PATHOLOGY AND LABORATORY MEDICINE
RESEARCH DAY 2016

APRIL 7, 2016

Program Guide

Schulich
MEDICINE & DENTISTRY

Western

Message From The Chair

Our Pathology and Laboratory Medicine Research Day is a day to celebrate our research and our people. It is a unique forum through which the key discoveries we have made in the last year are showcased to the entire community. It is wonderful to see all the great work that our students, residents, and faculty are doing. All of our trainees in various programs including undergraduate programs, thesis-based graduate studies, professional graduate program, dual Oral and Maxillofacial Surgery MSc program, and Pathology postgraduate programs are presenting their research work. As you listen to the platform presentations and view the posters, you realize our truly multi-disciplinary approach to studying health and disease.

This year, we are extremely fortunate to have Dr. Andrew Fire deliver the Paterson Lecture. Dr. Fire is Professor of Pathology and Genetics at Stanford University School of Medicine. His laboratory studies how organisms adapt to genetic changes and how genomes respond to changing conditions. Among many awards for his pioneering work, Dr. Fire shared the 2006 Nobel Prize in Physiology or Medicine with Craig Mello "for their discovery of RNA interference - gene silencing by double-stranded RNA".

The day would not be successful without the exceptional work of the organizing committee and many members of our department. I would like to personally thank Nancy Chan, Martin Duennwald, Manal Gabril, Zia Khan, Emily Goebel, Niamh Richmond, Jina Kum, Steffi Stephenson, Tracey Koning, Cheryl Campbell, Mellonie Carnahan, Kathilyn Allewell, and Mair Hughes. Lastly, I would like to thank the judges for interacting with our presenters, sharing their valuable experience, and offering insights. 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, Department of Pathology and Laboratory Medicine, Western University
Chief, Department of Pathology and Laboratory Medicine, London Health Sciences Centre and St. Joseph's Health Care

PATHOLOGY AND LABORATORY MEDICINE
RESEARCH DAY 2016

PROGRAM

9:05 - 9:15 am

Welcome and Opening Remarks
Dr. Subrata Chakrabarti
Chair/Chief, Pathology and Laboratory Medicine,
Schulich Medicine & Dentistry, Western University
and London Health Sciences Centre

9:15 - 10:15 am

Paterson Lecture
Dr. Andrew Fire
Professor, Pathology and Genetics
School of Medicine,
Stanford University

10:15 - 10:45 am

Nutritional Break

10:45 - 12:15 pm

Oral Presentations

12:25 - 12:45 pm

Group Photo
The Great Hall, Somerville House

12:45 - 1:45 pm

Lunch
The Great Hall, Somerville House

1:45 - 4:00 pm

Poster Presentations
The Great Hall, Somerville House

4:00 - 6:00 pm

Awards Ceremony
Windermere Manor

Paterson Lecture:

Dr. Andrew Fire

Professor, Pathology and Genetics

Stanford University School of Medicine

Biological Responses to Foreign Information

Dr. Fire is Professor of Pathology and Genetics at Stanford University School of Medicine. Dr. Andrew Fire is a brilliant scientist and received the Nobel Prize for Physiology or Medicine in 2006 together with Dr. Craig Mello for discovering a mechanism for controlling the flow of genetic information. Working with Dr. Mello, Dr. Fire helped discover RNA interference (RNAi), a mechanism in which genes are silenced by double-stranded RNA. The discovery has completely changed the landscape of biomedical research. In addition to the Nobel Prize, Dr. Fire has received several major awards including the Meyenburg Prize in 2002 from the German Cancer Research Centre, the Wiley Prize in 2003, and the National Academy of Sciences Award in Molecular Biology in 2003. Dr. Fire continues his pioneering work on how organisms respond to genetic changes. We are truly honoured that Dr Fire will deliver the Paterson Lecture.

