribution of reads across the genome, which can be used as a reliable predictor of enriched regions. Fixed-bandwidth KDE has been employed in the literature for this purpose; however, its main limitation is its inability to adjust to local density variations, thereby leading to poor accuracy. We propose a variable-bandwidth strategy, where the kernel bandwidth corresponding to a

given read is a function of the read's distances from a predetermined number of neighbouring reads. Consequently, a wide bandwidth is used for low-density regions and a narrow bandwidth is used for high-density regions, thereby resulting in a much more accurate PDF and, in turn, leading to potential improvements in the identification of enriched regions. Various aspects of the proposed technique are illustrated using a number of high-throughput NGS datasets.

95. **A Lymphocyte-Dependent Mode of Action for Imatinib Mesylate in Experimental Pulmonary Hypertension**

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BACKGROUND
Pulmonary arterial hypertension (PAH) is a progressive and ultimately fatal disease with limited treatment options. PAH is marked by significant pulmonary inflammation, increased right ventricular systolic pressure (RVSP), right ventricular hypertrophy (RV/LV+S), and muscularization of the pulmonary arterioles. Recently, small molecule broad spectrum tyrosine kinase inhibitors (sorafenib and imatinib) have proven effective in rodent models of PAH, and imatinib mesylate (Gleevec®) has shown some promise in early clinical trials. The therapeutic effect of these molecules in PAH has been attributed to antiproliferative effects on vascular smooth muscle via PDGF receptor inhibition. However, in other diseases, e.g. gastrointestinal tumours and collagen-induced arthritis, imatinib's influence on the immune system contributes to its efficacy. Here, we employ two immunocompromised rat models to assess the immune system's contribution to imatinib's mode of action in PAH.
OBJECTIVE
To investigate the immune system's response to imatinib in PAH.
METHODS
Two immunocompromised models were used: nude rats, and immunodepleted F344 rats. The severity of PAH induced by monocrotaline (MCT) injection was evaluated by measuring cardiac hypertrophy (RV/LV+S) and right ventricular systolic pressure (RVSP). Muscularization of arterioles was scored by immunohistochemical analysis. Pulmonary leukocytes were detected by immunohistochemical analysis of lung cryosections immunostained for CD3 (Ts), CD68 (macrophages) and CD161 (NKs). Cytokine concentrations in lung lysates were measured using a multiplex bead immunoassay.
RESULTS
1) F344 rats treated with MCT demonstrated increased RVSP, RV/LV+S, and pulmonary arteriolar muscularization at 28 d, signifying PAH. NK and T lymphocytes in the lung decreased in PAH while macrophages increased. These effects were blocked by imatinib treatment. IFN?, TNFa and IL-10 increased in the lung after imatinib treatment, associated with activation of NK and T cells.
2) In nude rats, imatinib decreased RV/LV+S and pulmonary arteriolar muscularization but not RVSP. Notwithstanding the absence of T cells, cytokine levels and NK populations increased in the lung.
3) In F344 rats, NK and T cells were ablated by injecting ASGM-1 antiserum. The beneficial effects of imatinib were blocked; it did not decrease RVSP, RV/LV+S and muscularization of arterioles.
CONCLUSIONS
Our results provide insight into imatinib's mode of action in PAH. We demonstrate that activation of NK and T cells is an important counterpart to imatinib's inhibition of smooth muscle cell proliferation. PAH patients often co-present with diseases that compromise the immune system, e.g. HIV. Our work indicates that the immune status of patients will influence the efficacy of imatinib in PAH.

96. **The Proteostasis Function of the Saccharomyces cerevisiae metacaspase Yca1**

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Background
The activation of caspase/metacaspase proteases has been largely regarded as a detriment to cell viability owing to the nascent ability of these proteins to induce programmed cell death/apoptosis. Despite these prevailing assumptions, our laboratory has shown that both mammalian caspase and yeast metacaspase activities are required for normal cell function, independent of regulating cell death. In the yeast model system we first observed that the single yeast

metacaspase, Yca1, regulates critical checkpoints associated with cell cycle progression to enhance overall fitness and cell survival; a novel non-death function . More recent investigations have shown that Yca1 co-localizes with markers of protein aggregates and acts to limit the accumulation of protein aggregates. However, the exact role of this multifunctional protease in limiting proteotoxicity is still not clear.

Objective

Investigate the role of Yca1 in maintaining proteostasis by determining the structural requirements within the protein and the mechanism by which it functions.

Methods

Using a mutagenesis approach we aim to identify the structure-function relationships within the protein that control the aggregate remodeling and protease activity of Yca1. C-terminal and N-terminal deletion mutants of Yca1 were fused to mRFP and expressed from centromeric plasmids in the null Yca1 strain and monitored for their distribution between soluble and insoluble fraction as well monitored for processing. Vacuolar morphology within these mutant strains was also observed to determine the fidelity of active proteostasis. Further, the protein levels within the insoluble fraction were assessed to determine overall ability to remove aggregates within the mutant Yca1 strains.

Also, we utilized to a proteomics approach to identify potential Yca1 targets and gain further insight into the mechanism by which Yca1 mediates proteostasis. We conducted a 2D LC-MS analysis on the insoluble protein fraction of the wildtype and Yca1 knockout strains and assessed the levels of Yca1-interacting proteins and conducted GO term analysis on the protein dataset.

Results

The N-terminal prodomain of Yca1 regulates autocatalytic processing and targeting to insoluble protein fraction. The loss of theYca1 prodomain results in the increased processing of Yca1. Cdc48 recruitment to the insoluble fraction is Yca1-dependant. Loss of Yca1 alters levels of proteins related to protein translation within the insoluble fraction.

Conclusion

Together, these results suggest that a previously uncharacterized sub-domain of Yca1p regulates autocatalytic processing of the protease, a feature that is essential to the aggregate remodeling activity of the protein. Yca1 may complex with Cdc48 in the insoluble fraction and with Hsp40/70 in the soluble fraction.

