2014 Annual Pathology Research Day

Friday, March 28, 2014

Program Guide

Schulich MEDICINE & DENTISTRY

Western

This event is an Accredited Group Learning Activity (Section 1) as defined by the Maintenance of Certification program of The Royal College of Physicians and Surgeons of Canada and approved by Continuing Professional Development, Schulich School of Medicine & Dentistry, Western University (4.5 hours). Each participant should claim only those hours of credit that he/she actually spent participating in the educational program.

This program has no commercial support.

Message From The Chair

Annual Pathology Research Day is a major celebratory event and one of the most rewarding days of our academic year. It is a day to celebrate the outstanding work of our students, residents, fellows, and faculty. The day showcases what we have accomplished in basic science, clinical science, and education research. Our department continues to play a leading role in areas of recognized excellence at Western. The broad range of topics being presented is a true reflection of the multi-disciplinary nature of our research.

We are very fortunate to have internationally recognized scholar, Dr. Tom Hudson, deliver the keynote address. Dr. Hudson is the President and Scientific Director of the Ontario Institute for Cancer Research (OICR). He is internationally renowned for his work in genomics and human genome variation. He has also been a leader in the development and applications of robotic systems and DNA-chip-based methodologies for genome research. He exemplifies the bridge between basic research and clinical medicine, which is integrated in our educational and research programs in the Department of Pathology.

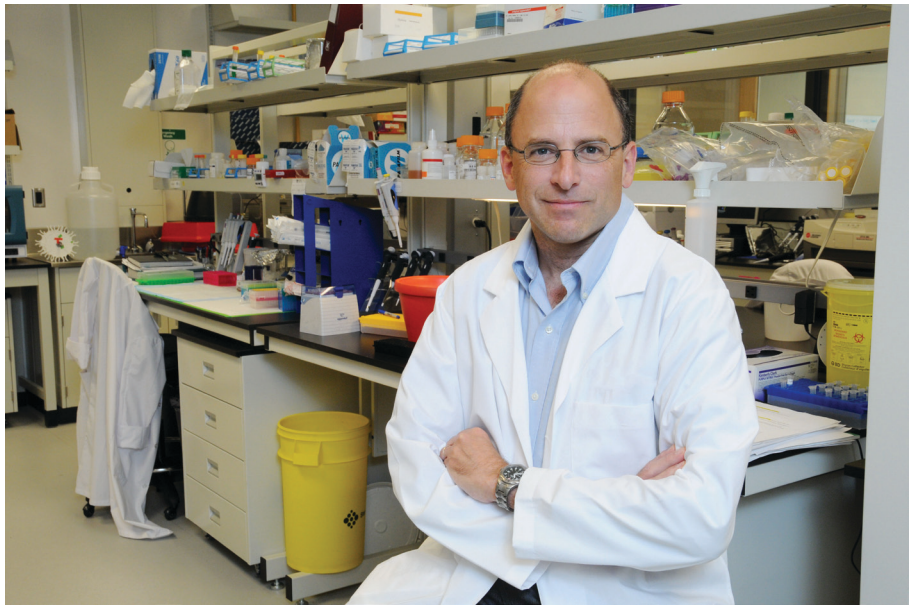
This event is made possible by the efforts of the organizing committee. I would like to personally thank Zia Khan, Nancy Chan, Manal Gabril, Martin Duennwald, Allison Osmond, Michael Ruiz, Matthew Riopel, Susan Stewart, Tracey Koning, Rodney Hagle and many others for making this day a success. I hope you enjoy the day and learn about the fantastic research being carried out in our department.

Subrata Chakrabarti, MBBS, PhD, FRCP(C)
Chair/Chief – Department of Pathology
Western University and London Health Sciences Centre

2014 Annual Pathology Research Day

PROGRAM

9:05am	**Welcome, Opening Remarks** **Dr. Subrata Chakrabarti** Chair/Chief, Pathology and Laboratory Medicine, Schulich Medicine & Dentistry, Western University and London Health Sciences Centre
9:15am	**Keynote/ Speaker** **Dr. Tom Hudson** President and Scientific Director, Ontario Institute for Cancer Research (OICR) Professor, Medical Biophysics, University of Toronto
10:15am	**Nutritional Break**
10:45am	**Platform Presentations**
12:00pm	**Pathology Faculty Presentation** Dr. Lisa Cameron "Understanding Genetic Susceptibility to Allergic Disease - Role of CRTh2"
12:30pm	**Lunch**
1:15pm	**Poster Presentations**
4:00pm	**Awards Ceremony** Ivey Spencer Leadership Centre



Featuring:

Dr. Tom Hudson

President and Scientific Director,
Ontario Institute for Cancer Research (OICR)
Professor, Medical Biophysics,
University of Toronto

Keynote / Address:
Genome Variation & Personalized Cancer Medicine

Platform Presentations

#	Last Name	First Name	Program	Title
1	Al Sufiani	Fahd	PGY4	(11;22) Translocation Negative Ewing sarcoma/peripheral primitive neuroectodermal tumour (EWS/PNET)
2	A. Tikhomirov	Ilia	MSc	Development of a Novel EGFR-Targeting Therapeutic Antibody-Drug Conjugate for the Treatment of EGFR-Expressing Malignancies
3	Ruiz	Michael	MSc	SUZ12 regulates glucose induced VEGF production through miR-200b regulation in retinal endothelial cells
4	A. Khan	Wahab	PhD	Differences in Chromatin Accessibility are present between Homologous Metaphase Chromosomes
5	Stecho	Will	PGY2	Assessment of Resident Grossing Skills in the Digital Age: Evaluation and Implementation of a Mobile Digital Assessment Tool

Platform Presentation #1

(11;22) Translocation Negative Ewing sarcoma/peripheral primitive neuroectodermal tumour (EWS/PNET)

Fahd Al Sufiani[1,2], Bret Wehrli[1,2] and Lee-Cyn Ang[1,2]

[1]Department of Pathology, Western University; [2]Pathology and Laboratory Medicine, London Health Sciences Centre

Introduction: Ewing sarcoma/peripheral primitive neuroectodermal tumour (EWS/PNET) is one of the primitive round cell sarcomas of children and adolescents. The vast majority of EWS/PNET are characterized by a t(11;22)(q24;q12) translocation, involving the EWSR1 gene. The aim of this study is to review the histopathology and the immunohistochemistry of EWSR1 negative EWS/PNET cases at London Health Sciences Centre (LHSC).

Methods: A total of 15 pediatric EWS/PNET cases were retrieved from the pathology department archive at LHSC between the years 2002 to 2013. The terms “Ewing's sarcoma” and/or “peripheral primitive neuroectodermal tumour” were used as key words in the search retrieval. The age, gender, location of tumor, histopathology, immunohistochemistry, and cytogenetic findings, both classical karyotyping and FISH analysis, were reviewed.

Results: Three out of the 15 EWS/PNET cases were excluded from the study due to the absence of cytogenetic testing. Nine cases (75%) of EWS/PNET were EWSR1 positive. The remaining EWSR1 negative cases were identified in 3 females. The age at diagnosis ranged from 2 to 12 years. In one case, the EWS/PNET was found in the rib (osseous) while the other 2 cases were extra-osseous. CD99 immunostaining was diffuse in 2 cases and multifocal in one. All 3 cases were poorly differentiated with minimal neuroectodermal differentiation. Despite the lack of a detectable EWSR1 translocation, two cases were categorized as classical EWS/PNET and the other one as belonging to the EWS family of tumors based on the histopathology and immunohistochemistry.

Conclusions: EWS/PNET is a heterogeneous tumor with an overlapping histologic, immunohistochemical, cytogenetic and molecular genetic features. An accurate diagnosis requires correlation between clinical findings, histopathology, immunostaining and molecular testing.

Keywords: Ewing sarcoma; peripheral primitive neuroectodermal tumour; pediatric sarcoma

Platform Presentation #2

Development of a Novel EGFR-Targeting Therapeutic Antibody-Drug Conjugate for the Treatment of EGFR-Expressing Malignancies

Ilia A. Tikhomirov[2,3,7], Maria L. Jaramillo[1], Traian Sulea[1], Myriam Banville[1], Rene Figueredo[2], Jason Baardsnes[1], Suzanne Grothe[1], Gregory P. Adams[4], Walter Blättler[5], Maureen D. O'Connor-McCourt[1], and James Koropatnick[2,3,6,7]

[1]Human Health Therapeutics Portfolio, National Research Council of Canada, Montreal, Canada; [2]Lawson Health Research Institute, London, Ontario, Canada; [3]AvidBiologics Inc., Toronto, Ontario, Canada; [4]Department of Microbiology and Immunology, Fox Chase Cancer Center and Temple University School of Medicine, Philadelphia, USA; [5]Morphosys AG, Planegg, Germany; [6]Departments of Oncology, Microbiology and Immunology, and Physiology and Pharmacology, Western University; [7]Department of Pathology, Western University

Introduction: Conjugation of antibodies to potent cytotoxics to create antibody-drug conjugates (ADCs) is an attractive strategy to improve anticancer activity of mAbs. However, development of EGFR-targeting ADCs has been blocked by concerns that already debilitating dermatologic toxicities with anti-EGFR mAbs may be aggravated with ADCs. Severe dermatologic toxicities of EGFR-targeting occur as a result of EGFR expression by keratinocytes. We sought to overcome this barrier by screening for EGFR-targeting antibodies for which conjugation to highly potent cytotoxic payload: (1) increases anti-cancer activity against EGFR-expressing cancer cells, and (2) does not increase toxicity against EGFR-expressing keratinocytes.

Methods: A library of EGFR targeting antibodies was conjugated to highly potent cytotoxic agent to create fully functional ADCs. The ADC were subsequently evaluated in vitro using SPR, and alamarBlue assays in a variety cell lines. NIH III mice were injected s.c. with tumor cells (MDA-MB-468; FaDu) and treated with a novel ADC selected on the basis of desirable in vitro activity.

Results: Screening identified a unique anti-EGFR antibody that when linked to a highly potent cytotoxic payload did not exhibit any observable potentiation of toxicity against keratinocytes compared to naked antibody. Conversely, the novel ADC exhibited significantly enhanced cytotoxic activity against multiple cancer cell lines compared to non-conjugated antibody.

MDA-MB-468 tumour xenograft studies demonstrated significant tumour growth delay and complete remission in over 90% of tumours after a single ADC dose (5 mg/kg) compared to 0% complete remission in vehicle or naked antibody controls. FaDu tumour xenograft studies demonstrated significant capacity of candidate ADC to reduce growth in vivo.

Conclusion: This research identified a novel anti-EGFR ADC that may be safe and effective in the treatment of EGFR-expressing malignancies.

Keywords: EGFR, antibody-drug conjugates, ADCs, antibodies, antibody screening, oncology

Platform Presentation #3

SUZ12 regulates glucose induced VEGF production through miR-200b regulation in retinal endothelial cells

Michael Ruiz[1], Biao Feng[1], Rokhsana Mortuza[1], Subrata Chakrabarti[1,2]

[1]Department of Pathology, Western University; [2]Pathology and Laboratory Medicine, London Health Sciences Centre

Introduction: Glucose induced augmented vascular endothelial growth factor (VEGF) production is a key event in diabetic retinopathy. We have previously demonstrated that downregulation of miR-200b causes overexpression of VEGF, mediating structural and functional changes in the retina in diabetes. However, regulation of miR-200b is not known. Polycomb Repressive Complex 2 (PRC2) is histone methyltransferase complex whose components EZH2, EED and Suz12 have been demonstrated to repress miRNAs in neoplastic process. We hypothesized that, in diabetes, PRC2 represses miR-200b through its H3K27 methylation mark.

Methods: Human retinal microvascular endothelial cells (HRMECs) were treated in high glucose (25mM) or normal glucose (5mM) for 24 or 48 hours. Expression of miR-200b, VEGF, EZH2, EED and Suz12 were measured by qPCR. EZH2 activity was measured using a commercial kit and miR- 200b promoter methylation was measured by ChIP-PCR. Loss-of-function experiments were also performed using a chemical inhibitor for EZH2, 3- Deazaneplanocin A (DZNep), and siRNA against various targets. Finally, gene expression was measured in the retinal tissues of diabetic animals by qPCR.

Results: When treated with high glucose, HRMECs showed significantly decreased miR-200b expression with increased VEGF expression. EZH2, EED and Suz12, were also overexpressed in both HRMECs in high glucose and diabetic retinal tissue. Increased EZH2 activity was detected globally and at the miR-200b promoter region, though additional replicates are necessary. Furthermore, inhibition of PRC2 with DZNep produced increased miR-200b expression with parallel decreased VEGF, demonstrating a causal link. Interestingly however, silencing of EZH2 directly produced no changes in miR-200b and VEGF, though silencing SUZ12 produced increased miR-200b and decreased VEGF.

Discussion: This research demonstrates a repressive relationship between Suz12 and miR-200b that appears independent of EZH2. These data further provide evidence of a novel mechanism of miRNA regulation through another epigenetic pathway, i.e, histone methylation. Understanding such pathways will potentially yield new treatment strategies.

Keywords: microRNA, histone methylation, VEGF, retinal endothelial cells, diabetic retinopathy

Platform Presentation #4

Differences in Chromatin Accessibility are present between Homologous Metaphase Chromosomes

Wahab A. Khan[1,3], Peter K. Rogan[2,3,4], and Joan H.M. Knoll[1,3,4]

Departments of Pathology[1], Biochemistry[2], Computer Science[3], Western University; [4]Cytognomix, London, Ontario, Canada

Introduction: Mitotic metaphase chromosomes are a product of complex chromatin restructuring during interphase. Defects in this organization have implications for physiological and pathological processes. We previously detected differences in chromatin accessibility, termed differential accessibility (DA), between human metaphase homologs by fluorescence in situ hybridization (FISH) using short (1.5-5 kb), single copy (SC) genomic sequence-defined DNA probes. This study maps inter-homolog DA at sub-optical resolution and determines whether DA is non-random.

Methods: To quantify DA, genomic-sequence defined probes were mapped onto chromosome topography by FISH correlated atomic force microscopy or super-resolution 3D-structured illumination. Next, SC FISH probes were hybridized to cytogenetically distinguishable homologs and the probe's chromatin accessibility at single cell resolution was tracked. Finally, epigenetic features of open chromatin were examined for genomic targets with and without DA.

Results: Reproducible DA has been observed in ~10% of 450 distinct genomic regions mapped by SC FISH. Genomic regions with no DA (NOMO1, NOMO3) hybridized to grooves in chromosome topography and exhibited similar probe depth ($\bar{x}$differences = 0.08 µm) and volume between homologs. In contrast, probe targets with DA had different depths ($\bar{x}$differences = 0.77µm) and volumes (~4-fold). For cytogenetically distinguishable homologs, DA was non-random ($p < 0.05$) for 8 genomic targets from different chromosomes among unrelated individuals. We further showed that within a three-generation family pedigree, the more accessible homolog was always paternal in origin. Genomic regions with no DA between homologs were also enriched in epigenetic features of accessible interphase chromatin (i.e. DNase hypersensitivity) relative to those regions with DA.