Platform Presentations

Time	Last Name	First Name	Title
10:45 am	Cecchini	Matthew	Loss of the retinoblastoma tumor suppressor in lung adenocarcinoma is associated with an increase in neuroendocrine markers and improved survival
11:00 am	Krstic	Milica	The transcriptional regulator TBX3 promotes progression from non-invasive to invasive breast cancer
11:15 am	Lin	Lilian	Hsp104 modifies TDP-43 toxicity in ALS
11:30 am	Richmond	Niamh	Transforming growth factor-β induces pluripotency genes in hemangioma stem cells through repressing T-box 2
11:45 am	Ruicci	Kara	Investigating mechanisms of innate and evolved resistance to PI3K-pathway inhibition in head/neck squamous cell carcinoma
12:00 pm	Stecho	Will	The role of the Nrf2 antioxidant pathway in cancer

Platform Presentation #1

Loss of the retinoblastoma tumor suppressor in lung adenocarcinoma is associated with an increase in neuroendocrine markers and improved survival

Matthew J. Cecchini[1], Frederick A. Dick[2], Christopher J. Howlett[1]

[1]Department of Pathology and Laboratory Medicine, London Health Sciences Centre, Western University; [2]London Regional Cancer Program; Children's Health Research Institute, London Health Sciences Centre; Department of Biochemistry, Western University.

Background and Objectives: Lung cancer is the number one cause of cancer related deaths in Canada with a projected 20,900 deaths in 2015 alone. We have recently identified that loss of expression of the retinoblastoma tumor suppressor protein (pRB) results in a significantly improved survival for patients with lung adenocarcinoma treated with surgery and chemotherapy. The mechanism by which pRB influences outcome in lung adenocarcinoma is not well understood as expression of proliferative and apoptotic markers are unchanged. Experimental models have suggested that pRB can influence differentiation of tumor cells and pRB loss has been associated with transformation to a neuroendocrine small cell lung cancer phenotype that results in resistance to EGFR targeted therapy.

Methods: Archival surgical pathology slides from lung cancers treated with chemotherapy were obtained along with clinical outcome data. Immunohistochemistry for neuroendocrine markers (synaptophysin and chromogranin) was performed. Staining was quantified and compared with pRB status and clinical outcome.

Results: We identified that loss of pRB expression is associated with an increase in expression of synaptophysin and chromogranin. Cases with loss of pRB and expression of neuroendocrine markers were found to have a significantly improved survival.

Conclusions: Loss of expression of pRB is associated with an improved survival in lung adenocarcinoma. We find that this loss is associated with an increase expression of neuroendocrine markers. This supports a potential mechanism for the observed survival benefit and a means to identify patients that will best respond to aggressive treatment regimes.

Platform Presentation #2

The transcriptional regulator TBX3 promotes progression from non-invasive to invasive breast cancer

Milica Krstic[1,2], Carl O. Postenka[2,3], Hon S. Leong[1,4], Muriel Brackstone[4,5], Ann F. Chambers [1,2,3], Alan B. Tuck[1,2,3]

[1]Department of Pathology and Laboratory Medicine, Western University; [2]The Pamela Greenaway-Kohlmeier Translational Breast Cancer Research Unit, London Health Sciences Centre; [3]Department of Oncology, Western University; [4]Department of Surgery, Western University; [5]Division of Surgical Oncology, London Health Sciences Centre

Introduction: We have shown that breast cancer cells that have gained the ability to invade adjacent tissue express high levels of the regulatory protein TBX3. In cell lines derived from the same breast cancer patient at different phases of progression, TBX3 is abundant in the invasive 21MT-1 cells, and minimally expressed in the non-invasive, DCIS-like 21NT cells.

Methods: Both TBX3 isoforms were overexpressed in 21NT cells and knocked down in 21MT-1 cells. Functional changes and gene expression changes were assessed. Cells were injected into the chick embryo vasculature to examine extravasation and invadopodia formation. Cells also were implanted into nude mice. In vitro angiogenesis (i.e. tubule formation) was assessed. Patient samples of early breast cancer cases (186) from the London Breast Cancer Database were examined for TBX3 expression by immunohistochemistry.