97. ## Periostin Induced Pancreatic Regeneration

Johnathan Smid , Sharlene Faulkes, Fan Xaio, Michael Rudnicki

We identified a novel splice isoform of Periostin (Postn) that is highly induced in the regenerating pancreas following partial pancreatectomy. Postn is a 90kDa secreted protein containing four fasciclin domains that have been shown to act through the binding of integrins. Notably, we found that mice lacking Postn were defective in pancreas regeneration both following the administration of streptozotocin or after partial pancreatectomy. Direct injection of Postn into the pancreas resulted in formation of tubular complexes that contain Ngn3- and Pdx1-expressing islet progenitors. This regeneration was not dependent on either the N or C-terminus of the protein, but on the presence of the four fasciclin domains. Intraperitoneal injection of Postn resulted in leaner mice with enhanced glucose tolerance and significantly increased numbers of islets. Furthermore, injection of Periostin into the pancreas ameliorated STZ-induced diabetes. Therefore, Postn is necessary and sufficient for the induction of pancreas regeneration and may represent a novel therapeutic candidate for the treatment of Type 1 Diabetes.

98. ## Effect of BMPR2 mutations on the kinome of late endothelial progenitor cells in heriditary pulmonary hypertension

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Background: Pulmonary arterial hypertension (PAH) is a debilitating disease, which is characterized by profound dysregulation in growth and survival of pulmonary endothelial cells resulting in elevated pulmonary vascular resistance. This obliterative remodelling leads to elevated pulmonary arterial pressures leading to right heart failure and eventually death. Mutations in the bone morphogenetic protein receptor 2 (BMPR2) gene, a serine/threonine kinase receptor in the TGF-B receptor superfamily have been identified in up to 80% of cases of hereditary PAH (HPAH) and 25% of idiopathic PAH (PAH). The mechanism of BMPR2-associated PAH appears to be complex, not only involving alterations in canonical Smad signalling, but also activation of other pathways such as TGF-ß and p38MAPK and ERK1/2.

Objective: To identify differences in kinase signalling pathways between late outgrowth endothelial progenitor cells (L-EPCs) isolated from HPAH patients and healthy controls using an unbiased phosphoproteomics approach.

Methods: L-EPCs derived from the peripheral blood from healthy control and HPAH patients were maintained in endothelial cell culture medium. Whole cell lysates from quiescent L-EPCs were resolved by 2D-PAGE, followed by in-gel phosphoprotein staining. Spots were excised and identified by LC-MS/MS for protein identification. Validation of phosphorylated candidates was carried out by Western blotting for specific phospho-residues. For proteins with unknown phosphorylation sites, validation of the proteomic results were carried out by immunoprecipitation of the candidate, followed by SDS-PAGE and phosphoprotein (ProQ Diamond) plus total protein (SYPRO Ruby) staining. Additionally, Western blotting was performed on lung tissue from rats with SU5416-induced PAH.
Results: Over 20 phosphoproteins were significantly increased ($p < 0.05$) in the HPAH (n=4) vs. control (n=4) group, while reduced phosphorylation was seen in only one. A subanalysis revealed 13 proteins detected solely in HPAH L-EPCs. Within the subset, p21-activated kinase 2 (PAK2) and serine-threonine kinase receptor associated protein (STRAP) were selected for further validation due to their role in mediating TGF-B signalling and regulating cell proliferation and apoptosis. At baseline, abundance of PAK2 and STRAP was not significantly affected by disease state or BMP-9 stimulation. Therefore, we have undergone further validation of phosphorylation status in STRAP and PAK2. An increase in phospho-PAK2 (Ser141) was observed in HPAH L-EPCs ($p < 0.05$). We have also demonstrated proof-of-principle that endogenous STRAP in L-EPCs can be immunoprecipitated for subsequent phosphoprotein gel staining. Also, we have shown for the first time that STRAP protein is increased in lungs of rats with SU5416-induced PAH.
Conclusions: A paradoxical increase in kinase signalling was observed in BMPR2 mutant L-EPCs. Both PAK2 and STRAP may play a role in the pathogenesis if HPAH.

99. **Severe pulmonary hypertension in transgenic mice lacking Sirt1 or Sirt1 catalytic activity in response to chronic hypoxia**

Mohamad Taha, Yupu Deng, Michael McBurney, Duncan J. Stewart

Background: Pulmonary hypertension (PH) is a devastating disease characterized by increased pulmonary artery pressure, leading to right ventricle hypertrophy and ultimately heart failure and death. Sirtuin (Sirt)-1 is an NAD+ dependent deacetylase that has been identified as a crucial link between cellular longevity, metabolism and response to stress. Sirt1 has been strongly implicated in maintaining endothelium homeostasis in systemic vessels, but little is known about its role in the lung vasculature.
Objective: The purpose of this study was to investigate the role of Sirt1 in PH induced by chronic hypoxia (CH).
Hypothesis: Cellular metabolic dysregulation caused by the absence of Sirt1 can potentiate pulmonary vascular response to CH and increase arteriolar remodeling, contributing to a more severe PH phenotype.
Methods: Male and Female sirt1-/-, sirt1Y/Y (H355Y point mutation lacking catalytic activity) and their wild type littermates were exposed to normoxia (N) or CH (9-10% O2). Hemodynamic assessments, lung tissue collection and hematocrit (percent RBCs of total blood volume) levels were obtained at three weeks.
Results: Exposure of sirt1Y/Y mutant mice to CH for 3 weeks resulted in a marked increase in right ventricle systolic pressure (RVSP) compared to their WT littermates exposed to the same conditions (41.5±1.8 sirt1Y/Y vs. 29.7±0.8 WT; n=27 both, $p < 0.001$) with significantly greater RV remodeling, measured by the RV/LV+S weight ratio (0.56±0.02 sirt1Y/Y vs. 0.43±0.01 WT, n=27 both; $p < 0.001$). Similarly, the sirt1-/- mice showed exaggerated responses to CH (RVSP: 42±4.5 sirt1-/- vs. 28 ±1.8 WT; n=7 both, $p<0.01$) (RV/LV+S 0.64±0.04 sirt1-/- vs. 0.43±0.01 WT, n=7-19; $p<0.001$). Hypoxia-induced pulmonary smooth muscle cell hyperplasia was also exacerbated in both mutant strains. Interestingly, there was a profound increase in the hematocrit in both strains compared to wild type animals (71±1.5% sirt1Y/Y, 73±4.8% sirt1-/- vs. 63±1.2% WT, n=7-17; $p<0.001$), which was strongly correlated with increases in RVSP.
Conclusions: The exaggerated PH response to CH in the Sirt1 mutant animals implicates Sirt1 as being part of a protective pathway against hypoxia-induced PH. It also provides an excellent model system to explore the mechanism and relevance of Sirt1, and its downstream targets, in the adaptation of the lung circulation to CH.