Discussion: We present several lines of evidence that differences in condensation between homologs are programmed during metaphase chromosome compaction. These differences are non-random and have an inherited parental chromosome bias. We suggest DA provides a form of chromatin memory, linking the epigenetic programs of parent and daughter cells.

Keywords: Differential accessibility, Homologous chromosome structure, Metaphase, FISH, Chromatin memory, Epigenetics

Platform Presentation #5

Assessment of Resident Grossing Skills in the Digital Age: Evaluation and Implementation of a Mobile Digital Assessment Tool

Will Stecho[1,2], Aaron Haig[1,2], Allison Osmond[1,2], and Michele Weir[1,2]

[1]Department of Pathology, Western University; [2]Pathology and Laboratory Medicine, London Health Sciences Centre

Introduction: The upcoming transition to competency-based medical education (CBME) within Canadian residency programs will require an increased frequency of high-quality trainee assessment. A robust and efficient assessment tool will enable supervisors to meet these new educational demands while minimizing the increased administrative burden. In preparation for this transition, our centre evaluated and implemented mobile computing technology for the assessment of resident grossing competencies.

Methods: Pre-existing paper-based grossing skill competency assessment tools were reformatted for easy digital data entry using Microsoft Word. Documents were then converted into digital fillable PDF forms using Adobe Acrobat XI. An Apple iPad running the PDF Expert app was utilized to allow evaluators to complete digital forms in the grossing room during residency competency assessments. Completed assessments were immediately digitally signed by the supervisor and resident before being secured to prevent further modification. Secured digital copies were emailed to the supervisor and resident for inclusion in their learning portfolios.

Results: The use of fillable PDF forms mimicked the intuitive ease of paper forms, improved data security, and minimized data entry and administration time. Utilization of a mobile platform minimized data replication and offered increased convenience over traditional computing devices. The entire process, from form completion to email distribution, took only minutes and was performed entirely on the iPad at the gross bench upon completion of the assessment. The supervisors were able to learn to use the device and software quickly and easily.

Conclusion: Through the use of mobile computing devices and digital PDF forms, we developed an efficient system which combines the ease-of-use and convenience of our previous paper-based assessment tool, with the speed and data security of computer and web-based assessment systems. Future obstacles to implementation include initial capital costs for the digital device and software, and a shallow software learning curve.

Keywords: education, assessment tools, mobile computing, competency-based medical education

Poster Abstracts

#	Last Name	First Name	Program	Title
1	Leung	Aaron	BMSc	The Effects of Human DnaJ Proteins on Huntingtin Aggregation and Toxicity
2	Pavlosky	Alexander	MSc	RIPK3 regulates microvascular endothelial cell death and cardiac allograft rejection
3	Osmond	Allison	PGY4	A Competency Based Assessment Tool for Pathology Resident Grossing
4	Pena	Ana M	MSc	Investigating the effects of modified human acidic Fibroblast Growth Factors treatment in Diabetic Nephropathy
5	Arifin	Andrew	BMSc	Influence of Desferrioxamine and Gallium Conjugates on Staphylococcus Aureus Infection
6	Thomas	Anu A.	PhD	Role of H19 in diabetic complications
7	Hennop	Anzel	BMSc	The potential role of RIPK3 in PARP-1-mediated memory T cell necrosis
8	Lau	Arthur	PhD	SPI-6 (Serpin Protease Inhibitor-6) inhibits granzyme B mediated injury of renal tubular cells and promotes renal allograft survival
9	Blanchard	Audrey	MSc	Ontario Growth Standards for Infants: A Retrospective Autopsy Study
10	Schick	Brian A.	PGY3	Negative Colorectal Polyp Biopsies: The Utility of Cutting Deeper Levels
11	Kwok	Cecilia	MSc	Characterizing the impact of membrane vesicles produced by apoptotic and necrotic tubular epithelial cells on renal transplant rejection and graft-versus-host disease
12	Garcia-Marques	David	PGY2	Follicular Mucinosis with a Clonal T-cell Pattern – A Case Report
13	Kagen	Dov B.	BMSc	Expression of the Growth Hormone Secretagogue Receptor in Human Cardiomyopathies
14	Cox	Jacqueline	MSc (OMFS)	Expression of Human tissue Kallikriens (KLKs) in Polymorphous Low Grade Adenocarcinoma (PLGA)

#	Last Name	First Name	Program	Title
15	Roos	James	BMSc	Trauma in adult pedestrians due to frontal motor vehicle collisions
16	Peart	Jason	MSc	The Effect of a Conditional β-cell Specific β1-Integrin Knockout on β-cell Survival, Proliferation and Function
17	Strong	Jenna	MSISc (PA)	Canada's First Accredited Pathologists' Assistant Graduate Program: A Self Study and Review
18	Hui Yan	Jin	BMSc	The Impact of Indoleamine-2,3-dioxygenase (IDO) Isoforms in Melanoma Cells
19	Kum	Jina	MSc	β-adrenergic receptor-independent action of propranolol in infantile hemangioma
20	Montgomery	Jule A.	MSISc (PA)	Pediatric Pathology Cases: Retrospective Review at London Health Sciences Centre for Training Purposes
21	Watson	Kelsey	BMSc	Interaction of Primary Human Trabecular Meshwork Cells with Metal Alloy Candidates for Microinvasive Glaucoma Surgery
22	Cheung	Kevin	MSc	The role of Rho Guanine Nucleotide Exchange Factor, an RNA-binding protein discovered in amyotrophic lateral sclerosis, in stress response
23	Siu	King Sun	PhD	Folate receptor targeted siRNA delivery with a novel non-covalent functionalization of single-walled carbon nanotubes for cancer therapy
24	Brennan	Lacey	BMSc	Determining Global Cytogenomic Changes in Classical Hodgkin Lymphoma
25	Piccinin	Meghan A.	MSc	Extracellular Matrix is Selectively Regulated Following Diabetes-induced Adipogenesis of Bone Marrow Mesenchymal Progenitor Cells
26	Zhou	Liangyi	MSc	Investigation of β-cell insulin receptor regulation of β-cell growth, function and survival
27	Woo	Mark	BMSc	Immunohistochemical Characterization of mTOR Pathway Activation in Gastroenteropancreatic Neuroendocrine Tumours
28	Chan	Matthew	BMSc	Tissue Engineering a Corneal Stroma for Transplantation through Keratocyte-mediated Mechanotransduction
29	Riopel	Matthew	PhD	Fibrin mediates Human Islet Cell Differentiation via p70s6k, promotes Integrin Expression while enhancing Vasculature during Transplantation
30	Piccinin	Meghan A.	MSc	Distinctive Expression of Niche Factors by Adipocytes and Osteoblasts May Mediate Diabetic Stem Cell Depletion
31	Wen	Mei	BMSc	The Effects of Mechanical Stress on Human Trabecular Meshwork Cells
32	Krstic	Milica	MSc	The role of transcriptional regulator TBX3 in early breast cancer progression
33	Brackstone	Muriel	PhD	Concurrent Neoadjuvant ChemoRadiotherapy Improves Pathological Complete Response But Not Survival in Locally Advanced Breast Cancer
34	Johnston	Nathan	MSc	miRNA Regulation of PD1, TIM3 and BTLA: Reverting T-cell Exhaustion in Melanoma
35	Henley	Phaedra	PhD	Hair Cortisol as a Biomarker of Stress in Sub-Saharan African Communities
36	Zhang	Qi	PGY3	Molecular characterization of gastrointestinal glandular-neuroendocrine mixed tumor
37	Mantha	Rebecca	MSISc (PA)	The Application of a Multitissue Spring-roll Control Block in Immunohistochemistry
38	Woodford	Rebecca	MSc (OMFS)	Selected Human Kallikrein Expression in Odontogenic Cysts and Tumors

#	Last Name	First Name	Program	Title
39	Ansari	Reem	MSc	ERK5 expression in Brain Tumors
40	Filek	Richie	MSc	Investigating the Structural and Functional Changes to the Retina Following PRP in Diabetic Retinopathy Patients
41	Asiry	Saeed	PEAP	Traditional and Electronic Ki-67 Quantitation in Oligodendrogliomas
42	Doshi	Samik	BMSc	The Role of MicroRNA-346 in Breast Cancer
43	Mekhaiel	Sandra	BMSc	Understanding the Mechanism of Carboplatin-Induced Vascular Dysregulation in Ovarian Serous Adenocarcinoma
44	Multani	Satwinder	MSISc (PA)	Lymph Node Dissection in Total Gastrectomy Specimens. A Retrospective and Prospective Institutional Review
45	Shekari	Shayan	MSc	Understanding Head and Cervical Spine Injuries in Pediatric Occupants Involved in Motor Vehicle Collisions
46	Diregorio	Sonja E.	MSc	Deciphering the role of RGNEF in ALS using a novel yeast model
47	Mok	Stephanie	MSc	Epithelial to mesenchymal transition in the pathogenic progression of small intestinal neuroendocrine tumours
48	Tejeda Saldana	Yadira	PhD	Validating Inclusivity/ Exclusivity of an Alternative Method for Detecting E. coli O157 According to AOAC Guidelines
49	Chang	Yuxin	BMSc	Effect of Dietary Modifications during Pregnancy With and Without Diabetes on Offspring Pancreas Development
50	Kerr	Zachary	MSc (OMFS)	Expression of kallikrein-related peptidases (KLKs) in Adenoid Cystic Carcinomas

Poster Abstract #1

The Effects of Human DnaJ Proteins on Huntingtin Aggregation and Toxicity

Aaron Leung[1], and Martin L. Duennwald[1]

[1]Department of Pathology, Western University

Huntingtin's Disease (HD) is a neurodegenerative disorder caused by an autosomal dominant mutation in the IT15gene, which encodes for the Huntingtin protein (HTT). This mutation results in the expression of HTT with an abnormally long polyglutamine (polyQ) region, thus defining HD as one of nine known polyQ expansion disorders. PolyQ expansion proteins misfold, aggregate, and cause the dysfunction and death of neurons.

All eukaryotic cells possess elaborate protein quality control mechanisms to guide proper protein folding, prevent protein misfolding and its cytotoxic consequences. Cellular protein quality control has also been established as a promising therapeutic target for the treatment of many neurodegenerative diseases. Key players in protein quality control are molecular chaperones, including DnaJ proteins. DnaJ proteins are expressed in all living cells and constitute the most diversified and substrate-specific class of molecular chaperones. Here, we explore the specific interactions of human DnaJ proteins with polyQ expanded HTT and its role in HD using yeast as a model system.

Yeast models have been established as powerful tools to explore the cellular mechanism underlying protein misfolding in neurodegenerative diseases, including polyQ expansion diseases. We have used yeast models to study the effects of DnaJ proteins on the aggregation and toxicity of polyglutamine (polyQ) expanded HTT. Two DnaJ proteins, Ydj1 and Sis1, modulate polyQ toxicity and aggregation. Both chaperones reduced polyQ aggregation: yet while Ydj1 reduced polyQ toxicity, Sis1 increased it. It remains unknown, however, if these DnaJ-mediated effects are unique to yeast. To address this knowledge gap, our study will focus on the human orthologs (DnaJA1 for Ydj1 and DnaJB1 for Sis1) and their effects on polQ toxicity.

Yeast strains expressing polyQ expanded HTT (tagged with CFP, cyan fluorescent protein) will be transformed with either DnaJA1, DnaJA2 (a negative control) or DnaJB1 with a carboxy-terminal YFP (yellow fluorescent protein) tag. We will monitor the effect of these human DnaJ proteins on polyQ toxicity using growth assays and fluorescent microscopy to assess the aggregation and of polyQ expanded HTT as well as the localizations of both HTT and DnaJ proteins.

While it is too early to draw any conclusions from our results, we expect the human DnaJ proteins to have similar effects on polyQ toxicity and aggregation as their yeast counterparts. Our studies will thus establish yeast as a model to explore cellular and molecular mechanisms underlying the interactions between human molecular chaperones and misfolded proteins in neurodegenerative diseases.

Poster Abstract #2

RIPK3 regulates microvascular endothelial cell death and cardiac allograft rejection

Alexander Pavlosky[1,2], Xuyan Huang[1], Arthur Lau[1,2], Ziqin Yin[1], Aaron Haig[2,3], Dameng Lian[1], Anthony M. Jevnikar[1,3], and Zhu-Xu Zhang[1,2,3]

[1]Matthew Mailing Centre for Translational Transplantation Studies, London Health Sciences Centre; Departments of Pathology[2] and Medicine[3], Western University

Introduction: Despite recent advances in immunosuppression, patients who have undergone allogeneic cardiac transplantation still suffer from poorly understood chronic graft loss. Previous studies have shown that Tumour Necrosis Factor Alpha (TNFα) contributes to cell death by activating apoptotic and newly identified Receptor Interacting Protein 1 and 3 (RIPK1/RIPK3) mediated necroptotic death pathways. These variations in cell death may be important for graft survival as necroptosis can lead to the release of chemotactic and activating danger molecules which have been shown to activate host immune cells. This pathway has yet to be studied in transplantation.

Methods: We isolated and treated microvascular endothelial cells (MVECs) from C57BL/6 hearts and treated them with TNFα in the presence and absence of RIPK1 inhibitor, necrostatin-1 (nec-1), and pan-caspase inhibitor, zVAD-fmk to inhibit and induce necroptosis, respectively. Release of pro-inflammatory cytokine HMGB1 was also measured in the supernatant of cells following treatment. In addition, in vivo heterotopic heart transplantation was also performed using wildtype C57BL/6 or C57BL/6-RIPK3-/- donors into fully MHC mismatched BALB/c mice following short term sirolimus treatment. Graft survival and viability was determined at several timepoints.

Results: Our data shows that sirolimus treatment (9 days) markedly prolongs cardiac allograft survival of B6-RIPK3 null as compared to wildtype donor heart grafts into Balb/c recipients (95 ± 5.8 vs 24 ± 2.6 days $p<0.001$). In vitro, MVEC cell death is reduced by the RIPK1/RIPK3 inhibiting small molecule nec-1 in the presence of zVAD-fmk following TNFα treatment (25.9 ± 2.73% vs 15.6 ± 2%, PI positive at 48 hours, n=3, $p<0.05$). As well, necrosis and release of the pro-inflammatory danger molecule HMGB1 are attenuated in vivo in RIPK3 null heart allografts and in vitro with VEC after RIPK1/RIPK3 inhibition. Finally, quantitative blinded scoring of graft infiltration was attenuated in RIP3 null hearts compared with wildtype allografts (0.8 ± 0.4 in RIP3 null vs 1.8 ± 0.4 in wildtype).