Results: Overexpression of TBX3 isoforms in 21NT cells resulted in increased survival, growth and invasiveness in vitro, with increased extravasation and invadopodia formation in vivo in the chick embryo. Extravasation and invadopodia were drastically reduced with TBX3 knockdown in 21MT-1 cells.
Gene expression changes suggest that TBX3 promotes the transition to invasiveness through altered expression of regulatory and EMT-related genes, common to both isoform transfectants. Differences in gene expression were also noted between isoforms. Interestingly, TBX3iso1 overexpressing cells have increased tumorigenic potential in nude mice compared to empty vector control and TBX3iso2 overexpressing cells, possibly due to the promotion of angiogenesis by TBX3iso1.
Within human patient samples TBX3 was highly expressed in columnar cells. TBX3 expression was also associated with low-grade DCIS lesions and ER/PR expression, suggesting that TBX3 may be implicated in progression involving the low-grade DCIS molecular pathway.

Discussion: This work may have clinical potential in identifying patients with high-risk lesions, and/or as potential direct/indirect therapeutic targets to prevent disease progression.

Keywords: TBX3, breast cancer, metastasis, ductal carcinoma in situ (DCIS), invasive mammary carcinoma (IMC), epithelial-mesenchymal transition (EMT), estrogen receptor (ER), progesterone receptor (PR)

Platform Presentation #3

Hsp104 modifies TDP-43 toxicity in ALS

Lilian Lin[1], Martin Duennwald[1]

[1]Department of Pathology and Laboratory Medicine, Western University

Introduction: In 97% of sporadic amyotrophic lateral sclerosis (sALS), TAR DNA-binding protein (TDP-43) is found in the pathological neuronal inclusions of patient brains and spinal cords. These pathological inclusions are a result of the clearance of TDP-43 from the cell nucleus and the formation of aggregates in the cytoplasm. This is known as TDP-43 proteinopathy. Hsp104 is a molecular chaperone that acts as a dissaggregase and promotes the refolding of denatured proteins. My study attempts to understand the role of Hsp104 in TDP-43 proteinopathy by using a yeast model. Although Hsp104 is not conserved in humans, we study the mechanism and effects of the Hsp104 in order to provide insight into potential therapeutic solutions relating to protein aggregation and misfolding.

Material and Methods: Yellow fluorescence protein (YFP) tagged TDP-43 plasmids were transformed into wild type yeast cells and cells with Hsp104 deletion (Δhsp104). Toxicity assays were performed to observe how the absence of Hsp104 affects TDP-43 toxicity compared to wild type. Fluorescence microscopy was also done to observe the aggregation pattern of TDP-43 in Δhsp104 and wild type yeast cells.

Results: Results from the toxicity assays show that the deletion of Hsp104 rescues TDP-43 toxicity. The microscopy results show that the deletion of Hsp104 leads to a more diffuse profile of TDP-43 present in the yeast cell.

Discussion: Although previous studies have shown Hsp104 as having no effect on toxicity and aggregation of TDP-43, our results show that the deletion of Hsp104 has a notable effect on the toxicity as well as the localization of TDP-43 aggregates. We suspect that Hsp104 breaks apart TDP-43 toxicity, thereby creating abundant toxic oligomeric TDP-43 protein species. We also speculate that Hsp104 may inhibit other molecular chaperones that effectively reduce TDP-43 toxicity and aggregation.

Keywords: ALS, amyotrophic lateral sclerosis, TDP-43, Hsp104, yeast models, protein aggregation

Platform Presentation #4

Transforming growth factor-β induces pluripotency genes in hemangioma stem cells through repressing T-box 2

Niamh Richmond[1], Zia A. Khan[1,2,3]

[1]Department of Pathology and Laboratory Medicine, Western University;
[2]Diabetes and Metabolism Research Program, Lawson Health Research Institute;
[3]Division of Genetics & Development, Children's Health Research Institute

Introduction: Infantile hemangiomas (IH) are benign vascular neoplasms characterized by the differentiation of multipotential stem cells (hemSCs) into endothelial cells during the early proliferative phase, and later into adipocytes during spontaneous involution. Transforming growth factor-β (TGFβ) has been shown to be significantly elevated upon IH involution and this coincides with repression of a developmentally-regulated transcription factor T-box 2 (TBX2). These findings implicate both TGFβ and TBX2 in mediating hemSC differentiation during IH transition. I hypothesize that TGFβ represses TBX2 in hemSCs, thereby modulating their differentiation-competence.