100. **PHF6, a nuclear/nucleolar protein that is mutated in Börjeson-Forssman-Lehmann syndrome and T-cell acute lymphoblastic leukemia, interacts with the nucleosome remodeling and deacetylation (NuRD) complex**

Matthew Todd

Mutations in the PHD finger protein 6 (PHF6) gene cause Börjeson-Forssman-Lehmann syndrome (BFLS), a developmental X-linked intellectual disability disorder. PHF6 mutations occur throughout the gene and include missense, nonsense, and inactivating mutations. Recently, we and others have also identified inactivating PHF6 mutations in patients with T-cell acute lymphoblastic leukemia (T-ALL) and acute myeloid leukemia (AML), suggesting the involvement of PHF6 in both developmental and acquired disease. The PHF6 protein contains two atypical PHD zinc fingers that share homology with chromatin remodelers including the MLL family of histone methyltransferases, although the precise

functions of PHF6 remain unknown. To elucidate a role for PHF6, we performed Flag purification of PHF6 in HEK 293T cells and co-purified several putative nuclear and nucleolar interacting partners. Interestingly, we were able to validate an interaction between PHF6 and multiple constituents of the NuRD complex, which contributes to lineage commitment in several mammalian developmental pathways, including T-cell development. We have since developed a PHF6 loss-of-function model in order to better assess functional implications of PHF6 loss and the contribution of NuRD-mediated transcriptional regulation with respect to the molecular mechanisms that link PHF6 mutations with the BFLS and T-ALL disease states.

101. ## Exogenous Hydrogen Sulfide (H2S) Protects Alveolar Growth In Experimental O2-Induced Neonatal Lung Injury

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INTRODUCTION: Bronchopulmonary dysplasia (BPD), the chronic lung disease of prematurity, is characterized by arrested alveolar development and complicated by pulmonary hypertension (PHT). Currently there is no specific treatment for BPD. Hydrogen sulfide (H2S), together with carbon monoxide and nitric oxide (NO), belongs to a class of endogenously synthesized gaseous molecules referred to as gasotransmitters. While inhaled NO is already used for the treatment of PHT and currently tested for the prevention of BPD, H2S has long been exclusively regarded as a toxic gas. Recent evidence suggests that endogenous H2S exerts beneficial biological effects, including vasodilation and anti-inflammatory properties.
HYPOTHESIS: H2S preserves normal alveolar development and prevents PHT.
METHODS AND RESULTS: All procedures were approved by the animal welfare committee. Statistical comparisons were made using student t-test or ANOVA, Fisher's post hoc analyses. We took advantage of a recently described slow-releasing H2S donor, GYY4137 (morpholin-4-ium-4-methoxyphenyl(morpholino) phosphinodithioate). In vitro, H2S preserved alveolar epithelial type 2 cell (AT2) viability during hyperoxic stress (95% O2), accelerated AT2 wound healing and vascular network formation of lung microvascular endothelial cells. To test the therapeutic potential of H2S in vivo, rat pups were randomly exposed from birth to normoxia, normoxia+GYY4137 (37.75 mg/kg/day intraperitoneally), hyperoxia (95% O2, BPD model), and hyperoxia+GYY4137. H2S preserved alveolar growth in hyperoxic rats and attenuated PHT in hyperoxic animals as determined by improved pulmonary arterial acceleration time on Echo-Doppler and decreased right ventricular hypertrophy as compared to untreated animals.
CONCLUSION: H2S prevents arrested alveolar development and PHT in experimental BPD. H2S warrants further investigation as a new therapeutic target for alveolar damage and PHT.

102. ## Wnt7a Treatment Ameliorates Muscular Dystrophy

J. von Maltzahn, J.M. Renaud, M.A. Rudnicki
Duchenne muscular dystrophy is a debilitating degenerative disease caused by the absence of the dystrophin protein. The muscle cannot compensate for increased susceptibility to damage resulting in cycles of regeneration and degeneration. Ultimately the muscle is replaced by fibrotic tissue concomitant with reduced muscle strength.
Wnt signalling plays an important role in the homeostasis of adult tissues. In skeletal muscle Wnt7a drives the expansion of the satellite stem cell pool through the PCP signalling cascade thereby facilitating regeneration. Furthermore, we discovered that Wnt7a activates the Akt/mTOR anabolic pathway in differentiated myofibers thereby inducing hypertrophy in concert with its receptor Fzd7. Notably the activation of the Akt/mTOR pathway through Wnt7a is independent of IRS-1 and IGF-receptor. Treatment of dystrophic mice with Wnt7a resulted in increased numbers of satellite cells and pronounced hypertrophy. In addition to significantly increased muscle size Wnt7a augmented the tetanic force in healthy and dystrophic mice thereby providing additional physiological relevance. Furthermore Wnt7a reduced contractile damage in mdx muscles through a shift in fiber types. Lastly we demonstrated that Wnt7a also induces hypertrophy and a switch in fiber types in human primary myotubes. These results suggest that Wnt7a is an effective ameliorative treatment for muscular dystrophy.