Conclusion: These data suggest that RIPK1/RIPK3 contributes to inflammatory injury in cardiac allografts through MVEC necroptotic death and the release of danger molecules. The ability of immunosuppression to provide rejection protection or permit tolerance is influenced by the level of cell death and inflammation. We suggest targeting RIPK1 and/or RIPK3 mediated necroptosis may be an important therapeutic strategy in solid organ transplantation.

Poster Abstract #3

A Competency Based Assessment Tool for Pathology Resident Grossing

Allison Osmond[1], William Stecho[1], David Garcia Marquez[1], Aaron Haig[1], Teresa Van Deven[1], and Michele Weir[1]

[1]Department of Pathology, Western University

Background: The Royal College of Physicians and Surgeons of Canada will be phasing in competency based learning objectives for resident training. The aims of the study are: 1) to examine the attitudes of pathology residents towards grossing specimens as a baseline and 2) to examine utility of a competency based assessment tool and log book for grossing specimens.
Design: A survey of residents' attitudes on grossing and competency based training was obtained for our program. A competency based checklist for grossing gynecological specimens & logbook with milestones were designed and tested on one senior resident and subsequently implemented on one junior resident during a 1 month gynecologic pathology rotation. Impact was assessed by examining comments from participants & supervisors; and variety of specimens grossed.

Results: The majority of our residents (86%) reports receiving minimal feedback on specimens grossed and not having clear grossing objectives on number & type of specimens. They (67%) report that grossing skills are not emphasized in our program. Advantages identified by residents for the assessment tool were: immediate feedback on grossing deficiencies with supervisors, greater emphasis on grossing objectives (skills, type of specimens) and increased awareness of grossing procedures. One realization was that the assessment process was less intense and more user friendly than anticipated by the residents. Supervisors identified opportunity to give feedback on and teach around grossing deficiencies as advantages. Supervisors' realizations were: checklists were not as time consuming or complicated as expected. A variety of specimens were grossed by the resident.

Conclusions: From the survey, our residents report some challenges regarding specimen grossing. The competency based assessment tool & logbook for grossing increased emphasis and feedback on resident grossing and allowed supervisors opportunity for timely feedback.

Keywords: Competency based medical education, Royal College of Physicians and Surgeons, Surgical pathology, resident grossing

Poster Abstract #4

Investigating the effects of modified human acidic Fibroblast Growth Factors treatment in Diabetic Nephropathy

Ana M Pena[1], Shali Chen[1], Biao Feng[1], and Subrata Chakrabarti[1]

[1]Department of Pathology, Western University

Introduction: Diabetic Nephropathy (DN) is thought to result from interactions between metabolic and hemodynamic factors activated by hyperglycemia within kidney tissues. One of the prominent causes for kidney derangement is oxidative stress. Acidic Fibroblast Growth Factor has been shown to confer protection from oxidative stress, function that is independent of its mitogenic activity. We have generated a modified human acidic FGF (MhaFGF), which is devoid of angiogenic activity with potent antioxidant activity. Since hyperglycemia-induced oxidative stress plays an important role in the pathogenesis of DN, we hypothesized that MhaFGF treatment has a protective effect in DN.

Methods: We aimed to investigate the effects of MhaFGF treatment on the biochemical, structural and functional changes in a mouse model of type I diabetes, induced by STZ. The respective groups were subjected to treatment (IP injections) with MhaFGF every day for 1 month or 6 months. The animals were clinically monitored. Blood, urine and kidney tissues were collected. Functional changes were determined through Urinary Albumin-Creatinine ratio levels. Experiments to test expression of vasoactive and fibrogenic factors, and oxidative stress were performed. In vitro studies will also be performed to understand the possible mechanistic pathways associated with this treatment.

Results: MaFGF treatment didn't show any effects in the body weight and blood sugar compared to the diabetic group at 1 month or 6 months. It prevented renal functional alterations in diabetes at both time points and prevented diabetes induced upregulation of vasoactive factors transcripts such as ANG and eNOS, and the molecular marker of oxidative stress HO1 compared to diabetic groups. On the other hand, it failed to prevent alterations of fibrogenic factors such as TGF β1 mRNA, and ECM protein e.g. FN and Col 1α (IV) mRNA expression. Confirmation experiments and the future direction of the investigation will be discussed.

Discussion: The maFGF is shown to be nonmitogenic because of its inability to translocate to the nucleus and act as a transcription factor. Nevertheless, it exerts the activation of the FGFR with all the downstream pathways associated with the tyrosine kinases which can act as a protective signal against oxidative stress. This research can shed light to one of multiple therapeutic strategies that could be used to treat DN: by influencing growth factors. Furthermore, this will help to understand the role of FGF in DN.

Keywords: basic FGF, acidic FGF, non-mitogenic acidic FGF, diabetic nephropathy, oxidative stress.

Poster Abstract #5

Influence of desferrioxamine and gallium conjugates on Staphylococcus aureus infection

Andrew Arifin[1], Mélissa Hannauer[2], Robert Lannigan[1], and David E. Heinrichs[2]

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Methicillin-resistant Staphylococcus aureus is the leading cause of skin and soft tissue infections worldwide. Gallium conjugates have been shown to inhibit the growth of S. aureus and other bacteria in vitro, yet in vivo studies are scarce. In contrast, desferrioxamine, a chelator used to treat iron overload, has been shown to promote S. aureus growth in vitro. However, there are no contraindications against the use of desferrioxamine in patients with S. aureus infections. This study investigates the effect of gallium conjugates against S. aureus infections, and the effect of administering desferrioxamine to an infected organism. The antibacterial activity of the gallium conjugates against S. aureus was assessed using growth and zone of inhibition assays. We found that these compounds were bacteriostatic, and had an additive effect when combined. We are now in the process of assessing the activity of these compounds in an in vivo model of systemic infection, in which we will determine the extent of infection by enumerating bacterial colony forming units in the heart, liver, and kidneys. We will also test the effects of desferrioxamine on S. aureus infections using the same model. We expect to find decreased bacterial burden when mice are administered gallium conjugates, and increased bacterial burden when mice are administered desferrioxamine. This will be the first study using gallium compounds against S. aureus in vivo, and may demonstrate the potential for gallium conjugates as anti-Staphylococcal agents. It may also indicate that S. aureus infections should be monitored or managed when administering desferrioxamine to a patient.

Poster Abstract #6

Role of H19 in diabetic complications

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Introduction: Non coding RNAs (ncRNAs) such as microRNAs(miRNAs) and long non-coding RNAs(lncRNAs) are emerging as key players in various diseases. In this project we plan to investigate a specific lncRNA, H19 along with its relationship to specific miRNA(mir-200). Recently H19 has been shown to alter several members of mir-200 family and enhance upstream histone acetylation in hepatocellular carcinoma. Our lab has previously shown a novel mir-200b mediated regulation of histone acetylator, p300 in diabetic retinopathy. Hence, modulating H19 may potentially lead to correction of abnormalities in several molecules altered during diabetic retinopathy, directly or through p300.

Methods: In this study, we plan to elucidate role of H19 and its interactions with members of miR-200 family and p300. These alterations will be studied using multiple models. In vitro experiments will utilize ECs, retinal cells, podocytes and cardiomyocytes exposed to hyperglycemia. In vivo models will include STZ mice models for chronic type1diabetes and db/db for type 2 diabetes.We will perform siRNA experiments to understand the mechanistic role of H19 and decipher its interaction with mir-200 and p300 in diabetic conditions.

Results: Preliminary results have shown increased expression of H19 in retina, heart and kidneys of diabetic mice compared to controls. We hope to see similar change in H19 expression levels in vitro and further understand its role in diabetic complications.

Conclusions: Elevated levels of H19 in tissue samples of diabetic mice indicate potential upregulation during diabetic complications. We hope to explicate its regulatory functions through potential interactions with mir-200 family and p300.

Keywords : H19, mir-200, p300, acetylation, diabetic complications

Poster Abstract #7

The potential role of RIPK3 in PARP-1-mediated memory T cell necrosis

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Alloreactive memory T (TM) cells have emerged as a major barrier to transplant tolerance. These cells are resistant to apoptosis and are not susceptible to traditional immunosuppressive therapies. Necroptosis, regulated necrosis that is dependent on receptor-interacting protein kinase 1 and 3 (RIPK1, RIPK3), has been newly identified as a form of programmed cell death. Interestingly, necroptosis becomes the predominant form of cell death when caspase 8-mediated apoptotic pathways are blocked. Furthermore, dysregulation of RIPK1 and RIPK3, in response to caspase 8 deletion, has been shown to result in T cell defect and loss. Pathways of necroptosis have yet to be studied in TM cells. In addition, genotoxic stress-induced poly-(ADP-ribose)-polymerase-1 (PARP-1) hyper-activation and subsequent cell necrosis has also been linked to RIPK1 and RIPK3 mediated necroptosis. However, debate still exists on whether or not these two pathways are in fact related. This present study aims to identify the role of RIPK3 in PARP-1 mediated TM cell necrosis.

Effector T (TE) cells and antigen specific TM cells, developed in vitro and in vivo respectively, from wild-type and RIPK3 null mice were treated with the DNA alkylating agent 1-methyl-3-nitro-1-nitroso-guanidine (MNNG), a potent activator of PARP-1. The effects of PARP-1 hyper-activation on the survival of TE and TM cells were determined by flow cytometry.

We found that PARP-1 hyper-activation results in mass TE and TM cell necrosis. Our preliminary results indicate that both TE and TM cell necrosis is caspase and RIPK3 independent. This finding is contrary to our hypothesis that RIPK3 deletion would attenuate necrosis in response to MNNG treatment. It appears that RIPK3 as well as caspases do not play an important role in MNNG-induced TM cell death. However, further investigation of necroptosis in TM cells is warranted as understanding this pathway may be important for the design of therapeutic strategies to control TM cells that mediate transplant rejection.

Keywords: memory T cells, cell death, necroptosis, PARP-1, RIPK3

Poster Abstract #8

SPI-6 (Serpin Protease Inhibitor-6) inhibits granzyme B mediated injury of renal tubular cells and promotes renal allograft survival

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Introduction: Proteinase inhibitor 9 (PI-9) is an intracellular serpin that inhibits Granzyme B (GrB), a serine protease found in the cytosolic granules of CD8+ T and Natural Killer (NK) cells. PI-9 functions to prevent “misdirected” apoptosis in GrB expressing cells. The murine homolog of PI-9 is serpin protease inhibitor 6 (SPI-6). Kidney tubular epithelial cells (TEC) are a principal targets for cytotoxic cells following transplant. Therefore TEC resistance to cell mediated injury may influence the graft survival and function. The expression and regulation of SPI-6 in TEC and kidney has not been studied.

Methods/Results: We demonstrate TEC express SPI-6 protein, the murine homolog of PI-9, basally with a modest increase following cytokine exposure. TEC expression of SPI-6 blocks granzyme B mediated death as TEC from SPI-6 null kidneys have increased susceptibility to cytotoxic CD8+ cells in vitro. We then tested the role of SPI-6 in a mouse kidney transplant model using SPI-6 null or wild type donor kidneys (H-2b) into nephrectomized recipients (H-2d). SPI-6 null kidney recipients had reduced renal function at day 8 post-transplant compared to controls (creatinine: 113+23 vs. 28+3 μmol/L, n=5, $P<0.01$) consistent with greater tubular injury and extensive mononuclear cell infiltration. Finally, loss of donor kidney SPI-6 shortened graft survival time (20+19 vs. 66+33 days, n=8-10, $P<0.001$).

Conclusion: Our data shows for the first time that resistance of kidney TEC to cytotoxic T cell, granzyme B induced death is mediated by the expression of SPI-6. We suggest SPI-6 is an important endogenous mechanism to prevent rejection injury from perforin/granzyme B effectors and enhanced PI-9/SPI-6 expression by TEC may provide protection from diverse forms of inflammatory kidney injury and promote long term allograft survival.

Poster Abstract #9

Ontario Growth Standards for Infants: A Retrospective Autopsy Study

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Background: There were on average 136,099 live births per year in Ontario between 1993 and 2008. The mortality rate for infants under one year of age for the same time period was 5.49 per 1000 live births. This predicts on average 747 infant deaths per year in Ontario. Of these, a significant proportion will require a post mortem examination to determine the cause and manner of death. To complete this task, the pathologist will compare the findings against the standardized population parameters. Existing growth assessment charts aid clinicians in monitoring infant growth parameters against normal value ranges. As these are prepared from cross-sections of normal live populations, their applicability for assessment of infantile deaths is questionable. The autopsied population should be considered abnormal to some degree. An additional comparison resource used by the pathologist is organ and body measurement charts. These charts have been created based on the autopsied populations and provide a more accurate comparison group.

Objectives: To create Ontario population-specific organ and body measurement mean charts for infants under one year of age; growth graphs for each age category; and organ weight prediction models based on body weight, sex and age categories.

Methods: To construct the charts, the data will be obtained by reviewing the postmortem examination reports and coroner's statements (where applicable), pertaining to infant deaths under one year of age, that occurred in Ontario during the time period 1993-2010. The data will be classified into region specific groups, age categories, sex and cause of death dependent subgroups, and will be further analysed using standard statistical methods.

Poster Abstract #10

Negative Colorectal Polyp Biopsies: The Utility of Cutting Deeper Levels

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Introduction: Microscopic examination of colorectal biopsies from lesions identified endoscopically as polyps sometimes fails to identify an abnormality on initial routine sections, but a polyp may be found if deeper levels are cut from the tissue block. The objective of this study was to determine the frequency with which deeper levels reveal a lesion, where none was found initially, and to identify clinical, endoscopic, and/or pathologic features that predict occult lesions.

Methods: All "polyp" biopsy specimens where no polyp was identified on the initial, standard sections were consecutively accumulated over an 18-month period, from the practice of two pathologists. Standard sections included preparation, from one block, of three slides, each with two serial sections, with each pair of serial sections cut at deeper levels, 50μM apart. In each case where no polyp was identified initially, three to ten additional levels were cut, 50μM apart. The presence of any lesion, the level at which it was found, the location, number and size of fragments, number of levels obtained, presence of any lymphoid aggregate, endoscopic size and appearance, and bowel preparation quality were recorded.

Results: There were 214 specimens accrued from 203 patients (104 females, 99 males). The mean age was 61.4 years (range 27-86 years). Deeper levels revealed a lesion in 52/214 (24.3%) cases; of these, 40 (76.9%) were tubular adenomas (TA), 11 (21.2%) were hyperplastic polyps (HP), and 1 was a leiomyoma. All TAs were negative for high-grade dysplasia and malignancy. The mean level at which the TAs were found was 4.85 (range 4-12). Male sex ($p = 0.021$) and right-sided location ($p = 0.0075$) were statistically significant predictors of an occult TA. The presence of a prominent lymphoid aggregate was predictive of a negative biopsy ($p = 0.097$). Endoscopic size, endoscopic appearance, and bowel preparation quality were not predictors of deeper lesions, but were unknown in 48.1%, 82.7%, and 52.8% of all cases, respectively.