Methods: HemSCs isolated from IH patient lesions were characterized for TBX2 expression through mRNA analysis. TBX2 protein localization in sectioned IH tissue and isolated cells was investigated via immunofluorescence staining. HemSCs were cultured in adipogenic, endothelial, neuronal, and hematopoietic (MethoCult) media following TBX2 knockdown via siRNA to examine the effect on cellular differentiation. Potential downstream targets were studied by conducting expression analysis via qPCR for cell cycle regulators, pluripotency factors, and T-box family members. To investigate potential upstream regulators of TBX2, hemSCs were cultured with the addition of exogenous TGFβ, and inhibitor. To confirm functional response of hemSCs to the treatments, up-regulation of collagen I and IV was assessed via qPCR analysis.

Results: TBX2 was found to be localized primarily in the nuclei of hemSCs; the level of expression varied between cultures of different patient-derived hemangiomas. HemSCs showed morphological changes upon differentiation towards mesenchymal, endothelial, and neurogenic lineages. TBX2 knockdown caused upregulation of hematopoietic lineage markers CD34, CD45, and ckit, as well as produced burst-forming unit colonies in MethoCult medium. Pluripotency factor and cell cycle regulator p16 expression was also upregulated upon TBX2 knockdown. The addition of TGFβ significantly repressed TBX2 mRNA expression, which also correlated with upregulated pluripotency factor expression.

Discussion: Our studies suggest that TBX2 modulates the differentiation-competence of hemSCs. TBX2 may in turn be regulated by TGFβ during IH involution. Understanding the signaling mechanisms regulated by TBX2 may provide a novel target pathway to promote premature involution of IH as a therapeutic option for patients.

Keywords: Infantile hemangioma, T-box 2, TGFβ, stem cells, differentiation, pluripotency

Platform Presentation #5

Investigating mechanisms of innate and evolved resistance to PI3K-pathway inhibition in head/neck squamous cell carcinoma

Kara M. Ruicci[1,2], Morgan Black[2], Ren Sun[3,4], Nicole Pinto[2,5], Laurie Ailles[6], Paul Boutros[3,4], John W. Barrett[2,7,8], Anthony C. Nichols[1,2,5,7,8,9]

[1]Department of Pathology & Laboratory Medicine, Western University; [2]Department of Otolaryngology – Head & Neck Surgery, Western University; [3]Department of Medical Biophysics, University of Toronto; [4]Informatics & Biocomputing Platform, Ontario Institute for Cancer Research, Toronto; [5]Department of Anatomy & Cell Biology, Western University; [6]Division of Stem Cell and Developmental Biology, Ontario Institute for Cancer Research, Toronto; [7]London Regional Cancer Program; [8]Department of Oncology, Western University; [9]Lawson Health Research Institute

Introduction: A significant limitation of targeted therapies is the inevitability of resistance. The PI3K-AKT network is the most frequently mutated axis in head/neck squamous cell cancer (HNSCC) and targeted agents demonstrate efficacy. Patient response to monotherapy is variable however, and resistance is almost inevitable. Presently, little is known about resistance to PI3K inhibitors. I hypothesize that persistent drug targeting of the PI3K-pathway in HNSCC promotes resistance to PI3Kα-inhibitor BYL719 and mTORC1-inhibitor RAD001, and that resistance occurs by PI3K-independent activation of mTOR.

Methods: HNSCC cell lines (n=28) were screened for BYL719 sensitivity and correlated with genetic profiles. To determine if HRAS mutations promote resistance, constructs expressing WT-HRAS or HRAS-G12V were transfected into BYL719-sensitive lines. Cell viability was examined following HRAS knockdown. PI3K pathway signalling was interrogated by immunoblotting. BYL719 and RAD001-resistant HNSCC lines were developed by treating and passaging subclones. Resistant lines were sequenced with a custom gene panel. Patient tumours were engrafted into NOD/SCID mice and randomized into control or treatment (BYL719, RAD001, combination) arms. Primary tumours, xenografts and patient blood were compared by STR-profiling.