103. **Disease Modeling of Tuberous Sclerosis Complex Using Patients' Specific Induced Pluripotent Stem Cells**

Ying Wang, Sean Delaney; William L. Stanford

Tuberous sclerosis complex (TSC) is a rare multi-system genetic disease caused by loss of function mutations to the tumor suppressor proteins TSC1 or TSC2, nodes of the mTOR signaling network. Patients are born as heterozygous for TSC1 or TSC2. Non-malignant tumors initiate upon loss of heterozygosity in the brain and other vital organs such as the kidneys, heart, eyes, lungs, and skin. Patients may be developmentally delayed and/or suffer from seizures, psychiatric disorders, skin abnormalities, lung and kidney disease. Interestingly, only women of child bearing age develop lung tumors, known as lymphangioleiomyomatosis (LAM). The cell of origin and the molecular mechanisms underlying the generation of the various tumors TSC has not been elucidated. We hypothesize that resident neural crest progenitors are the cell of origin in part because TSC tumor cells are histologically similar to undifferentiated smooth muscle cells and express various neural crest markers. Unfortunately, there is no effective treatment for TSC. Part of this reason is that there are no good models to study TSC. TSC null cells do not grow well in tissue culture and knockout mice share only some of the phenotypes of patients. A milestone in stem cell research was the development of reprogramming technologies that enable the generation of induced pluripotent stem (iPS) cells from somatic cells by ectopic expression of four defined transcription factors (OCT4, SOX2, KLF4 and c-MYC). The derived iPS cells are similar to human embryonic stem cells in morphology, transcriptome, epigenome, expression of pluripotency-associated genes, and differentiation capacities in vitro and in vivo. Moreover, iPS cells can be derived from a patient's own cells. Therefore, iPS cells have enormous potential to be used in disease modeling, drug development and transplantation therapy. In our project, iPS cells have been respectively derived from a TSC patient's TSC2 heterozygous dermal fibroblasts and TSC homozygous tumor cells using a non-integrating technique (episomal vectors) in defined culture conditions. Through differentiating the iPS cells into neural crest stem cells and smooth muscle cells in vitro and in vivo, the disease model will be created. To model LAM and angiomyolipoma, we will deliver differentiated TSC tumor iPS cells to the lungs and kidneys of immunocompromised (NSG) mice. The disease model will be used to elucidate the mechanisms underlying TSC and develop the therapies for this often fatal disease.

104. **Screening for molecular regulators of muscle stem cells in their niche**

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In muscle regeneration, the activation of resident muscle satellite cells to proliferate, differentiate, and fuse is necessary to lead to formation of new myofibers. However, prolonged regenerative states due to degenerative disorders, such as Duchenne's muscular dystrophy, lead to the depletion the satellite cell population and attenuate regenerative capacity. Our lab has identified the satellite stem cell population (Kuang et al., 2007; Cell) and demonstrated that increasing the tissue content of these cells results in a direct enhancement on regeneration kinetics (Le Grand et al., 2009; Cell stem cell). However, in vitro culture of satellite cells leads to their spontaneous differentiation into myoblasts; thus the maintenance and regulation of satellite stem cells is not well understood. To study the cell signalling network controlling the homeostatic levels of satellite stem cells and their response in regeneration, we have developed a novel drug screen platform to examine the proliferation kinetics of satellite stem cells on ex-vivo cultured muscle fibers. Screening against small library of compounds (~400 compounds), we have identified compounds capable of modulating the activation/proliferation kinetics of satellite stem cells and their committed progeny. Using pathway analysis, we have identified families of compound targeting known regulatory pathways of satellite stem cell homeostasis. As well, we identified Aurora A Kinase and Erbb2-EGFR pathways as a novel modulator of asymmetric satellite stem cell divisions.

105. **Histological and Immunofluorescent Analysis of Skeletal Muscle and the Satellite Cell**

David H.L. Wilson, J. Von Maltzahn, M.A. Rudnicki

Research into debilitating muscle related diseases will continue to generate high profile public interest due to the percentage of the population affected by such issues. Despite much work, the detailed mechanisms of muscle development and regeneration after injury are not yet clearly defined. The use of mouse models of disease combined with histological and immunofluorescent techniques provides essential insights, however, variation in methodology and analysis across the research spectrum can result in inconsistencies between laboratories and debate with regards in interpretation. The continuing research into debilitating muscle diseases requires clear cut guidelines for the investigation and analysis of muscle tissue. Here we present a methodological demonstration of histology and immunofluoresence in the investigation and quantification of mouse hind limb skeletal muscle tissue, with a particular focus on the satellite cell,

the stem cells of skeletal muscle. We show methods for inducing injury and challenging muscle, histological procedures for the identification of tissue and immunofluorescent procedures for the identification of different cell types and visualisation of proteins of interest. Finally, we discuss techniques for the analysis, quantification and interpretation of data obtained. Development and refinement of these procedures is essential for further study of muscle tissue and will lead to improved and standardised analysis and improved comparability of results obtained in different settings. These techniques are not just relevant to the research environment but also are applicable in the laboratory medicine field where muscle biopsy investigation is key to diagnosis and treatment.

106. **Treatment of cancer cells with an adenovirus vector encoding the p14 FAST protein causes cell fusion and enhances sensitivity to the chemotherapeutic drug bleomycin**

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Background: Adenoviruses (Ad) have been used in many preclinical and clinical studies for delivery and expression of anti-cancer genes or as an oncolytic vector. However, Ad has limited ability to spread throughout the tumour mass. The p14 fusion-associated small transmembrane (FAST) protein has the ability to mediate cell-cell fusion and apoptosis of fused cells, leading to enhanced membrane permeability. The FAST protein enhanced the efficacy of oncolytic viruses such as vesicular stomatitis virus and vaccinia virus.
Objective: To determine if Ad-mediated FAST protein expression will promote virus spread and/or enhance cell death in cancer cells.
Methods: 293 human embryonic kidney and A549 human lung adenocarcinoma cells were infected with early region 1(E1)-deleted adenoviral constructs expressing the p14 FAST protein. Immunoblotting was performed to confirm FAST protein expression. Infected cells were observed for fusion using fluorescence microscopy. Immunoblots probing for full length BID were conducted to determine whether there was enhanced cell death. Fluorescence based live/dead assays and MTS assays were conducted to assess cell viability. Cell membrane permeability was assessed using lactate dehydrogenase release assays while cell survival was determined using crystal violet assays.
Results: An E1 and E3-deleted Ad expressing FAST protein caused extensive cell fusion in replication permissive 293 cells and also at high multiplicity of infection, in A549 cells. Although FAST protein expression did not affect A549 cell viability directly, it did lead to decreased cell membrane integrity. Consequently, Ad-mediated FAST protein expression enhanced cell death following administration of the membrane impermeable chemotherapeutic drug bleomycin.
Conclusion: FAST protein expression can enhance uptake and action of chemotherapeutic drugs and improve Ad spread through tumour cells through cell-cell fusion.