Conclusions: Despite cutting three levels from the block initially, in approximately one-quarter of negative cases, deeper levels revealed a lesion, the majority of which were adenomas. As the presence of an adenoma affects subsequent colonoscopic screening of average-risk individuals, pathologists should consider "pursuing" polyps when initial sections reveal no lesion. Individual pathology laboratories should consider ascertaining their own incidence of occult lesions on initially non-diagnostic colorectal biopsies, as histotechnologist practices may vary.

Keywords: colon cancer, colorectal polyp, biopsy, quality assurance

Poster Abstract #11

Characterizing the impact of membrane vesicles produced by apoptotic and necrotic tubular epithelial cells on renal transplant rejection and graft-versus-host disease

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Introduction: Tubular epithelial cells (TECs) are highly susceptible to death from ischemia-reperfusion injury (IRI) that is associated with kidney dysfunction and graft rejection. Previous studies have shown that necroptosis, a receptor-interacting protein kinase (RIPK)3-mediated form of programmed cell death that occurs in the absence of caspase 8, can promote inflammation and impact IRI and transplant survival. Necroptosis lead to membrane permeabilization and result in release of death-associated molecular pattern molecules (cDAMPs). Membrane vesicles (MV) can be released following necroptosis and can contain molecules that modulate immune responses. We hypothesize that necroptosis will result in efficient release of MV containing molecules from the cell cytosol which will enhance inflammatory responses following IRI.

Methods: Necroptosis in TECs was induced by TNFα with addition of cycloheximide and zVAD-fmk. Cell death was confirmed by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. Ultracentrifugation and flow cytometry methods were used to isolate MVs for extraction, quantification and RNA and DNA analysis. In vivo experiments with wild type C57BL/6 and RIP3-/- mice will measure the effects of isolated MVs on TECs and immune cells.

Results: TNFα-induced necroptosis in TECs was confirmed by MTT assay (0.366±0.177 fold change vs. untreated; $p<0.001$). Immunoblot for high-mobility group box 1 (HMGB1) confirmed necroptotic death. MV release was isolated and measured using ultracentrifugation and flow cytometry. Total protein release was enhanced following necroptosis (1.48±0.09ug/uL) compared with apoptosis (1.06±0.02ug/uL, $p<0.05$) using Bradford protein assay.

Conclusions: We have found that TEC undergo necroptosis after TNFα treatment in presence of pan-caspase inhibition, which resulted in more cDAMP and cell content release than apoptosis.

Keywords: necroptosis, kidney injury, graft-versus-host disease, kidney transplantation, membrane vesicles, danger-associated molecular pattern molecules

Poster Abstract #12

Follicular Mucinosis with a Clonal T-cell Pattern – A Case Report

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Introduction: Follicular Mucinosis (FM) is an uncommon cutaneous inflammatory condition that presents clinically as follicular papules, patches, or plaques. The face, neck and scalp are the most frequently affected sites. Histologically it is characterized by mucin deposition within the folliculosebaceous units. It can be primary (idiopathic) or secondary, with the latter frequently associated with mycosis fungoides or Sezary syndrome ("lymphoma associated" FM).
Case report: We present a 48 year-old otherwise healthy male who presented with a solitary plaque on the back of his neck. The skin excision showed histological features of FM including follicular degeneration and abundant mucin deposition within pilosebaceous units, and a lymphocytic infiltrate centred around these follicles. Immunohistochemical studies confirmed a T-cell proliferation positive for immunomarkers CD4, CD5 and CD3, with partial loss of CD7. The T-cell receptor Beta (TCRβ) and T-cell receptor Gamma (TCRγ) gene rearrangement assays showed a reproducible clonal pattern in a single reaction (TCRβ). Although this result was considered inconclusive, the possibility of a T cell neoplasm was suggested with follow up recommended.

Discussion: There is considerable overlap between primary and secondary FM in terms of clinical presentation, histology and molecular findings, although solitary lesions at presentation are more common in primary FM. Progression of idiopathic FM to cutaneous T cell lymphoma has been well documented in the literature. In addition, monoclonal rearrangement of T cell receptors has been demonstrated in approximately 50% of tested cases in each group in previous studies. It is unclear whether solitary FM with a clonal T cell pattern represents a form of localized cutaneous T cell lymphoma. Long term clinical follow up is necessary in patients where the differential diagnosis is difficult. To the best of our knowledge our patient has no clinical evidence of mycoses fungoides or any other lymphoproliferative disorder after 6 months follow up.

Keywords: Follicular Mucinosis, Mycoses Fungoides

Poster Abstract #13

Expression of the Growth Hormone Secretagogue Receptor in Human Cardiomyopathies

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Introduction: Ghrelin is a peptide hormone that binds to the growth hormone secretagogue receptor 1a (GHS-R1a). Both ghrelin and GHS-R1a are expressed in cardiomyocytes, and ghrelin signalling through GHS-R1a results in enhanced survival and decreased apoptosis. We are determining the pattern of GHS-R1a expression in human cardiomyopathies using our previously developed Cy5-ghrelin analog for fluorescence imaging of GHS-R1a.

Hypothesis: We hypothesize that GHS-R1a expression will be elevated in diseased tissues versus newly implanted hearts.

Methods: Tissue samples were collected from left ventricles, right atria, aortae and pectorialis major muscles from 5 cardiomyopathy patients during routine endomyocardial biopsy. Samples were also collected from explanted hearts and newly implanted hearts at 1 and 2 weeks post-transplant from 2 patients. Samples were fixed in 4% paraformaldehyde, perfused in 30% sucrose, snap-frozen and cryosectioned at 10µm. Sections were then thawed and incubated with 10 µM Cy5-ghrelin for 30 min. Images were acquired using a fluorescence microscope with consistent exposure times. Five fields of view were taken per slide, with three slides per cardiac region per patient. ImageJ was used to determine mean fluorescence intensity. Data were analysed using one-way ANOVA and Student's t test.

Results: In tissue samples from cardiomyopathy patients, fluorescence intensity in the left ventricle (529 ± 95, n=5) was significantly greater ($p < 0.05$) than in the right atria (307 ± 21), aorta (159 ± 10) and pectorialis major (179 ± 3). In the transplant patients, there was a 2-fold increase ($p < 0.05$) in fluorescence intensity in the explanted hearts compared to the newly transplanted hearts at weeks 1 and 2 post-transplant.

Conclusion: In human cardiomyopathy patients, GHS-R1a expression is hightest in the left ventricle. GHS-R1a expression is elevated in the explanted hearts of transplant patients, indicating that it may be a biomarker for end-stage heart disease.

Poster Abstract #14

Expression of Human tissue Kallikriens (KLKs) in Polymorphous Low Grade adenocarcinoma (PLGA)

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Introduction: Polymorphous low-grade adenocarcinoma (PLGA) is the second most common malignant salivary gland tumor of the minor salivary glands. Recognized as its own identity in 1983, PLGA can pose diagnostic difficulties to the pathologist secondary to its polymorphic pattern. It is characterized by asymptomatic, indolent growth and low propensity for metastatic disease; however, delayed recurrence is not uncommon, warranting careful clinical follow up.

Human tissue kallikriens (KLKs) are a family of highly conserved serine proteases encoded by 15 genes. They are expressed by various tissue and organ systems throughout the body and are involved in the regulation of both physiologic and pathologic processes. Specifically, KLKs have been linked to different stages of cancer development and progression, and as a result, have become powerful tumor markers for the diagnosis, prognosis and management of the cancer patient. The literature demonstrates a link between KLKs and salivary gland neoplasms.

Objective: The aim of this project is to determine which KLKs are expressed in polymorphous low grade adenocarcinoma (PLGA). It is our hypothesis that the expression pattern of KLKs for PLGA will be markedly different compared to benign salivary gland tumors, such as pleomorphic adenomas, which have been previously studied. The consequences of altered expression will be explored with hopes of predicting tumor behaviour and its implications on diagnosis and treatment.

Methods: mRNA was isolated from formalin-fixed, paraffin-embedded tissue samples of PLGA. Following conversion to complementary DNA via reverse transcription, synthesized DNA primers were added to target kallikrein DNA. Through polymerase chain reaction the quantitative level of expression for the 15 KLKs was recorded.

Results & Conclusion: Pending data analysis.

Poster Abstract #15

Trauma in adult pedestrians due to frontal motor vehicle collisions

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Introduction: Various injury patterns have been described for upright adult pedestrians struck by the front end of a motor vehicle. “Bumper” fractures of the lower extremity are commonly observed in upright adult pedestrian collisions. In this study, we aimed to determine the type and distribution of lower extremity injuries, and other injuries unique to motor vehicle frontal impacts. We hypothesize that lower extremity injuries are invariable when an upright pedestrian is struck by the front of a vehicle and that certain trauma due to secondary impacts with the vehicle is unique to the speed-dependent pedestrian trajectories.

Methods: To test this hypothesis, we collected and analyzed human, environmental, and postmortem data related to adult pedestrian fatality cases contained within the coroner’s reports of postmortem examinations from the Office of the Chief Coroner for Ontario in order to identify injury patterns and possible hallmark indicators of upright pedestrian collisions.

Results: Our results show that bumper fractures are variable and sometimes absent in upright adult pedestrians. Brain injuries, pelvic fractures, and sacroiliac joint dislocations were observed to occur more frequently and with increased severity when bumper fractures were not observed. Bumper fractures were found more likely to occur when the impacting vehicle was a car than a truck-type-vehicle. We also found that injuries of the lower extremity, including bumper fractures, were more common in victims pronounced dead at the collision than victims who later died in medical care facilities.

Conclusions: These findings show that the incidence and severity of brain, pelvis, and sacroiliac joint injuries that are secondary to the initial bumper impact can serve as an indicator of upright pedestrian orientation in frontal motor vehicle collisions when bumper fractures are not observed.

Keywords: “Bumper” fracture, pedestrian collision, lower extremity injury, frontal impact, postmortem examination, secondary impact

Poster Abstract #16

The Effect of a Conditional β-cell Specific β1-Integrin Knockout on β-cell Survival, Proliferation and Function

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Introduction: Integrins are cell adhesion receptors involved in cytoskeletal-to-extracellular matrix (ECM) attachments, as well as cell-cell interactions. Research has shown that β1 integrin is essential for pancreatic β-cell development and maintenance throughout life in rats, human fetal islets, and mice, and that it functions through the FAK/ERK signaling cascade. Our hypotheses are that the β 1 integrin receptor within β-cells is required for islet formation and remodeling during pancreatic development in the prenatal and postnatal life, and that the interaction of β 1 integrin with the extracellular matrix is essential for maintaining islet vasculature, β-cell function, and survival.

Methods: We have successfully generated C57BL/6 mice with CreER recombinase specific to the mouse insulin promoter (MIP) that were crossed with mice expressing β1 integrin floxed by two LoxP sites for our experiments (MIP-CreER+/+:Itgβ1fl/fl). This allows us to generate an inducible β-cell specific β1KO upon injection of tamoxifen. The β1 integrin knockout in β-cells will be determined by qRT-PCR, western blot and immunofluorescence analysis. For in vivo studies we will be conducting intraperitoneal glucose tolerance tests, intraperitoneal insulin tolerance tests and assessing glucose stimulated insulin release using an enzyme-linked immunosorbent assay. Additional techniques will be used to examine changes in β-cell size, survival, differentiation, transcription factor expression, function, and islet vasculature.

Results: N/A

Conclusion: Further evidence showing β1 integrin is essential for islet cell growth, vasculature, maturation, survival and function could aid in further therapeutic techniques involving Islet/β-cell growth and maintenance in vitro and subsequently in islet or β-cell transplants in diabetics.

Keywords: integrin β1, pancreas, β-cell, diabetes, mouse insulin promoter

Poster Abstract #17

Canada's First Accredited Pathologists' Assistant Graduate Program: A Self Study and Review

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Background: Our program at Western University is the first Pathologists' Assistant (PA) graduate program in Canada to earn accreditation by the National Accrediting Agency for Clinical Laboratory Sciences (NAACLS). This is an emerging profession with bountiful employment opportunities where graduates are an important part of the health care team.

Aim: To evaluate the effectiveness of our program in graduating highly qualified PAs.

Methods: We solicited feedback from former graduates through a confidential, voluntary, electronic survey that asked graduates about their current employment, experience during their training and suggestions for improvement.

Results: The survey response rate is 15 out of 19 total graduates (79%), with representation from all 5 years of graduating students. The survey indicates that after graduation, 3 respondents (16%) entered medical school; all of the remaining respondents (84%) found employment as a PA. Most graduates found employment before the end of the program. Graduates are employed throughout Canada where their duties have included examination of surgical specimens (100%), assisting in postmortem examinations (58%), administrative duties (58%), and research (25%). Only 1 respondent (5%) has completed the American Society for Clinical Pathology (ASCP) certification examination. Some respondents specified suggestions for improvement during their clinical rotations. All of the respondents noted that our program met their expectations.

Discussion: The study shows that our program has been effective in training PAs and that employment opportunities are plentiful. We gained invaluable insight on how to improve our program. For example, graduates should be further encouraged to write the ASCP certification examination. Also, hospital staff who teach our students need further support in their mentorship role. This study has been an important tool in reviewing the effectiveness of our new program and for its continued improvement.

Keywords: Pathologist Assistant, Accredited, NAACLS, Canada, Graduate Program, Self-study

Poster Abstract #18

The Impact of Indoleamine-2,3-dioxygenase (IDO) Isoforms in Melanoma Cells

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Introduction: Many cancer types highly express the tryptophan-metabolizing enzyme indoleamine-2,3-dioxygenase (IDO), leading to cancer T cell suppression and immune tolerance. Though IDO1 and its effects have been known for many years, a second isoform of IDO (IDO2) was recently discovered. This study is aimed at studying the effects of the two isoforms of IDO (IDO1 and IDO2) on cancer cell growth in vitro and determining whether the two isoforms present any differences in their action.

Methods: To test the impact of the two IDO isoforms in melanoma cells, BL6 melanoma cells will be induced to express high levels of IDO (either IDO1 and/or IDO2) through cDNA transfection or knocked down to suppress IDO expression using siRNA transfection. Both IDO1 and IDO2 isoforms are expressed in the BL6 strain of mice melanoma cells at basal levels. The BL6 cells will then be studied on the basis of their proliferation, cell cycles, and migration to determine the effects of high vs. low IDO1 and IDO2 expression on cancer cell phenotypes. This study will show whether IDO-mediated tryptophan depletion through both IDO isoforms will inhibit 4T1 cancer cell growth in vitro.

Results: Preliminary results show high IDO1 expression has inhibited the growth, migration of BL6 cells. High IDO2 expression exhibits a much lesser inhibition in growth and migration.