Results: PIK3CA-altered lines were BYL719-sensitive, while HRAS-G12V mutants were innately resistance. Transfection of activating HRAS constructs resulted in increased BYL719 resistance. HRAS knockdown decreased proliferation in HRAS-mutant lines and immunoblotting revealed increased pAKT308/473. Cell lines with >5xIC50 resistance to BYL719 and RAD001 were phenotypically comparable to parentals. Genetic analyses highlighted alterations in key effectors (PIK3R1, PTEN) and chromosome 3-encoded genes (SOX2). After initially responding, BYL719 or RAD001-treated tumours resumed growth, while combination therapy remained effective. STRs confirmed genetic identities of all samples.

Conclusions: HRAS-G12V mutations are associated with intrinsic BYL719 resistance. Following prolonged treatment, both in vitro and in vivo, resistance to BYL719 and RAD001 develops. This study will elucidate resistance mechanisms to help guide the design of combination treatments to improve HNSCC patient outcomes.

Platform Presentation #6

The role of the Nrf2 antioxidant pathway in cancer

Will Stecho[1,3], Sechiv Jugnundan[1], Krupal Patel[2,3], Shannon Baker[1], Martin Duennwald[1], Brett Wehrli[1,3], Bekim Sadikovic[1,3], Anthony Nichols[2,3], Christopher Howlett[1,3].

[1]Department of Pathology and Laboratory Medicine, Western University; [2]Department of Otolaryngology, Western University; [3]London Health Sciences Centre

Introduction: Nuclear factor erythroid 2-related factor 2 (Nrf2) is the master transcription factor that coordinates the cellular response to oxidative stress. Aberrant Nrf2-dependent gene activation is vital for protecting cells against carcinogens. Yet, paradoxically, aberrant Nrf2 activation has been identified in non-small cell lung cancer where it promotes cell survival and resistance to chemo and radiation therapy. Furthermore, aberrant Nrf2 expression has been associated with activating mutations in Nrf2 and genes regulating Nrf2 expression and/or activity. Our objective is to evaluate the prevalence of Nrf2 activating mutations and aberrant Nrf2 expression in a variety of other cancer types.

Methods: The Cancer Genome Atlas (TCGA) dataset was analyzed using cBioPortal to identify tumor types with a high mutation frequency in Nrf2 and Nrf2 regulatory genes. Tissue microarrays were constructed from 282 cases of pretreatment head and neck squamous cell carcinoma (HNSCC) for evaluation by Nrf2 immunohistochemistry.

Results: Mutations in Nrf2 and associated regulatory genes were prevalent in a wide array of cancer types including: HNSCC (11% and 10% respectively), urothelial carcinoma (10% and 7%), endometrioid endometrial carcinoma (7% and 10%), and gastric adenocarcinoma (2% and 7%). Aberrant nuclear Nrf2 expression was also detected in a subset of HNSCC cases by immunohistochemistry.

Conclusions: Using the publically available TCGA dataset, we have detected Nrf2 mutations in numerous different cancer types. We further confirmed aberrant Nrf2 expression in HNSCC by immunohistochemistry. Future studies to detect the prevalence of aberrant Nrf2 expression in other tumor types will help to further elucidate its role in oncogenesis.

Keywords: Molecular, Genomics, Immunohistochemistry, Microarrays, Nrf2

Poster Abstracts

Session 1
1:45 - 2:45 pm

#	Last Name	First Name	Title
1	Zhang	Xusheng	Prevention of allograft rejection in heart transplantation through concurrent gene silencing of TLR and kinase signaling pathways
2	Yeung	Cynthia	Cul3 and βTrCP in the Nrf2/Keap1 pathway
3	Thomas	Anu A.	Long noncoding RNA ANRIL regulates VEGF mediated angiogenesis in diabetic complications
4	Tenn	Adam	Mitochondrial-targeted calpastatin reduces hypoxia-reoxygenation injury in cardiomyocytes in vitro
5	Tavolieri	Michael	Identification of a nuclear localization signal (NLS) within the PH domain of RGNEF
6	Su	Zijun	Up-regulation of junctophilin-2 prevents ER stress and apoptosis in hypoxia/reoxygenation-stimulated H9c2 cells
7	Stephenson	Steffi L.	Role of Wnt Pathway in regulating infantile hemangioma stem cell phenotype
8	Robinson	Brianne	Effects of olive oil as a nutritional intervention on β-cell development in offspring of diabetic rats
9	Osmond	Allison	Pediatric focal active colitis. A retrospective review.
10	Oakie	Amanda	Analysis of c-Kit and insulin receptor signaling interplay in regulating β-cell proliferation and function
11	Ngo	Vy	Nrf2 interactions with p21, prothymosin alpha, and Keap1 in cancer
12	Montwill	Natalie	Double-stranded RNA mediates microvascular endothelial cell death through toll-like receptor 3 following cardiac allograft transplantation