107. **The Smn-independent beneficial effects of Trichostatin A on an intermediate mouse model of SMA**

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Background: Trichostatin A (TSA) is a histone deacetylase inhibitor with beneficial effects in SMA mouse models that carry the human SMN2 transgene. Whether TSA specifically targets the upregulation of the SMN2 gene or whether other genes respond to TSA and in turn provide neuroprotection in SMA mice is unclear. We have taken advantage of the Smn2B/- mouse model, which does not harbor the human SMN2 transgene, to test the hypothesis that TSA has its beneficial effects through a non-Smn mediated pathway.
Results: Daily intraperitoneal injection (IP) of TSA (10 mg/kg body weight) or vehicle DMSO from postnatal day (P) 12 to P25 was performed in the Smn2B/- mice and littermate controls. Treatment with TSA increased the median lifespan of Smn2B/- mice from twenty days to eight weeks. As well, there was a significant attenuation of weight loss and improved motor behavior. Pen test and righting reflex both showed significant improvement following TSA treatment. Motor neurons in the spinal cord of Smn2B/- mice were protected from degeneration after TSA treatment. Both the size and maturity of neuromuscular junctions were significantly improved in TSA treated Smn2B/- mice. qPCR analysis revealed no changes in the level of Smn transcripts in TSA-treated SMA mice compared to control mice. Furthermore, western blot analysis revealed no significant upregulation in Smn protein levels in the brain or spinal cord of the TSA treated Smn2B/- mice. Therefore, we suggest that the beneficial effects on the SMA phenotype are likely Smn-independent. We are

currently investigating whether TSA alters regulation of actin cytoskeleton dynamics.
Conclusions: Serial TSA treatment significantly extends lifespan, attenuates weight loss and improves motor function in Smn2B/- mice. TSA treatment protects spinal cord motor neuron number, improves myofiber size in TA muscle, and promotes maturity of NMJs in Smn2B/- mice. TSA did not increase the level of Smn transcripts or Smn protein in various tissues of Smn2B/- mice. The beneficial effect of TSA is therefore likely through an Smn-independent manner. Identification of these pathways will be of therapeutic value for the treatment of SMA.
Acknowledgements: Funding for this research was from Canadian Institutes of Health Research and Muscular Dystrophy Association (USA).

108. MicroRNA-133 Controls Brown Adipose Determination by Targeting Prdm16

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The transcription factor Prdm16 governs brown-adipogenic lineage determination from myogenic progenitors during embryogenesis. However, it has not been established whether myogenic progenitors in adult skeletal muscle can be similarly specified. Here, we report that the expression of microRNA-133 in satellite cells in adult skeletal muscle prevents brown adipose determination by directly targeting the 3'UTR of Prdm16. Loss-of-function of microRNA-133 during muscle regeneration elicits brown adipogenic commitment of satellite cells in vivo and induces their differentiation into interstitial brown adipocytes, which increases local uncoupled respiration, glucose uptake and thermogenesis. Local antagonism of microRNA-133 during muscle regeneration also augments energy expenditure, improves glucose tolerance and impedes the development of diet-induced obesity. Notably, miR-133 similarly controls brown adipose determination in adipogenic progenitors isolated from brown adipose tissue or skeletal muscle. Our data reveal a central role of miR-133 in regulating brown adipose determination and suggest a therapeutic approach for obesity by inducing brown adipose tissue in vivo.

109. Functional Modification of Outgrowth Human Endothelial Progenitor Cells with Growth on Osteopontin Substrates and eNOS Transfection

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Background and Objective:
Complete endothelial coverage of the blood contacting surfaces of cardiovascular biomaterials has been difficult to achieve. A readily available autologous source of endothelium would improve the chances of developing functional and biocompatible surfaces.
Methods and Results:
Here we describe methods to derive high quantities of Endothelial Progenitor Cells (EPCs) from Peripheral Blood Monocytes (PBMCs) obtained by leukapheresis. These cells are morphologically and largely phenotypically indistinguishable from Human Umbilical Endothelial Cells; however, their expression of the key vascular factor, endothelial nitric oxide synthase (eNOS), is markedly lower than that observed in human umbilical vein derived endothlelium. We demonstrate here that eNOS levels can be elevated with transient plasmid based transfection. To further enhance EPC adherence and function we examined Osteopontin (OPN), as a substrate showing dose and time dependent responses of OPN in EPC adhesion and spreading. OPN also promoted haptotactic migration of EPCs in Boyden chamber assays. In addition, the OPN coating successfully enhanced the adhesion of eNOS overexpressing EPCs (39.6±1.7 and 49.4±2.4 cells/field for 0 and 1 nM OPN) and spreading (84.7±3.5% and 92.1±3.9% for 0 nM and 1 nM OPN). Microencapsulation of EPCs in agarose supplemented with OPN results in enhancement of cell survival (0.216 relative absorbance value (RAV) vs. 0.141 RAV for LEPCs encapsulation with OPN and without encapsulation, respectively).
Conclusions:
These data confirm the direct effects of OPN on EPCs adhesion, migration as well as increase of cell survival, suggesting that OPN works by mediating cell adhesion during vascular injury and the combination of eNOS transfection of autologous EPCs and OPN coatings could be a promising method of developing highly functional endothelialized biomaterials.