Discussion: These results suggest that IDO1 has greater level of activity than IDO2. Whether this difference is solely due to the enzymes affinity for tryptophan or some secondary actions needs to be further explored in future studies.

Keywords: melanoma, tryptophan, cDNA, siRNA, immune tolerance, T cells, indoleamine-2,3-dioxygenase, IDO2

Poster Abstract #19

β-adrenergic receptor-independent action of propranolol in infantile hemangioma

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Introduction: Infantile hemangioma is the most common vascular tumour of infancy. Propranolol, a non-selective β-adrenergic receptor antagonist, has quickly become the first-line treatment; however, its mechanism of action still remains ambiguous. Although propranolol shows promising results for patients with hemangiomas, recent reports suggest that significant number of cases where hemangiomas regrow following cessation of treatment. Recent studies show that propranolol causes caspase-3 mediated apoptosis in vascular endothelial cells. However, our laboratory has shown that hemangiomas arise from multipotential stem cells (hemangioma stem cells); therefore, hemangioma stem cells are the cells of interest in our study. The purpose of our study is to characterize cellular and molecular mechanisms of propranolol action in these hemangioma initiating stem cells. We hypothesize that hemangioma stem cells do not undergo apoptosis upon propranolol treatment and are responsible for hemangioma regrowth.

Methods: Primary stem cells from human hemangioma specimens were isolated and cultured in the presence of propranolol. Vascular endothelial cells were used as controls to determine selectivity of propranolol action.

Results: Our results revealed that propranolol causes caspase-3 mediated apoptosis in normal vascular endothelial cells, whereas hemangioma cells exhibited growth arrest along with induction of anti-apoptotic genes. The contrasting responses in normal vascular cells and hemangioma cells could be due to differential expression of β-adrenergic receptor subtypes. We show that normal vascular endothelial cells predominantly express β1-receptor subtype, whereas hemangioma cells express β2 and β3. Our results further indicate that propranolol does not alter the activity level of β-adrenergic receptors in hemangioma cells.

Conclusions: Our findings suggest that propranolol mediates its effects in hemangioma through a β-adrenergic receptor-independent mechanism. We are currently investigating these potential indirect mechanisms of propranolol action in hemangioma. The findings may lead to better and fast acting therapies for children with devastating hemangiomas.

Keywords: Infantile hemangioma, propranolol, β-adrenergic receptor, stem cells, recurrence, apoptosis

Poster Abstract #20

Pediatric Pathology Cases: Retrospective Review at London Health Sciences Centre for Training Purposes

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Pediatric pathology is a specialized area in pathology that often does not receive sufficient focus in training of residents and pathologists' assistant (PA) graduate students. This is due to the relative rarity of many of the specimens.

It is critical, however, that trainees in surgical pathology receive adequate training and exposure to achieve a level of competence in this area. For example, the Royal College of Physicians and Surgeons of Canada requires two blocks in pediatric pathology or equivalent longitudinal experience during residency.

Our goals are to ensure adequate exposure during training, and the capture of less common cases for future trainees.

At our institution, rather than have a dedicated pediatric pathologist, the majority of the pediatric material is distributed according to subspecialty. The challenge has been to ensure adequate exposure, as well as appropriate documentation, for anatomical pathology residents.

As part of a quality assurance initiative, we examined the number, distribution and type of pediatric surgical cases in 2013. London Health Sciences Centre received 1873 surgical cases from the pediatric population in 2013. The distribution was as follows: breast (1.4%), eye (1.6%), GI (59%), GU (5%), gynecology (2%), head and neck (9%), hematology (4%), lung (1%), muscle/bone (4%), skin (10%) and other (3%).

There are no specific quotas designated for residents by the Royal College or for PA students by the National Accrediting Agency for Clinical Laboratory Sciences (NAACLS).

We found that our institution has a significant number of pediatric surgical cases covering a wide spectrum of disease, allowing for adequate exposure for our trainees. In addition, our trainees are required to complete a log of their pediatric case experiences in order to ensure adequate longitudinal experience. Select cases were chosen to make a teaching set in order to assist in future training.

Keywords: pediatric pathology, residency training, residency education, pathologists' assistant (PA) graduate students

Poster Abstract #21

Interaction of Primary Human Trabecular Meshwork Cells with Metal Alloy Candidates for Microinvasive Glaucoma Surgery

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Introduction: As a novel therapy for glaucoma, small metallic stents are inserted into the trabecular meshwork to increase the drainage of aqueous humour. It has been predicted that this microsurgical stent approach may become the first line therapy in glaucoma management due to its efficacy, safety and lack of compliance issues associated with medications. However, as devices can be approved without the rigor of Phase I-III trials seen with medications, there have been no published studies on the effect of the metal alloys used in the stents on human trabecular meshwork cells (HTMCs). Our study will determine the morphological and functional response of HTMCs to metal alloys used in these stents and control materials.

Methods: HTMCs were cultured on the surface of titanium and titanium-nickel (50%) alloy, with glass and tissue culture plastic as control substrata. Titanium samples with different surface textures (sand blasted vs. machine polished) were also compared. Fluorescent imaging studies were conducted to measure cell attachment and spreading. Finally, a BrdU proliferation assay was conducted.

Results: Our results show that there is no significant difference in the number of cells adhered to each surface or the amount of cell spreading between all metals tests after 24 hours. However, the cells cultured on the sand blasted titanium surface had more prominent stress fibers than the cells cultured on the machine polished titanium. Also, there was significantly more cellular proliferation on the titanium metal ($p<0.04$) compared to the titanium-nickel alloy after 48 hours of incubation.

Conclusion: These findings suggest that the elemental composition and texture of a metal surface impact the functional and morphological properties of HTMCs. The results of this study identify cellular effects that may influence short- and long-term function of microinvasive glaucoma shunts.

Keywords: trabecular meshwork, glaucoma, biomaterials, stent, titanium alloy

Poster Abstract #22

The role of Rho Guanine Nucleotide Exchange Factor, an RNA-binding protein discovered in amyotrophic lateral sclerosis, in stress response

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Background: Amyotrophic Lateral Sclerosis (ALS) is a degenerative disorder of motor neurons which causes death within 5 years of onset. A common pathology observed in ALS is aberrant aggregation of protein, although the exact cause is unknown. Recent studies implicate aberrant RNA metabolism important in the disease, while other studies show a role of oxidative stress in neurodegeneration. Stress granules (SG), a specific type of RNA granule, are of interest in ALS due to increased oxidative stress within afflicted neurons. Currently, our lab is examining a novel, ALS-related RNA-binding protein: Rho Guanine Nucleotide Exchange Factor (RGNEF). RGNEF co-localizes with neuronal cytoplasmic inclusions, and we identified a novel mutation in ARHGEF28 (encodes RGNEF) in a family with familial ALS. We have also observed upregulation of RGNEF in neuronal injury in mice, so we hypothesize RGNEF is involved in stress response.

Methods: To evaluate RGNEF's protective effects, we subjected HEK293T cells overexpressing myc-tagged RGNEF to heat shock, oxidative (arsenite), or osmotic (sorbitol) stress. Cell death was measured using MTT assay. We also examined RGNEF's protective domain using several truncated constructs transfected in stressed HEK293T cells. The localization of RGNEF was observed by confocal microscopy.

Results: For arsenite and sorbitol treatments, myc-RGNEF overexpression increased survival compared to controls (arsenite: 65% vs 49% survival; sorbitol: 64% vs 51% survival; $p<0.05$). We observed the protective domain of RGNEF lies within the N-terminal region and may involve the leucine-rich domain. No difference in response to heat shock was observed. Under arsenite or sorbitol, RGNEF did not co-localize with TIA-1 (marker for SG), but occasionally co-localized with Staufen (marked transport granules).

Discussion: Our findings demonstrate that RGNEF has a role in stress response, that the protective effect of RGNEF may be mediated through a leucine-rich region, and that this effect does not require the participation of RGNEF in stress granules.

Poster Abstract #23

Folate receptor targeted siRNA delivery with a novel non-covalent functionalization of single-walled carbon nanotubes for cancer therapy

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Introduction: RNAi can specifically regulate the gene expression, but efficient and targeted delivery of siRNA in vivo to pathological cells or tissue is difficult. It has been shown that modified CNT protect siRNA and facilitate its entry into cells. Folate receptor (FR) is overexpressed in cancer and it has a high binding constant with folic acid.

Method: SWCNT were functionalized by non-covalent association with a folic acid conjugated PEI. PEI was modified with polyethylene glycol (PEG) and succinic acid. This product was used to disperse CNT and water soluble CNT were isolated for siRNA delivery. In vivo siRNA delivery was done by intravenous injection to melanoma bearing mice and mTOR siRN
A (si-mTOR) was used to test in vivo gene silencing and anti-cancer therapy since mTOR is usually overexpressed in cancer.

Results: The polymers as well as the CNT with folic acid (FGIS/C) and without folic acid (GIS/C) were characterized. The structural, biophysical, and biological properties of FGIS/C and GIS/C and their complexes formed with siRNA were investigated. We found significant uptake of siRNA as well as gene silencing in tumor by FGIS/C. Treatment with FGIS/C/si-mTOR resulted in attenuation of tumor growth in a murine melanoma model.

Conclusions: In conclusion, a novel functionalized targeted CNT was developed for cancer siRNA delivery, which siRNA was delivered in vivo to a murine melanoma model. The new delivery method has provided a possibility for cancer treatment, which could provide insight into the potential application and development of CNT-based antisense-based therapy.

Keywords: RNAi, siRNA delivery, folate receptor, SWCNT, nanomaterials, melanoma, mTOR

Poster Abstract #24

Determining Global Cytogenomic Changes in Classical Hodgkin Lymphoma

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Introduction: Classical Hodgkin lymphoma (cHL) is the most prevalent lymphoma in the western world. While this disease is generally curable, approximately 20% of patients have refractory or recurrent disease, suggesting a need for more targeted therapies. However, the pathogenesis of cHL remains largely unknown. The scarcity of neoplastic cHL cells, called Hodgkin Reed-Sternberg (HRS) cells, in a background of abundant inflammatory cells has made it difficult to study the mechanisms of Hodgkin lymphoma oncogenesis. To overcome this hurdle, we are examining the pathogenetic mechanism of HRS cell derivation by using laser-capture micro-dissection technology from formalin-fixed paraffin-embedded (FFPE) samples. This will allow us to obtain an enriched population of HRS cells for genomic studies.

Methods: Five cases have been selected based on FFPE storage of less than 5 years and the presence of greater than 2% HRS cells. We have previously attempted copy number analysis on two cases of cHL using the Affymetrix Cytoscan HD array; however, analysis was hampered due to difficulties obtaining sufficient quantity of dsDNA. In this study, following micro-dissection and DNA isolation, we employ whole genome amplification. High-resolution cytogenomic analysis using the Affymetrix Cytoscan HD array will provide both copy number and genotype (i.e. loss of heterozygosity) changes in HRS cells in comparison to normal lymphocytes from the same specimens. Results from the array will be analyzed using Affymetrix's Chromosome Analysis Suite.

Results: Micro-dissection, DNA isolation, and whole genome amplification steps are complete. Preliminary results of 2 cases will be presented.

Discussion: FFPE samples are an underutilized and important resource for genomic studies, however are challenging to work with due to poor quality of nucleic acids. Enrichment of HRS cells is essential in order to study genomic changes in Hodgkin lymphoma. Our findings will contribute to the current body of knowledge regarding the molecular genetics of cHL and may have future implications for safer and better-targeted therapies.

Keywords: Hodgkin Lymphoma, Reed-Sternberg cells, micro-dissection, microarray, DNA, cytogenomics

Poster Abstract #25

Extracellular Matrix is Selectively Regulated Following Diabetes-induced Adipogenesis of Bone Marrow Mesenchymal Progenitor Cells

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Introduction: The components of the extracellular matrix (ECM) play a profound role on the direction of mesenchymal progenitor cell (MPC) fate. In diabetes, an increased development of bone marrow (BM) MPCs along the adipogenic lineage leads to a differential cellular composition within the BM stroma. We aimed to delineate ECM protein changes following adipogenic conversion of MPCs in an attempt to identify therapeutic targets to reverse increased BM adiposity in diabetes.

Methods: MPCs were derived from fresh human BM samples and expanded in culture. We subjected MPCs to adipogenic differentiation in culture for seven days. We then conducted real-time polymerase chain reaction-based assays to determine the relative mRNA levels of ECM component genes.

Results: Our results show that adipogenesis in MPCs is associated with selective modulation of ECM proteins. The expression of fibronectin and collagen subtypes I, III, and VI were significantly reduced. Matrix metalloproteinases (MMPs) -1, -2, -9, -10, -11, -14, and -16 displayed substantial down-regulation and MMP-7 and -13 levels were increased. We then tested the effect of collagen and fibronectin on adipogenesis by plating cells on ECM-coated plates. Collagen I significantly inhibited adipogenic differentiation as determined by level of adipogenesis-specific transcription factors. Fibronectin, on the other hand, only delayed the differentiation and showed higher levels of early transcription factors, though late adipogenic marker, C/EBPα, was down-regulated.

Discussion: Our findings show that MPC adipogenesis selectively regulates ECM proteins and remodelling factors. The observed changes in microenvironment correspond with modifications in factors known to be involved in directing MPC function, migration, and differentiation potential. These results represent the first step in identifying obligatory ECM proteins to reverse BM adipogenesis in diabetes.

Keywords: mesenchymal progenitor cells, adipogenesis, diabetes, bone marrow, extracellular matrix

Poster Abstract #26

Investigation of βcell insulin receptor regulation of βcell growth, function and survival

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Introduction: Diabetes mellitus is characterized by a decline in pancreatic β-cell mass and the development of insulin resistance in muscle, fat, liver and β-cells, resulting from multiple molecular defects including a decrease in insulin receptor concentration. It is well established that insulin-producing β-cells of the endocrine pancreas express functional insulin receptor (IR). Studies have shown that β-cell specific IR knockout (βIRKO) adult mice were shown to develop age-dependent glucose intolerance, leading to eventual loss of β-cell mass, suggesting that insulin signaling via IR in β-cells is essential to maintain β-cell function and survival. However, the molecular mechanisms involved have yet to be studied. We propose to elaborate upon these previous studies by investigating the temporal role of the β-cell IR autocrine/paracrine signaling in pre- and postnatal islet development and function.

Methods: We utilized tamoxifen-inducible Cre recombinase under control of the mouse insulin promoter to drive temporal β-cell specific IR knockout in our mouse model. We will be conducting intraperiotoneal (IP) glucose tolerance tests, IP insulin tolerance tests, and glucose stimulated insulin release in vivo. Pancreases will then be dissected for morphological analysis including islet size, proliferation, apoptosis, and expression of downstream IR signaling pathways.