#	Last Name	First Name	Title
13	Martinsons	Kristina	Selective upregulation of kallikreins in mucoepidermoid carcinoma
14	Kum	Jina J.Y.	Role of DNA methylation in adipogenic differentiation potential of marrow progenitor cells
15	Kubica	Matthew	Comparative utility of distal and mid esophageal biopsies in the diagnosis of eosinophilic esophagitis: a retrospective analysis
16	Kleinsteuber	Derek	Pathologic resolution based on treatment of Grade II/III arteritis in post renal transplant biopsies
17	Kim	Yohan	Microparticle release during cancer extravasation: a novel anti-metastasis target
18	Kerr	Zachary	Expression of kallikrein-related peptidases (KLKs) in adenoid cystic carcinomas
19	Kanagalingam	Tharsan	The effect of glucocorticosteroids on CRTh2$^+$ Th2 cells
20	Jugnundan	Sechiv	Nrf2-Keap1 antioxidant pathway activation in urothelial carcinoma
21	Gordon	Andrew	Long non-coding RNA Malat-1 regulates inflammatory cytokines in chronic diabetic complications
22	Garcia-Marquez	David	Implementation of a peer to peer learning initiative in Anatomical Pathology residency training
23	Evetts	Audrey-Ann M.	Ontario growth standards for infants: A retrospective autopsy study
24	DiGregorio	Sonja	The role of RGNEF in cellular scaffolding in ALS

#	Last Name	First Name	Title
25	Dang	Brian	Bio-modulation of primary human tenon's capsule fibroblasts using a novel application of coated magnesium
26	Biswas	Saumik	Understanding MIC-1: A new diagnostic biomarker in prostate cancer
27	Almeida	Emily	Utilization of Immunohistochemistry and Special Stains at Autopsy
28	Aldhafeeri	Hamad	In vivo CRISPR screen for the identification of kinases that regulate prostate cancer metastasis

Poster Abstracts

Session 2
2:45 - 3:45 pm

#	Last Name	First Name	Title
29	Saldana	Yadira	Development of a single chain antibody for detection of Escherichia coli O157
30	Peart	Jason	Inducible beta-cell specific β1-integrin knockout affects islet architecture, beta-cell survival and function
31	Cecchini	Matthew	Molecular testing from cytology samples turning promise into practice
32	Roos	James	Etiology of motor vehicle collision fatalities in southern Ontario
33	Gan	Ingrid	Mitochondrial dependent necroptosis mediates microvascular endothelial cell survival following cardiac allograft transplantation
34	AlGhefari	Huda	Cortical ependymoma presenting with long-term refractory epilepsy: case report and review
35	Osmond	Allison	Choriocarcinoma arising within Crohn's related neoplastic lesions: report of a rare post-partum pitfall!
36	Tellios	Nikoleta	TGFβ regulates collagen deposition by modulating PTEN expression and activity in trabecular meshwork cells
37	Molko	Sharice	Effect of glucocorticoids on CRTh2 protein expression in Th2 cells in allergic asthma
38	Morrison	Matthew D.	Preliminary evidence suggests human tissue kallikreins are dysregulated in pleomorphic adenoma - a benign salivary gland tumor
39	Sullivan	Rebecca	Expression of the growth hormone secretagogue receptor 1a and ghrelin in human heart failure