Vision Program

110. ## Investigating the Role of Sortilin in Sonic Hedgehog Trafficking

Charles Campbell , Shawn Beug, Chantal Mazerolle, Valerie Wallace

Background: Sonic Hedgehog is a secreted morphogen, involved in patterning numerous tissues during development. Secreted Shh can act short and long range, affecting tissues dozens of cell bodies away. In the optic nerve, the range of Shh signaling is even greater, as Shh produced by the Retinal Ganglion Cells (RGCs) is trafficked via the regulated secretory pathway down RGC axons, though the mechanisms involved in this transport remain largely unknown. Sortilin (Sort), a multifunctional sorting receptor specializing in targeting ligands to the regulated secretory pathway, was identified in an affinity purification screen as a novel interacting candidate for Shh.
Objective: To characterize the role of Sort in Shh trafficking.
Methods: To determine the impact of Sort on Shh trafficking, Shh distribution was examined in Sort gain and loss of function (GOF and LOF) cortical neurons (CNs). For GOF, Shh was coexpressed with wild type Sort or dominant negative, truncated Sort (tSort); for LOF , Shh was coexpressed with short hairpins targeting Sort mRNA (with Scrambled control), or in CNs from Sort nullizygous embryos (with wild type littermate controls). CNs were counterstained for Shh, Sort, and various subcellular organelles, including synaptic vesicles and axons. To determine the affect of Sort on Shh secretion, Shh-responsive LIGHT2s were cultured and exposed to conditioned media from fibroblasts expressing Shh in Sort GOF and LOF. Shh pathway activation was determined as the activity of Shh-responsive Firefly-Luciferase normalized to constitutively active Renilla-Luciferase.
Results: As Sort targets its ligands to the regulated secretory pathway, we expected Sort GOF to increase Shh axonal targeting. Unexpectedly, Sort GOF decreased Shh in the axons and in synaptic vesicles, and coexpression with tSort, which is not actively trafficked, abolished Shh signal in the axon. Consistent with this observation, Sort LOF increased Shh in the axons and in synaptic vesicles. Rescue of the LOF condition (expression of Sort in null cortical neurons) reduced Shh signal in the axons and synaptic vesicles, reversing the Sort null phenotype. In LIGHT2 cells, control conditioned media was able to activate the Shh pathway, while GOF conditions decreased pathway activation.
Conclusions: Sort appears to be a negative regulator of Shh trafficking to the regulated secretory pathway. GOF and LOF studies in CNs and LIGHT2s suggests that Sort functions to limit Shh targeting to secretory pathways, and thus limit the release of biologically active Shh.

111. ## Investigating agrin, a sonic hedgehog interacting protein

Faduma Jama, Charles Campbell, Jacqueline Tokarew, Shawn Beug, Chantal Mazerolle, Valerie Wallace

Background: Sonic hedgehog (Shh) is a secreted signaling protein that regulates patterning and cell fate in a variety of tissues though activation of a signaling cascade that culminates in altered gene expression. The effects of Shh signaling can occur over short (1-2 cell diameters) and longer range (300μm) distances. In the retina, for example, Shh is expressed in neurons and it signals over a short range to adjacent progenitor cells and over longer distances to glial cells in the optic nerve. This long range signaling is associated with anterograde transport of Shh down retinal ganglion cell axons via the regulated secretory pathway, though the molecular basis of this mode of Hh trafficking remain relatively unknown. Through an affinity purification screen, we identified Agrin as a novel Shh interacting protein, and confirmed the interaction via reciprocal co-immunoprecipation (co-IP). Agrin is a heparan-sulfate proteoglycan, of which the secreted isoform is released from the nerve terminal to cluster acetocholine receptor (AChR) of the motor end plate, at neuromuscular junction (NMJ). This role of Agrin in directing the development of the NMJ has been well documented, although its role in modulating Hedgehog signaling is not known. Objective: To investigate the nature of the Agrin-Shh interaction and its impact on the secretion and biological function of each respective protein. Methods: To determine the impact of Agrin on Shh secretion, media from cells co-transfected with Agrin and Shh was tested for Hh levels by ELISA and for Hh activity by measuring luciferase activity in the Hh-responsive LIGHT2 cells. Results: As measured by ELISA, Shh secretion into the medium was increased following co-transfection with constructs encoding for the secreted form of Agrin. Previous work in the lab has shown that Agrin and Shh can be co-immunoprecipitated from the medium. Furthermore, in LIGHT2 cells, conditioned media from cells co-transfected Agrin and Shh stimulated a higher fold activation of the Shh pathway in comparison to cells transfected with Shh alone. Conclusions: From the ELISA assays, and LIGHT2 data, secreted Agrin appears to increase the release of biologically active Shh from a cell. Future directions include investigating the impact of Shh on Agrin-induced receptor clustering through AChR aggregation assays performed with cultured C2C12 myotubes.

112. ## The role of Gli in Hedgehog dependent neural progenitor proliferation

Randy Ringuette, Michael Atkins, Alan J. Mears, Chantal Mazerolle, Valerie A. Wallace

Background: The Sonic hedgehog (Shh) signaling pathway is a critical regulator of growth and patterning in a variety of tissues and organs. In the developing retina, a tractable model system of central nervous system development, Shh signaling from neurons targets the adjacent neural progenitor cells. Studies of conditional Shh inactivation in vivo and gain of function in vitro have demonstrated that Shh is required to maintain progenitor proliferation and control cell fate.

How this important signaling pathway affects these different developmental processes is largely unknown, but is likely to involve the downstream mediators of the Shh signaling pathway, the Gli transcription factors. In the retina, Shh target gene expression and proliferation require Gli2, but not Gli1. We hypothesize that transcriptional and posttranscriptional regulation of Gli2 is essential for neural progenitor self-renewal and differentiation.

Objective: To determine the levels of Gli2 at the protein and message level over the course of retinal development and investigate how interactions with known Gli2 interacting proteins regulate Gli2 function and stability in neural progenitor cells.

Methods: Acutely dissected and cultured neonatal retinal tissues were subjected to western blot or quantitative real time PCR. To over express Gli2, explants were transfected using electroporation.

Results: During retinal development Gli2 message is initially expressed in the developing neuroblast layer and becomes restricted to a thin row of cells in the inner nuclear layer, likely the Müller glia, the only cell type in the adult retina that can re-enter the cell cycle. Similarly, Gli2 protein is detectable only at early stages of retinal development and loss of Gli2 protein correlates with loss of retinal progenitor cells, suggesting that down regulating Gli2 transcription may be part of the neuronal differentiation program. Consistent with the inverse correlation between neuronal differentiation and the presence of Gli2 protein, we find that ectopically expressed Gli2:GFP fusion proteins in retinal explants only exert a transient effect on proliferation and are down regulated after several days, which corresponds to the timing of cell cycle exit and neurogenesis. In addition, ectopic Gli2 expression in retinal explants resulted in only minimal Shh target gene induction (compared with activation of the Shh pathway upstream of Gli2 transcription factors), suggesting that additional regulators are required for full Gli2-mediated transactivation of target genes.

Conclusion: Based on results thus far, restricting Gli2 activity might be a mechanism utilized by RPCs to activate the differentiation program and forced expression overcomes this process, resulting in proliferation.