Results: N/A

Discussion: This study utilizes a novel inducible βIRKO mouse model to unravel the critical role of autocrine/paracrine insulin signaling on β-cell development, function, proliferation, and survival. Furthermore, we will investigate the pathogenic link between β-cells IR and diabetic progression.

Keywords: β-cells, insulin receptor knockout, insulin signaling, pancreatic development

Poster Abstract #27

Immunohistochemical Characterization of mTOR Pathway Activation in Gastroenteropancreatic Neuroendocrine Tumours

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Gastroenteropancreatic neuroendocrine tumours (GEP-NETs) are an increasingly prevalent and highly heterogeneous group of neoplasms with suboptimal patient outcomes. NETs of the small bowel (SI-NETs) are a subtype that displays worse outcomes than other GEP-NETs. This stems from the lack of useful prognostic and predictive markers, reflecting our lack of understanding of their molecular pathogenesis. A number of mutations have been associated with these tumours, including in the mTOR pathway. This pathway has ample evidence suggesting a role in GEP-NET tumourigenesis, and moreover appears to correlate with suboptimal outcomes.

This study aimed to better elucidate mTOR pathway activation in SI-NETs, in order to better characterize the pathogenesis of this poorly studied subtype of GEP-NETs, as well as provide evidence for the use of the mTOR pathway as a predictive biomarker. We used immunohistochemistry to investigate the phosphorylation, as a surrogate for activation, of pathway molecules 4EBP1, mTOR and Akt, as well as the deletion of repressor protein PTEN, on tissue microarrays of primary and metastatic SI-NETs.

P-4EBP1, p-mTOR and PTEN were characterized in both the primary tumour and liver metastasis microarrays, with highly variable expression. Certain cases had very strong effector expression and/or PTEN loss, agreeing with the idea that these tumours are known to have heterogeneous presentation. It is also worth noting that in some cases there was phosphorylation of mTOR and 4EBP1 in patterns suggesting an association with progression, such as within mitotic figures and at the leading edges of the lesions. Further investigation to characterize other pathway molecules and correlate this data with patient outcomes is ongoing, and could have implications on the use of mTOR inhibitors to treat these tumours.

Poster Abstract #28

Tissue Engineering a Corneal Stroma for Transplantation through Keratocyte-mediated Mechanotransduction

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Over 10 million people worldwide are blind due to various corneal diseases and injuries, making cornea-related problems the second leading cause of blindness in the world. Due to its avascularity and relatively simple organization, the cornea has been a target for early applications of tissue engineering. However, mechanical instability in the stroma is a major problem tissue engineers face when attempting to generate a viable human cornea. To explore this problem, we use classic tissue engineering techniques to model a human corneal stroma by seeding human keratocytes into a collagen gel scaffold. Previous studies by our group have demonstrated the importance of keratocyte/integrin-mediated mechanotransduction in normal corneal development; when mechanotransduction is inhibited, resultant corneas in mice models are weakened and thinned. TGF-β2 has been shown to be important in the production of type I collagen and integrins in keratocytes, and as such, is believed to be a mediator of mechanotransduction. Therefore, we expose our corneal stromata to TGF-β2 during their development to elicit mechanotransduction and determine its effect on the mechanical strength of our model stromata.

Poster Abstract #29

Fibrin mediates Human Islet Cell Differentiation via p70s6k, promotes Integrin Expression while enhancing Vasculature during Transplantation

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Introduction: Extracellular matrix (ECM)-integrin interactions influence β-cell differentiation, function, proliferation and survival, yet when ECM proteins are used during long-term culture, loss of islet function and structure occurs. Fibrin is a provisional ECM protein and contains Arg-Gly-Asp motifs that can be bound by integrin αvβ3. Fibrin supports human and rat adult islet function, but the mechanisms by which this occurs are unknown. The current study analyzed whether fibrin can promote human fetal pancreas differentiation and proliferation in vitro and in vivo, and the mechanisms behind the resultant effects.
Methods: Human fetal pancreatic progenitor cells isolated from 2nd trimester of pregnancy were cultured up to 2 weeks on tissue culture polystyrene (TCPS) and with fibrin in 2D and 3D, or injected into nude mice. Cells were analyzed by scanning electron microscopy, quantitative RT -PCR, western blot, and immunofluorescence.

Results: The human fetal pancreas contains extensive ECM fibers made up of collagens, fibronectin and laminin. Fibrin culture of human pancreatic progenitors lead to enhanced expression of glucagon, insulin, and PDX1 when compared to TCPS ($p<0.05$). The fibrin-cultured group also had significantly higher expression of integrin αvβ3, phosphorylated-FAK, VEGF-A, and phosphorylated-p70s6k ($p<0.05$). Rapamycin treatment of cells cultured on fibrin lead to significantly decreased p70s6k phosphorylation as well as reduced PDX1 and cyclin D1 expression ($p<0.05$). Subcutaneous injection of fibrin mixed with human fetal pancreatic progenitor cells in mice resulted in enhanced vascularization compared to the collagen controls, with maintenance of islet gene expression.

Conclusions: The human fetal pancreas contains a complex ECM environment that stimulates integrin receptors for proper cell function. Fibrin stimulates αvβ3 integrin receptors leading to increased p70s6k activity, which promotes expression of islet-specific genes, including PDX1. The enhanced vasculature observed in fibrin-mixed human fetal pancreatic cells after transplantation suggests that fibrin may improve graft health during islet transplantation.

Keywords: Human fetal pancreas, fibrin, integrins, signaling pathway, subcutaneous transplantation, vasculature

Poster Abstract #30

Distinctive Expression of Niche Factors by Adipocytes and Osteoblasts May Mediate Diabetic Stem Cell Depletion

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Introduction: Maintenance of quiescent stem cells (SCs) is the main role of bone marrow (BM) SC niche. Cellular makeup of the BM as well as cytokines, growth factors, and adhesion molecules are involved in anchoring SCs in the BM and maintaining SC phenotype. We have recently shown that chronic diabetes leads to enhanced adipogenic differentiation of marrow mesenchymal progenitor cells (BM-MPCs), thus altering the niche structure and function. We aimed to identify genes that display differential expression patterns in adipocytes and osteoblasts in order to understand whether this change in cell fate and BM composition may have an effect on resident SCs.

Methods: BM-MPCs were isolated from fresh human samples and expanded in culture. We induced adipogenesis and osteogenesis through the application of the appropriate differentiation media. We then conducted a large-scale profile of various niche genes.

Results: Our studies have identified thrombopoietin as an osteoblast-specific niche factor as the factor was virtually absent in adipocytes. Upon enhanced adipogenesis, which is seen in diabetic bone marrow, this loss may play an important role in the disruption of SC maintenance. In addition, we found increased ratio of angiopoietin-2 (Ang2) to angiopoietin-1 mRNA in adipocytes compared to osteoblasts. Culture of BM-MPCs in media containing exogenous Ang2 led to selective alterations in the expression levels of several fundamental adipogenic factors.

Discussion: Our studies have identified two potential targets for restoring bone marrow SC niche in diabetes. We have shown that changing cellular composition of the BM may be associated with loss of thrombopoietin and altered Ang/tie signaling pathway. The selective changes in adipogenic factors highlight a potential role for Ang2 in modulating the differentiation of BM-MPCs and the depletion of the marrow stem cell niche. We are currently undertaking studies to investigate the functional significance of altering these two signalling pathways in diabetes.

Keywords: mesenchymal progenitor cells, adipogenesis, diabetes, bone marrow, angiopoietin-2, adipocytes, osteoblasts

Poster Abstract #31

The Effects of Mechanical Stress on Human Trabecular Meshwork Cells

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Introduction: High intraocular pressure (IOP) is a major risk factor for glaucoma. Resistance to outflow of aqueous humor through the trabecular meshwork cells (HTMCs) is believed to cause high IOP. However, the exact mechanism is unknown. Pilot studies from our laboratory show that mechanical stretch, conditions mimicking pressure-induced stretch in HTMCs, causes a decrease in viability. The aim of the present study is to develop a clear dose- and time-response relationship between degree of stretch and HTMCs viability. We have also attempted to study specific downstream effects of mechanical stretch such as changes in gap junction Connexin43.

Methods: Primary HTMCs various donors were obtained and cultured. Upon reaching confluency, HTMCs were stretched at 5%, 10%, and 15%, each for 24hr, 48hr, 72hr. Cell health was then measured using vital Trypan blue stain, lactate dehydrogenase (LDH) assay, and cytoplasmic nucleosome detection. Expression of connexin43 was measured using real time qPCR and Western Blotting.

Results: Our results show no significant changes in viability upon stretching HTMCs as measured by Trypan blue stain. Interestingly, LDH levels decreased in a dose- response manner with increasing % stretch and duration of stretch. We also detected a change in connexin43 expression under high % stretch conditions.

Conclusions: Our studies have the potential to provide key insight into the contribution of mechanical stretch to impaired trabecular meshwork in glaucoma. To date, the cause of glaucoma remains elusive and there is scarce information on ocular cell response to mechanical stress. In addition, if a stretch-stressor response is identified, perhaps the knowledge will uncover potential new targets for drug therapy targeting the trabecular outflow.

Keywords: Trabecular meshwork, glaucoma, mechanical stress, intraocular pressure, gap junction, cyclic stretch

Poster Abstract #32

The role of transcriptional regulator TBX3 in early breast cancer progression

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Introduction: Our recent studies show that breast cancer cells that have gained the ability to invade adjacent tissue express high levels of the regulatory protein TBX3. Through the inhibition of p14ARF and/or p21CIP1 and up-regulation of E2F and NF-kB, cells with elevated levels of TBX3 have alterations in cell cycle proliferation and progression, promoting the bypass of cellular senescence. The 21T series cell lines have been proposed as a model for the human breast cancer progression series. 21PT cells mimic ADH (non-tumorigenic), 21NT cells mimic DCIS (tumorigenic, non-metastatic), and 21MT-1 cells depict characteristics of IMC (tumorigenic and metastatic). Based on our previous studies with 21T cell lines, high levels of TBX3 appear to be correlated with invasiveness of breast cancer.

Methods: Cell lines 21PT, 21NT, 21NT+EV, 21NT+TBX3iso1, 21NT+TBX3iso2, and 21MT-1 were grown in Matrigel. Quantification of Ki67 positive and caspase3 positive cells was conducted through immunofluorescent staining and confocal microscopy. Colony formation ability was quantified for each cell line, along with various morphological characteristics. qPCR of 84 breast cancer related genes was conducted to investigate altered gene expression with TBX3iso1 or TBX3iso2 up-regulation. MT-1 shLUC and MT-1 shTBX3 stably transduced cells have been constructed for future experiments. TBX3 up-regulated cell lines will be injected into athymic nude mice to investigate resultant pathology.

Results: The up-regulation of TBX3 in DCIS-like 21NT cells resulted in an IMC-like phenotype; cells exhibited an increased Ki67/caspase3 ratio, increased colony-forming ability, and less spherical, irregular-shaped colonies. The expression of several genes was altered with the upregulation of TBX3iso1 and TBX3iso2; down-regulation of SLIT2 is of particular interest.

Conclusions: Preliminary findings suggest that TBX3 promotes the transition from in situ to invasive breast cancer through the altered expression of key regulatory and EMT-related genes. These mechanisms will be further examined.

Keywords: TBX3, breast cancer, metastasis, atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS), invasive mammary carcinoma (IMC)

Poster Abstract #33

Concurrent Neoadjuvant ChemoRadiotherapy Improves Pathological Complete Response But Not Survival in Locally Advanced Breast Cancer

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Introduction: Locally advanced breast cancer (LABC) represents 15% of all breast cancers, with a 5-year survival ranging from 30-45%. Standard treatment includes neoadjuvant chemotherapy followed by mastectomy then adjuvant radiotherapy. Since taxane class of chemotherapies are radiosensitizers, and since concurrent taxane-radiotherapy has been used to improve clinical outcomes in other disease sites, we proposed to test whether breast cancer surrogate outcome (pathological complete response, pCR) or survival outcome, could be improved by combining taxane-radiotherapy pre-operatively. We also sought to determine whether MIBI Spect/CT imaging, tumour biomarker osteopontin (OPN) levels or 3D ex vivo human tumour culturing could predict patient outcome.

Methods: Thirty-two LABC (stage III) newly diagnosed patients were recruited to participate in this pilot study. Patients received 3 cycles q3 weekly of 5-fluorouracil, cyclophosphamide and epirubicin, followed by weekly docetaxel (35 mg/m2) concurrent with 45Gy in 25 fractions of 4-field radiotherapy. A modified radical mastectomy was performed 5 weeks later. Patients received pre-mid- and post-chemotherapy biopsies for culture in matrigel and MIBI Spect/CT as well as q3weekly plasma samples. Patients were matched to a historical cohort 2:1 by tumour histology (luminal subtype), age and menopausal status for survival analysis using SAS.

Results: Thirty-one patients were included for analysis, one patient died mid-treatment. The pCR rate was double what clinicians have historically seen (22% vs 10-12%), however in matched analysis, there was no significant difference in median survival at 3 years (Hazard Ratio (95% CI) = 1.44 (0.52,4.02)). Neither serial MIBI Spect/CT nor serial tumour ex vivo response to chemotherapy could predict which patients would respond to chemoradiotherapy. Baseline plasma OPN levels did significantly predict for clinical outcome (p=0.025).

Conclusions: Although combining neoadjuvant chemotherapy with radiotherapy for LABC does appear to increase pCR rates, there is no improved 3-year survival in these patients. OPN appears promising as a predictive tumour marker, but larger trials are required to validate its response to neoadjuvant therapy.

Keywords: LABC, neoadjuvant, pathological complete response, breast cancer

Poster Abstract #34

miRNA Regulation of PD1, TIM3 and BTLA: Reverting T-cell Exhaustion in Melanoma

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Introduction: T-cell exhaustion is when T-cells, in response to constant antigen stimulation, fail to proliferate and exert effector functions such as cytotoxicity and cytokine secretion. Recent studies have shown that T-cell exhaustion may explain how cancerous cells can evade the immune system and proliferate. Inhibitory cell receptors that can induce a state of T-cell exhaustion include PD1, CTLA-4, TIM3 and BTLA. These receptors have become popular targets to block in cancer therapy, however little research has been done on a synergistic blockade therapy. One way to circumvent the issues of cost and drug-drug interactions with antibodies is by using miRNAs, which no one has attempted against T-cell exhaustion. The objective for this project is to investigate the miRNA regulation of PD1, TIM3 and BTLA, and to discover a novel RNAi therapy using miRNAs that bind to these receptor transcripts.

Methods: I injected C57L/B6 mice with B16F10 cells and after 16 days tumors were excised and PD1+ T-cells were isolated using MACS beads and flow cytometry. Global miRNA expression was compared between PD1+ and PD1- T-cells using an Affymetrix GeneChip 3.0 miRNA array and confirmed with qPCR. I will determine the silencing efficiency of the candidate miRNAs using a dual luciferase assay then use an animal model to test their therapeutic effects.