113. ## Transplantation of cone photoreceptors into the murine retina as a therapy for retinal degenerative diseases

Sheila Smiley, Sherry Thurig, Adam Baker, Alan Mears, Catherine Tsilfidis, Valerie Wallace

Background: Loss of photoreceptors, due to injury or disease, results in visual impairment or blindness. Retinal transplantation aims to replace these lost photoreceptors with stem cells that will differentiate and form connections with the existing retinal cells, thereby recovering visual function. While many advances have been made in the field of retinal transplantation, much of the work has been done on rod photoreceptor transplantation. Although rod photoreceptors mediate vision in dim light, cones photoreceptor function is essential for colour vision and high visual acuity. Additionally, cone function is typically lost in many retinal degenerative conditions, including macular degeneration. Therefore, we have developed the Nrl-/-Ccdc136+/- mouse model to use as a donor line in cone transplantation studies. The retinas in this model are enriched with cone photoreceptors that express GFP under the control of the Ccdc136 locus. Objective: Our study aims to characterize the GFP+ve cells in the Nrl-/-Ccdc136+/- model and investigate their integration potential in retinal transplantation. Methods: Histological sections of eyes and dissociated retinal cells were prepared from Nrl-/-Ccdc136+/- mice and were analyzed with immunohistochemistry for the characterization of GFP+ve cells. For transplantation, retinas from donor mice were dissociated and 200,000 cells were injected sub-retinally into adult recipient mice. Injected eyes were harvested at three weeks post injection and were analyzed for integrated cells using confocal microscopy. Results: In Nrl-/-Ccdc136+/- retinas, GFP was expressed in 60% of retinal cells, with expression observed primarily in the cells in the outer nuclear layer (ONL) and in a subset of cells in the inner nuclear layer (INL). GFP+ve cells in the ONL co-expressed cone markers, including PNA, S and M opsin, confirming their identity as cone photoreceptors. GFP+ve cells in the adult retina co-localized with bipolar markers, such as Chx10 and PKC, identifying these cells as bipolar neurons. This co-expression is found in adult retinas and does not initiate until after P6. Using this model for transplantations, cells were found to survive for at least three weeks post injection. Typically, it was observed that 100 to 200 transplanted cells had migrated into recipient retinas. Conclusion: Our analysis identifies the Nrl-/-Ccdc136+/- mouse model as a strain with a large population of GFP-marked cone photoreceptors. These cells are able to survive transplantation and migrate into a recipient retina. Future studies aim to improve integration numbers to rates that will allow for visual recovery in degenerated retinas.

114. **The Role of Norrie Disease Pseudoglioma (Ndp) in cerebellar development and its relationship with the sonic hedgehog pathway**

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Background
Norrie Disease Pseudoglima (Ndp) is an X-linked cysteine-rich secreted protein that is best known for its role in regulating angiogenesis in the developing retina. Mutations in the Ndp gene cause Norrie disease, a congenital condition that is associated with congenital blindness, deafness and in some cases, cognitive delays. Recently, we showed that Ndp is a target of the Hedgehog signaling pathway in neural progenitors in the developing retina. The Sonic hedgehog ligand is an essential regulator of neural progenitor proliferation in several regions of the brain, including the retina and cerebellum. In the retina, Ndp is a downstream mediator of Hh-driven proliferation. Our objective was to investigate how Hh-dependent Ndp function extends to other neural progenitor pools by studying its role in cerebellar development.
Objectives
Our objective is to investigate the function of Ndp in neural progenitor development in the cerebellum.
Methods
We will examine the spatial and temporal expression profile Ndp as well as explore the role of Ndp in neuronal proliferation and survival. This investigation will be carried out in vivo in transgenic mice and in vitro, by using primary granule neuron progenitor (GNP) cell cultures.
Results
Our data shows that Ndp is expressed in the neural progenitor pool of the cerebellum.
Conclusions
The Hh pathway plays a causal role in the development of medulloblastoma, a tumor of the cerebellum and one of the most common pediatric solid tumors. Investigating the relationship between Hh and Ndp in cerebellar development has the potential to identify novel therapeutic targets for the treatment of these tumors.

115. **Characterizing The Dedifferentiation Response In The Regenerating Newt Limb**

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The newt, Notophthalmus viridescens, has the ability to regenerate lost structures, including the limbs and tail, throughout its adult life. This phenomenon has been studied for over 250 years, but relatively little is known about the molecular events involved in the regeneration process.
To learn more about limb regeneration in the newt, we have identified transcripts that are potentially over- or underexpressed in 3-day regenerating limb tissue via subtractive hybridization. Approximately 500 unique genes (or EST contigs) were spotted onto microarrays which were then hybridized with RNA from regenerating limb tissue at various time points to yield a temporal expression profile for these genes during the regeneration process. Using quantitative RT-PCR, we have validated the differential expression of approximately 70% of the genes identified by subtractive hybridization and/or microarray analysis. Finally, RNA from various time points of tail regeneration was also used to probe the microarrays to determine if any of the genes display a similar response in the different tissues which may be indicative of a more general role in regeneration.
Current efforts are underway to clone and characterize interesting candidates identified by the subtractive hybridization and microarray screens.

Other

116. ## Gating false alarms from a commercial myocardial ischemia detection system through real-time signal quality estimation of ambulatory ECG monitoring

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BACKGROUND: Around the world, approximately 100 million people undergo non-cardiac surgery each year. Of these more than 1 million will experience a major cardiac complication as a result of increased blood chemicals from the surgery. Prophylactic ß-blockade has been shown to reduce the incidence of these cardiac complications in at-risk patients, however a large scale study of the treatment has demonstrated that it also increases mortality rates. This is likely due to administration of ß blockers to less at-risk patients. Changes in a patient's electrocardiogram (ECG) due to myocardial ischemia (lack of blood flow to the heart) are typically visible prior to cell damage. PROSE seeks to selectively administer ß blockers to only those patients exhibiting myocardial ischemia by employing real-time ECG monitoring. The environment surrounding postoperative patients following non-cardiac surgery subjects the ECG to high levels of noise. As such, myocardial ischemia detection techniques still return significant numbers of false alarms despite supplementary engineered "white box" solutions. OBJECTIVE: This research seeks to establish and validate real-time signal quality estimation techniques to enhance the supplementary parallel "white box" solution, which will be used to control a commercially supplied detection system. METHODS: Signal quality estimation algorithms leverage the repeatability of ECG waveforms to form an updating approximation of the true signal of an electrical heartbeat. This updating approximation of the true heartbeat is used to calculate the signal to noise ratio (SNR) of incoming ECG data and provide a real-time estimation of signal quality. Validation of the estimation algorithms and true signal approximation is being performed through offline analysis with signals of varying known quality levels. This real-time quality measure will then be used to control alarm output from the commercial system with the aim of suppressing false alarms generated during periods of poor signal quality. RESULTS: Preliminary testing of the estimation algorithms indicate correlation between estimated SNR values and the SNR values of known signals. CONCLUSION: Preliminary testing indicates that it is possible to estimate SNR from noisy ECG data. This suggests feasibility for using a derived real-time signal quality measure as a gating feature to control false alarm rates from a commercial myocardial ischemia detection system. Consequently, sensitivity and specificity rates may be balanced such that PROSE can more accurately identify patients experiencing myocardial ischemia.

117. ## Dopaminergic Neuron Regeneration In The Goldfish Brain

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Background: Abnormalities in the functioning and signaling pathways of dopaminergic (DA) neurons can lead to neurodegenerative diseases such as Parkinson's disease. A parkinsonian syndrome can be induced in vertebrates by injecting the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Goldfish (Carassius auratus) injected with MPTP (50µg/g Bwt) had severe depletion of DA neurons in the telencephalon, optic tectum, and the midbrain as determined using tyrosine hydroxylase (TH) immunohistochemistry. Other evidence indicates that these DA neurons regenerate, suggesting the existence of neuronal stem cells in the adult brain.
Objective: My project focuses on investigating the neuroanatomical influence of MPTP on DA neurons in the goldfish brain, identifying regenerating DA neurons following MPTP injection and assessing the upregulation of stem cell markers during neuronal regeneration.
Methods: The neurotoxic effects of MPTP in the goldfish brain are investigated using immunohistochemistry with anti-TH and anti-nestin antibodies at various time-points following injection. Testing this hypothesis required the generation of the first rabbit anti-goldfish nestin antibody. Nestin is an intermediate protein involved in neuronal generation and is a good neuronal stem cell marker. I have located proliferating cells in the goldfish forebrain marked by the presence of bromodeoxyuridine and proliferating cell nuclear antigen immunoreactivity following MPTP injection. I also sequenced the coding region of the goldfish nestin mRNA using a genome-walking strategy.
Results: Depletion of DA neurons in the telencephalon, optic tectum, and the midbrain following MPTP injection was observed and determined using immunohistochemistry and light microscopy. The antigen I used to generate the nestin antibody was a multi-antigenic peptide composed of a 23 amino acid fragment of nestin. Western blotting experiments indicate that the predicted ~143 kDa nestin precursor protein is processed to smaller fragments of 30, 37, and 70 kDa in

vivo. Preliminary experiments indicate an unpregulation of nestin at the time when DA-depletion begins.
Conclusion: The study has explored the location of MPTP-affected DA neurons in the goldfish brain by localizing and comparing TH-immunoreactive cell bodies and fibers in non-treated and MPTP-treated goldfish brain. These studies will provide further insight into DA neuronal circuitry and regenerative mechanisms in teleost. My research project provides a venue to studying neuronal regeneration, neuronal circuitry, and brain remodelling following MPTP injection and highlights important comparisons between various vertebrate models.

OHRI Core Facilities

118. **Proteomics @ OHRI**
Lawrence Puente
The OHRI Proteomics Core Facility provides an advanced technology platform for biological mass spectrometry, enabling the identification and quantification of proteins from biological samples.

119. **StemCore Laboratories Genomics Facility**
Katayoun Sheikheleslamy, **Caroline Vergette**, Atieh Jalali-Pakdaman, Pearl A. Campbell.
StemCore Laboratories www.stemcore.ca is OHRI's high-throughput genomics facility located at the General Campus of the Ottawa Hospital Research Institute. Our mandate is to provide access to research-enabling technologies which are beyond the scope of individual laboratory operations, thereby facilitating biological and medical research through the use of high-end technology and state of the art equipment. StemCore is continuously developing a world-class genomics services infrastructure, and is capable of facilitating large-scale scientific research and biotechnology projects. StemCore provides DNA sequencing services, Affymetrix Genechip microarray solutions, and a recently acquired Next-Generation sequencing platform. StemCore seeks out projects that are challenging, cutting-edge, extend the boundaries of biological knowledge, and will positively impact the state of human health

OHRI IMPACT (Identification of Marketable Products, Applications and Commercializable Technologies) Award Posters

120. **Improving extubation decisions through multiorgan variability analysis**
Andrea Bravi, André Longtin, Andrew JE Seely, on behalf of the WAVE (Weaning and Variability Evaluation) Research Team.

121. **Viral Sensitizer Compounds for Enhancing Vaccine Manufacturing**
Fabrice Le Bœuf, Jean-Simon Diallo.

122. **X-linked Inhibitor of Apoptosis (XIAP) Gene and Protein Therapy to Prevent Retinal Degeneration**
Sarah Wassmer, Catherine Tsilfidis

Evaluation Instructions

Once again we are offering prizes for the best trainee presentations at OHRI's Research Day. Participants will be judged in the following categories:

Best Poster ($500 for 1st, $250 for 2nd and $100 for 3rd in each category)

- Masters
- PhD
- Postdoctoral

Best Oral Presentation ($500 for 1st, $250 for 2nd and $100 for 3rd)

The following criteria have been given to all the evaluators to guide their judging.

All presentations will be evaluated with a score from zero to 100 for each of the following categories:

- Introduction (clearly presented rationale and hypothesis)
- Methodology (sufficiently clear with appropriate details)
- Results (quality and clearly explained)
- Discussion (summary, interpretation and relevance)
- Visual appearance of poster/slides
- Ability to answer questions

The scale should be applied as follows:

50 – 59	Below average: unclear methodology and results
60 – 69	Average: many presentations will fall into this category
70 – 79	Good: most presentations will fall into this category
80 – 89	Very good: clearly above average; only a few fall into this category
90 – 100	Excellent: Best possible!! Wow!! Top 5%.

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