Results: PD1 expression was significantly upregulated in the lymph node, spleen and tumors of injected mice. Various miRNAs that are associated with many cancers were significantly upregulated in PD1+CD4+ T-cells. However, based off the in silico search, miRNA array and qPCR data; miR-150, miR-28, miR-103 and miR-107 were chosen as candidates.

Conclusions: These findings show that miRNA may silence multiple T-cell exhaustion markers at once. This study will provide valuable data on a novel cancer therapy, supporting the use of RNAi to confer T-cell immunity against cancer.

Keywords: PD1, TIM3, BTLA, Melanoma, Cancer Immunology, T-cell Exhaustion, RNA Interference, miRNA

Poster Abstract #35

Hair Cortisol as a Biomarker of Stress in Sub-Saharan African Communities

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Introduction: Stress is known to contribute to overall health status. Many individuals in sub-Saharan Africa are believed to be stressed about their employment status, income and health. Hair cortisol is increasingly being used as a biomarker of chronic stress. This study aimed to investigate hair cortisol as a biomarker of stress in settlement communities in Kenya.

Methods: Hair samples were collected from 107 volunteers from settlement communities in Naivasha and Mogotio, Kenya and compared to a European reference group (N=15). An enzyme-linked immunosorbent assay (EIA) technique was used to measure hair cortisol concentrations. In parallel, a health status survey was completed.

Results: Median hair cortisol concentration (range) in volunteers from the settlement communities in Kenya was 588 ng/g (1,411) which was significantly higher than the European reference group [279 ng/g; (475)] (Kruskal-Wallis test; $P < 0.0001$). Hair cortisol concentrations also were significantly higher in females, volunteers who were divorced, those who made below minimum wage and those who reported feeling unsafe collecting water or using sanitation facilities.

Discussion: The increased hair cortisol concentrations among volunteers from the settlements in Kenya compared to our reference group as well as among women compared to men suggest higher levels of chronic stress and/or poorer hair hygiene habits. The causes for this apparent increased stress appear to be due to factors including socio-economic, socio-cultural, poorer health and increased maternal responsibility and are worthy of further evaluation. Hair washing habits could be contributing to increased hair cortisol content in females vs. males and in volunteers from the settlements.

Keywords: hair, cortisol, stress, sub-Saharan Africa

Poster Abstract #36

Molecular characterization of gastrointestinal glandular-neuroendocrine mixed tumor

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Introduction: Glandular-neuroendocrine mixed tumor (GNMT) of the gastrointestinal tract is an uncommon neoplasm. The histogenesis and clinicopathological features are not well understood. Previous studies, using a limited panel of molecular markers, showed that both the glandular and neuroendocrine components shared concurrent loss of heterozygosity (LOH) of chromosomes 17p and 5q, which harbor p53 and APC genes respectively, suggesting a shared carcinogenesis mechanism.

Methods: We have identified 12 cases of GNMT from the surgical pathology files of the Pathology Department at London Health Sciences Centre (January 2003 to June 2013). Neuroendocrine and glandular components of formalin-fixed paraffin-embedded (FFPE) tumor samples will be separated using laser-capture microdissection. Genomic DNA will be extracted from these components, analyzed and compared using the Affymetrix CytoScan HD microarray. This array utilizes both polymorphic and non-polymorphic oligonucleotide probes which will provide high-resolution copy number changes and genotypic changes such as LOH.

Results: Of the 12 GNMT cases, 7 cases are composite tumors and 5 are collision tumors; 9 of the glandular components are adenomas, and 3 are invasive adenocarcinomas; 5 of the neuroendocrine components are low grade neuroendocrine tumors and 7 are high grade neuroendocrine carcinomas. Four recent cases containing sufficient material for molecular analysis were selected. To date, laser-capture microdissection has been performed on one of the cases; results to follow.

Conclusions: This study will utilize a novel microarray technology to examine genetic alterations at much higher resolution than has been previously performed on GNMT; this should provide a more in depth understanding of the molecular relationship between the different histologic components of GNMT.

Keywords: gastrointestinal glandular-neuroendocrine mixed tumor; carcinogenesis; microarray

Poster Abstract #37

The Application of a Multitissue Spring-roll Control Block in Immunohistochemistry

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Introduction: The modern practice of pathology involves the use of immunohistochemistry (IHC) for the detection of tumour cell markers with prognostic and therapeutic significance. The presence of external positive and negative control tissue mounted on the same slide as a patient's sample, and thus subjected to the same treatment, is important to demonstrate the validity of the test. The current methodology used to prepare these external control tissues at LHSC is somewhat of a cumbersome process with preparation of these control tissues needing to be done on a fairly continuous basis. The preparation and application of a multitissue spring-roll control block and its purported benefits have been documented previously. In the present study, we have prepared a multitissue spring-roll control block designed for sufficient coverage of immunoreactivities to act as a general control in our lab.

Methods: Twelve tissue types were selected for inclusion in the spring-roll control block. The majority were non-tumoural (colon, kidney, liver, lung, placenta, pancreas, tonsil, salivary gland, adrenal gland, spleen) and readily obtained in our surgical pathology laboratory. Metastatic melanoma was less commonly available. Amnionic membrane was chosen as a wrapping material for its convenience as well as to itself provide a positive control. After initial paraffinization, the multitissue spring-roll was sectioned transversely and these thin (3 mm) cross-sections were again embeded to act as external IHC controls.

Results: The pattern of staining in the constituent tissues provided an equal degree of quality assurance to our current method in comparative immunostaining procedures.

Conclusion: While the prospective collection of tissues was time-consuming, it is still feasible at a large, tertiary centre such as LHSC and, with regular use, the multitissue spring-roll control block would potentially save time and resources while meeting most laboratory demands.

Keywords: immunohistochemisty, quality control, multitissue control block, surgical pathology laboratory

Poster Abstract #38

Selected Human Kallikrein Expression in Odontogenic Cysts and Tumors

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Introduction: Non-inflammatory lesions of odontogenic epithelium show a spectrum of clinical behavior, ranging from indolent cysts of limited growth potential (lateral periodontal cyst), to potentially destructive cysts (dentigerous cyst), cystic neoplasms (keratocystic odontogenic tumors), and aggressive tumors (ameloblastoma). To better understand this behavioural cascade, in terms of molecular biology, we investigated the expression of human kallikrein-related peptidases (KLKs) 6,7,8,10,13 and 14 in these lesions.

KLKs are a group of 15 serine proteases, the best known of which is KLK3 (prostate specific antigen), widely used in the screening and monitoring of prostate carcinoma. KLKs are involved in many different processes, including keratinization and desquamation, amelogenesis, cell proliferation, migration and invasion. They are also implicated in many cancers, including ovarian cancer, endometrial cancer, cervical cancer, prostate cancer, breast cancer, and salivary gland cancer.

Methods: Archived paraffin embedded samples obtained from the Division of Oral Pathology at the University of Western Ontario were assessed for the presence of KLKs 6,7,8,10,13, and 14 using a standard immunostaining technique, utilizing a polyclonal antibody for each. Sixty-one lesions were investigated including lateral periodontal cysts (n=10), dentigerous cysts (n=10), keratocystic odontogenic tumors (KOT) (n=11), ameloblastomas (n=10), nasopalatine duct cysts (n=10, non-odontogenic cystic control), and odontomas (n=10, neoplasm control). A scoring system assessing intensity and proportion of epithelial cells stained was used. These scores were combined to yield an overall score for each sample. Data were analyzed using the Kruskal-Wallis and Dunn's multiple comparison tests.

Results: The expression of KLKs 6,7,8,10, and 13 differed among controls, cysts and tumors, while only KLK14 was expressed uniformly. The results suggest that KLKs 6, 7, 8, 10, and 13 are involved in the progression of aggressive odontogenic tumors (viz. ameloblastoma). Specifically, KLKs 10 and 13 appear to have a role in the development of KOTs and KLKs 6, 10, and 13 appear to have a significant role in the development of ameloblastomas. KLKs 6, 10, and 13 appear to work together in a cascade or pathway.

Conclusions: Overall, for the first time, it has been shown that KLKs 6,7,8,10,13, and 14 are present in the epithelium of odontogenic cysts and tumors. With future research, we hope to further define the specific roles of these KLKs in these cysts and tumors, and to determine if they can serve as biomarkers in early diagnosis and treatment monitoring.

Keywords: Odontogenic cyst, Odontogenic tumor, Human Tissue Kallikreins, Tumor Biomarkers

Poster Abstract #39

ERK5 expression in Brain Tumors

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Introduction: Due to the invasive nature of certain types of brain tumors, such as Glioblastoma, efforts to battle this disease must be focused on its invasive nature and effectively inhibiting signaling pathways that control cell migration and invasion. We have previously shown in breast cancers, ERK5 as a downstream effector of Cdc42, which is known to regulate tumour cell migration and invasion. Previously, we analyzed ERK5 expression in various brain tumours using a tissue microarray (TMA). The findings suggested that ERK5 signaling plays a role in regulating the progression of certain types of brain tumours, including Glioblastomas and Astrocytomas. Next, we will use banked brain tumour specimens to further investigate the relationship of phosphorylated ERK5 with brain tumour progression. Through the proposed experiments, we expect to better understand the role of ERK5 in controlling the progression of these brain tumours, as well as identify a novel function of ERK5 in glioblastoma cell migration/invasion. We hypothesize that there is a presence of a novel mechanism of ERK5 regulation of Glioblastoma progression.

Methods: Banked brain tumour specimens will be stained with ERK5 antibody, and the expression of ERK5 will be analyzed by immunohistochemistry. The role of ERK5 in the migration/invasion of these cells will be examined using a microscope. The Immunohistochemical reactivity for ERK5 will be evaluated, and scored according to the percentage of staining astrocytic nuclei and the intensity of staining. The data will then be analyzed by statistical analysis.

Results: The differential expression of ERK5 using banked brain tumour specimens and IHC will be reported.

Conclusions: These findings may suggest that ERK5 signaling plays a role in regulating the progression of certain types of brain tumours, including Glioblastomas and Astrocytomas. Future research includes further investigating the relationship of ERK5 with brain tumour progression.

Keywords: ERK5, phosphorylated ERK5, Glioblastomas, Astrocytomas

Poster Abstract #40

Investigating the Structural and Functional Changes to the Retina Following PRP in Diabetic Retinopathy Patients

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Introduction: Diabetic retinopathy is the leading cause of blindness worldwide and is estimated to affect more than 100 million adults. Over the past 30 years, epidemiological studies and clinical trials have shown that timely laser photocoagulation could prevent visual loss. Laser therapy has been well proven to stabilize and control proliferative disease. Questions however on the long-term effects on the nerve fiber layer and optic disk from these treatments remain to be answered. The objective of the study is to evaluate changes in structural and functional diagnostic tests at defined period of times up to 12 months in diabetic retinopathy patients.

Methods: Patients will undergo pre-laser and 3,6 and 12 month post-laser visual fields, HRT and OCT tests. OPTOS fluorescein angiography will be performed at the pre-laser baseline, 6 and 12 months post-laser.

Results: From baseline (n=20), average RNFL thickness did not significantly change (P>0.05) by 6 months post-treatment (3 months +2 µm; 6 months +1.8 µm). Trend analysis observed a nonsignificant thickening in nerve fiber layer of the macula (+3µm) at 3 months with a nonsignificant thinning (-5.4µm, P>0.05) at 6 months compared to baseline. Visual field MD showed a nonsignificant (P=0.14) decreasing trend compared to baseline (6 months -1.07 dB). Average cup/disk ratio decreased by -0.014 at 3 months but has shown an increasing non-significant trend (+0.008, P=0.54) from baseline.

Discussion: Trend analysis has shown decrease in the cube average thickness, increase in optic nerve cupping and decrease in mean deviation which is consistent with the hypothesis. Further correlation will be made as patients continue to undergo their 12 month testing. Development of a software to measure ischemia is also in development.

Keywords: Diabetic retinopathy, ophthalmology, retina, structural and functional changes, panretinal photocoagulation

Poster Abstract #41

Traditional and Electronic Ki-67 Quantitation in Oligodendrogliomas

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Introduction: The KI-67 proliferative index has become a useful, objective, immunohistochemical tool that can aid in grading and prognostication for patients with oligodendrogliomas. Previous studies have described the prognostic significance of the Ki-67 index for such patients. According to the WHO classification of tumors of the central nervous system (2007) "mitotic activity is low in WHO grade II oligodendroglioma, and labeling indices for proliferation markers are accordingly low, usually below 5%". Furthermore, the predictive value of the Ki67 index appears to be independent of age, tumor site, and histological grade. What is less well described is the relative accuracy of traditional vs. semi-automated methods of enumeration for a test where small differences can influence grading, prognosis and treatment. Tang et al. (2012), studying gastroenteropancreatic neuroendocrine tumours, found high concordance between two semi-automated methods for Ki-67 quantitation whereas "eyeballed estimates" were far less reliable. Proposed method: We will compare the reported proliferative index estimates to those calculated by digital image analysis of 35 recent oligodendrogliomas from the LHSC Pathology archives.

Summary: The objective of the present study is to compare the accuracy of traditional estimates of Ki-67 expression in oligodendrogliomas with those calculated by digital image analysis. Our aims include: i) discovering the technical limitations of semi-automated Ki67 calculations, ii) optimizing sampling methodologies, and iii) describing any discrepancy between manual and semi-automated methods.

Keywords: oligodendroglioma, Ki-67, proliferative index, image analysis

Poster Abstract #42

The Role of MicroRNA-346 in Breast Cancer

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Introduction: Breast cancer is a complex neoplasm that in most cases has a genetic component. In the recently developing field of microRNA research, many microRNAs are being implicated in cancer pathogenesis. miR-346 is an intronic microRNA that has been associated with thyroid and prostate cancer, and has been shown to target genes that are related to breast cancer. The present study is aimed at exploring the role of miR-346 in breast cancer tumorigenesis and the mechanisms by which this may occur using an in vitro model.

Methods: Mouse mammary carcinoma cells (4T1) were transfected with miR-346 mimic and inhibitor. The effect of miR-346 on tumor cell biological functions were characterized through migration, viability, and cell-cycle assays. We explore the mechanisms for these effects through analysis of target gene (in particular, Leukemia Inhibitory Factor) expression and downstream apoptosis and proliferation pathway associated gene expression.

Results: Our results show that miR-346 mimic transfection increases proliferation and migration of 4T1 breast cancer cells in vitro, while miR-346 inhibitor has the opposite function. Early experiments do not show significant effects and are currently being repeated. Cell-cycle assays do not show clear differences in phase distribution. Interestingly, the effect of miR-346 to induce tumorigenic processes is in contrast to what is expected based on the function of predicted target genes (such as LIF and RIP140). An analysis of target gene expression is yet to be done and may address this discrepancy.

Conclusions: Our results indicate for the first time that miR-346 expression level may be related to breast cancer tumorigenesis. This finding suggests a novel mechanism that may be relevant in the development of novel diagnostic/prognostic disease biomarkers or therapeutic approaches in breast cancer. We hope to perform further research to yield more conclusive results and determine the mechanism for these effects.

Keywords: microRNA, miR-346, breast cancer, leukemia inhibitory factor (LIF), 4T1 cells, proliferation, tumorigenesis

Poster Abstract #43

Understanding the Mechanism of Carboplatin-Induced Vascular Dysregulation in Ovarian Serous Adenocarcinoma

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Introduction: Ovarian cancer is one of the leading causes of cancer-related mortality among women. Although advances have been made in surgical and chemotherapy strategies, long-term survival remains poor. Carboplatin, a platinum-based drug, has become the chemotherapy agent of choice in ovarian cancer due to its favorable therapeutic profile. However, there remains a high rate of resistance to chemotherapy treatment in ovarian cancer. Since, angiogenesis has been linked to tumour progression and chemotherapy resistance in several malignancies including ovarian cancer, we aim to examine whether carboplatin treatment can induce the expression of angiogenic factors and subsequently promote angiogenesis in ovarian tumours. We hypothesize that carboplatin treatment alters angiogenesis signalling pathways in tissue samples of ovarian serous adenocarcinomas.

Methods: To test our hypothesis, we first extracted RNA from samples of ovarian serous adenocarcinomas obtained from patients treated with carboplatin and those without such treatment. We then used quantitative RT-PCR to determine the gene expression profiles of angiogenic factors in the tissue samples.

Results: Our findings show that carboplatin-treated tumours had higher expression levels of several genes involved in regulating angiogenesis and vascular permeability. These genes included matrix proteins such as fibronectin, and secreted growth factors such as vascular endothelial growth factor-A and angiopoietin 1. In addition, we noted high mRNA levels of several chemokines including CCL2, CCL11, and CXCL5 in samples obtained from patients treated with carboplatin.

Conclusion: These findings to date show that carboplatin treatment modulates the tumour microenvironment in ovarian carcinomas by increasing the expression of various angiogenic factors. To further investigate the effects of carboplatin, we will perform immunohistochemistry staining in order to detect and localize the chemokines and the growth factors that showed higher expression in the PCR assay.

Keywords: Ovarian cancer, angiogenesis, carboplatin, chemokines, VEGF

Poster Abstract #44

Lymph Node Dissection in Total Gastrectomy Specimens. A Retrospective and Prospective Institutional Review.

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Introduction: Gastric cancer (GC) is the third leading cause of cancer death worldwide. Recent recommendations suggest that at least 16 lymph nodes (LNs) be pathologically assessed to ensure adequate staging in gastric cancer resections. This study aims to address the issue of pathological assessment of LNs resected in total gastrectomy specimens at a specialist center; firstly to quantify the mean number of LNs per specimen, and secondly to determine if this could be improved by providing an education session to pathologist's assistants (PAs), and pathology residents.

Methods: A retrospective review of GC resections was performed by searching the LHSC archive for total gastrectomies performed between January 2010 and August 2013. Total number of LNs and number of positive LNs per case were recorded. An education session was then provided to emphasize to gross room staff the importance of retrieving a minimum of 16 LNs per case. The grossing protocol was amended to include the use of GEWF (glacial acetic acid, ethanol, distilled water, and formaldehyde) to attempt to identify additional LNs in cases where 16 nodes were not identified. LN counts in total gastrectomies performed subsequent to the education session were then assessed.

Results: Between January 2010 and August 2013, 20 total gastrectomies were performed at LHSC. The mean number of LNs per case was 20.6 (range 3-64). Following the education session 2 total gastrectomies were performed with a mean node count of 16.5 (range 14-19).

Conclusion: To date, the number of post-education session total gastrectomies is too small to draw definite conclusions; however, the routine LHSC grossing protocol yields a mean of 20.6 LNs per case. The lack of a significant increase in LN count immediately following the education session suggests that the routine protocol of thorough gross examination at our institution is optimal and meets recommended adequacy criteria.

Keywords: Gastric cancer, lymph node dissection, total gastrectomy

Poster Abstract #45

Understanding Head and Cervical Spine Injuries in Pediatric Occupants Involved in Motor Vehicle Collisions

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Introduction: Pediatric spinal cord injury is a rare (2.7% to 9% of spinal injuries), but has a high morbidity and mortality (Ramrattan et al, 2012; Li et al, 2011). The pediatric spine is a unique and different entity of that compared to adults. Compared to the adult spine, the immature spine is hyper-mobile secondary to ligamentous laxity, shallow facet joints, underdeveloped spinal process, and physiological wedging of the vertebral body. These combined with a higher head-to-torso ratio and immature/poorly developed cervical musculature predispose younger children to high torque forces (Li et al, 2011; Mathison et al, 2008). Pediatric spine injuries (60%-80%) occur in the cervical spine region. Younger children (8 years and under) have injury in the occiput to C4 regions.

Methods: In our study we collected deceased victims, ages 12 and under, from vehicle collisions (n=96). Since the subjects were deceased we obtained coroners', police and pathologists' autopsy reports from the Office of the Chief Coroner located on 200-26 Grenville St, Toronto, ON M7A 2G9. The program used to collect information is called FileMaker. For each victim injury we used AIS coding to label the injuries. Major variable that I used were age in months, initial impact location, medical cause of death, Specific injuries. Variables are compared for association using odds ratios and common odds ratios.

Results: Review of the Office of the Chief Coroner data base with review of the medical literature will be done to determine predictive factors for cervical spine injury.

Conclusions: Pediatric cervical spine injury is rare, but has a high mortality and morbidity rate for younger children. The most common cause of cervical spine trauma is due to various types of motor vehicle collisions.

Keywords: Pediatric, Cervical Spine, Motor Vehicle Collisions, Injury, Fatal, Children, Atlas, Axis

Poster Abstract #46

Deciphering the role of RGNEF in ALS using a novel yeast model

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As the Canadian population continues to age, neurodegenerative disorders are on the rise. Amyotrophic lateral sclerosis (ALS) is a mid-to-late life onset motor-neuron degenerative disease. Despite considerable research efforts the underlying pathogenesis of the disorder is poorly understood and there are currently no treatments to cure or delay the onset of symptoms. Mounting evidence indicates that aberrant RNA metabolism observed in both familial and sporadic ALS may be a major contributor to the disease. Several proteins involved in RNA binding activity, such as TDP-43 and FUS/TLS, are well documented to have genetic mutations and appear misfolded within aggregates of diseased neurons. Recent genetic, biochemical, and pathological findings indicate a potential role of a protein called Rho Guanine nucleotide exchange factor (RGNEF), in aberrant mRNA metabolism in ALS. ALS-related mutations in the gene encoding RGNEF have been documented and the protein has been shown to be isolated from pathological aggregates in ALS. In addition, it has also been found that RGNEF modulates the mRNA for stability of low molecular weight neurofilaments (NFL) which appear decreased in ALS. Also, RGNEF contains a guanidine nucleotide exchange factor domain thus indicating a link between cell signaling and RNA metabolism in ALS. The mechanisms by which RGNEF may cause messenger RNA instability and contribute to the ALS pathogenesis remain unknown. We have established a yeast model expressing RGNEF. Our model will allow the exploitation of powerful yeast genetic tools to systematically investigate RGNEF misfolding, subcellular localisation, and its impact on RNA metabolism. Our study thus aims to elucidate novel interactions of RGNEF and provide insight into how these interactions may be linked to the pathological mechanism of ALS and possibly neurodegeneration in general.

Keywords: Amyotrophic Lateral Sclerosis ALS, Rho Guanine Nucleotide Exchange Factor (RGNEF), Protein misfolding, Protein aggregates, Neurodegenerative disorders, Yeast model

Poster Abstract #47

Epithelial to mesenchymal transition in the pathogenic progression of small intestinal neuroendocrine tumours

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Introduction: Neuroendocrine tumours (NETs) are a rare type of epithelial cancer arising from the diffuse neuroendocrine system. Advances in the treatment for NETs are hindered by our limited understanding of the pathogenesis of NETs. In this pilot study, we have performed gene expression profiling along with immunohistochemistry to highlight key molecular pathways underlying the pathogenesis of NETs.

Materials and Methods: We used formalin-fixed paraffin-embedded tissue samples of small intestine NETs (siNETs), the most common malignancy of the small bowel, to extract RNA for real-time PCR-based gene profiling, and to construct tissue microarrays (TMAs) for immunohistochemistry (IHC). Differences in gene expression were studied in samples of primary (n=9) and metastatic (n=4) samples using normal small bowel epithelium (n=3) as a control. We then performed IHC for E-cadherin, β-catenin and vimentin to confirm our findings.

Results: Our results show elevated expression levels of vascular endothelial growth factors (VEGF) A, B and C, placental growth factor, and VEGF receptors (R1 and neuropilin1). We also found abundant expression of transforming growth factor beta-receptor 1 (TGF□R1) in both primary and metastasis samples. Variations in the expression patterns of matrix metalloproteinase (MMP) 14 and 9, and tissue inhibitor of metalloproteinases (TIMP) 1 and 2 in both sample groups were also noted. Our IHC results highlighted some cases with unusual expression patterns where there is a loss of E-cadherin and β-catenin expression in the membrane along with strong vimentin reactivity.

Discussion: Our results suggest an important role of an epithelial to mesenchymal transition (EMT) phenotype in the pathogenesis of siNETs. Specifically, VEGF receptor expression pattern, ECM profile, and TGF□R1 point to global cellular behavior associated with EMT and cell guidance. Our IHC data confirms the presence of EMT events in NETs. We plan to expand these studies and extend them using a siNET cell line system.

Keywords: Neuroendocrine tumours, epithelial to mesenchymal transition, small intestine, immunohistochemistry, gene expression

Poster Abstract #48

Validating Inclusivity/ Exclusivity of an Alternative Method for Detecting E. coli O157 According to AOAC Guidelines.

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Although Canada is known to have a safe food supply, recent reports from the Public Health Agency (PHAC), show that one in eight Canadians get sick due to foodborne diseases each year. Thirty known pathogens, including E. coli O157, are responsible of 40% of the annual number of illnesses. Therefore, eliminating pathogens from food is one of the compelling objectives of any food processor. An important component for ensuring food safety is the method used for pathogen detection, which have evolved rapidly in the past century. Despite conventional culture methods still being the golden standard, new Alternative Methods (AM) are offering faster, cheaper and sometimes more sensitive alternatives to processors. However, it is essential for an AM to be validated against a Reference Method (RM) to guarantee their performance and reliability of the results. Several standards have been developed worldwide that provide protocols for the validation and further certification of new microbiological methods (AOAC, ISO, AFNOR, NordVal). The AOAC Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces is the most recognized standard used in US and also suggested on the Canadian Compendium of Analytical Methods. In this paper we are briefly introducing the first phase of the study known as Developer Validation Study as described by AOAC protocols. Our design includes evaluation of parameters such as probability of detection (POD), with main focus on inclusivity and exclusivity assessment. We have determined the ability of the AM to detect E. coli O157 and to avoid interference with a variety of non-target organisms, using so far 26 different strains. The analysis has been done using pure cultures as established in the AOAC guidelines. Preliminary results have shown that the AM is able to detect all of the inclusive strains, while excluding the non-target strains used.

Keywords: E. coli O157, pathogen, AOAC, detection method, Food Microbiology, Food Safety, Method Validation, inclusivity, exclusivity, Alternative Method

Poster Abstract #49

Effect of Dietary Modifications during Pregnancy With and Without Diabetes on Offspring Pancreas Development

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Maternal type 1 diabetes mellitus (T1DM) and maternal diets high in saturated fats impact the overall health of the fetus and may result in disease later in life. Previous research has shown that olive oil given to diabetic mothers increased the numbers of term births by reducing the inflammatory intrauterine environment. In contrast, a maternal diet high in saturated fatty acids can lead to fetal hyperglycemia and predisposes the offspring to health complications. Although it is known that variations in maternal diet have an impact on offspring development, it is unclear how this affects pancreatic development in the offspring. In this study, we seek to understand the role of these diets on neonatal pancreatic development and their impact on glucose metabolism in young adulthood. We established the maternal T1DM model by injecting rats with either streptozotocin (90 mg/kg) in a citrate buffer or citrate buffer alone and fed with a control chow diet or supplemented with 6% of olive oil. Dual immunohistochemistry was used to detect alpha and beta cells within the islets of the offspring. Morphometric analysis was carried out to study this problem. Our preliminary results showed that olive oil supplementation had a restorative effect on the islet area on the offspring at 4 months compared to the offspring of diabetic mother fed with the standard diet. Olive oil also altered the beta cell to alpha cell ratio in the offspring compared to the standard diet. These findings may provide important information that may be used as dietary recommendations for mothers, especially those with type 1 diabetes.

Poster Abstract #50

Expression of kallikrein-related peptidases (KLKs) in Adenoid Cystic Carcinomas

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Introduction: Human kallikrein proteins (KLKs) are a group of 15 serine proteases implicated in a wide variety of signalling and regulatory roles. KLK overexpression has been associated with the development of certain cancers, and epithelial-mesenchymal transitions leading to metastasis. Quantification of mRNA has identified specific KLKs associated with unfavourable clinical outcomes in a number of different cancers. The clinical application of KLK3, known as prostate specific antigen, as a biomarker highlights the potential clinical utility of KLKs in the diagnosis and monitoring of tumors. However, the role of KLKs in salivary tumors has not been extensively studied. The aim of this project is to determine if there is dysregulated gene and protein expression of KLKs in adenoid cystic carcinomas (ACC).

Methods: Formalin fixed paraffin embedded (FFPE) tissue specimens were obtained from the Oral Pathology archives of the London Health Sciences Centre. mRNA was then extracted from a total 40 FFPE samples, which included 25 adenoid cystic carcinomas and 10 from normal salivary tissue. Complementary DNA, obtained by reverse transcription, was then combined with gene specific kalikrein primers (KLK1-KLK15) to allow for quantitative real-time PCR.

Results: Normal salivary tissue and adenoid cystic carcinomas both expressed all 15 KLKs. However, ACC show a significant decrease in the expression of KLK 1, 8, 11, 14.

Conclusion: The genetic expression of KLKs in adenoid cystic carcinomas differs from that of normal salivary tissue. These results are consistent with the aberrant expression of kallikreins in other cancers. Specifically, downregulation of KLK 1, 11, 14 expression was also seen in the investigation of mucoepidermoid carcinomas in our laboratory. Current studies in our laboratory are aimed at understanding the role of these differentially expressed KLKs.

Keywords: kallikreins, adenoid cystic carcinoma, biomarker, salivary cancer, RT-PCR, gene expression, formalin fixation and paraffin embedding (FFPE)

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