#	Last Name	First Name	Title
40	Schenkel	Laila C.	Clinical utilization of genome-wide methylation testing in pediatric patients
41	Difazio	Kyle	Educational value and lessons learned for videos of grossing gynecological pathology specimens
42	Haddad	Faraj	The effect of selective inhibition of cholinergic Pedunculopontine nucleus neurons on attention as measured through the 5-Choice Serial Reaction Time Task in rats
43	Filek	Richie	Structural and functional changes to the retina and optic nerve following anti-VEGF treatments in diabetic retinopathy
44	Kum	Jina	Role of high glucose-induced matrix metalloproteinases in adipogenesis
45	Alharbi	Hajed	Immunological impact of CLI095 on ischemia reperfusion
46	Wallis	Julie	Quality management for the autopsy service – London Health Sciences Centre
47	Lac	Vivian	Presence of glypican-1 on extracellular vesicles fails to discern pancreatic cancer from benign pancreatic disease
48	Shin	Alice E.	Role of Inflammation on Dclk1+ Tuft Cell's Initiation of Colon Cancer
49	McLeod	Chelsea M.	Development of an Educational Module for Grossing Colorectal Specimens
50	Gaed	Mena	MRI signaling to non-cancerous abnormalities that could mimic cancer in prostate

#	Last Name	First Name	Title
51	Topiwala	Ishita	The regulation of miRNA-711 by HIF-1 and DNA methylation in cardiomyocytes during ischemia-reperfusion injury
52	McLean	Lachlan	Evaluating the utility of protein biomarker, S100A7, as a predictor for the transformation of oral dysplasia
53	Kahramanoglu	Zeynep G.	Mechanisms underlying chemotherapy-induced vascular proliferation in ovarian cancer
54	Good	Hayley	Role of inflammation in transformation of Dclk1+ colon cancer initiating cells
55	Goebel	Emily A.	Mitotically active sclerosing stromal tumour of the ovary: Report of a case series
56	Tan	Alice	Expression levels of growth hormone secretagogue receptor 1a and ghrelin in early diabetes

Poster Abstract #1

Prevention of allograft rejection in heart transplantation through concurrent gene silencing of TLR and kinase signaling pathways

Xusheng Zhang[1, 2], Hongmei Wang[1], Zhu Lan[2], Jifu Jiang[2], Xiufen Zheng[1], Terry Zwiep[1], Zhu-Xu Zhang[1, 2], Qing Li[1], Douglas Quan[1,2], Wei-Ping Min[1, 2]

[1]*Departments of Surgery, Pathology and Laboratory Medicine, and Oncology, Western University;* [2]*Multi-Organ Transplant Program, London Health Sciences Centre, Department of Pathology and Laboratory Medicine & Surgery, Western University*

Background: Toll-like receptors (TLRs) act as initiators and conductors responsible for both innate and adaptive immune responses in transplantation. The mammalian target of rapamycin (mTOR) is one of the most critical signaling kinases that affects broad aspects of cellular functions, including metabolism, growth, and survival. Concurrent gene silencing of multiple TLR adaptors, MyD88/TRIF and downstream kinases mTOR may enhance DC-mediated tolerance induction, thereby preventing graft rejection in heart transplantation.

Methods: Recipients (BALB/c) were treated with MyD88 , TRIF and mTOR siRNA vectors, 3 and 7 days prior to heart transplantation and 7, 14 and 21 days after transplantation. After siRNA treatment, recipients received a fully MHC-mismatched C57BL/6 heart. Control groups that included untreated mice, scrambled siRNA, mTOR siRNA monotherapy groups.

Results: Treatment with mTOR siRNA significantly prolonged allograft survival (32.5 ± 3.2 days) in heart transplantation. Moreover, the combination of mTOR siRNA along with MyD88 and TRIF siRNA further extended the allograft survival (89.5 ± 5.6 days); Flow cytometry analysis showed an up-regulation of FoxP3 expression in spleen lymphocytes and a concurrent down-regulation of CD40, CD80 and PD-L1 expression in splenic dendritic cells in MyD88, TRIF and mTOR treated mice. MLR, using splenic lymphocytes isolated from tolerant recipients, showed a significantly lower T cell proliferation capacity to the donor original antigen. There is significantly upregulated T cell exhaustion in T cells isolated from tolerant recipients.

Discussion: This study is the first demonstration of preventing immune rejection of allogeneic heart grafts through concurrent gene silencing of TLR and kinase signaling pathways, highlighting the therapeutic potential of siRNA in clinical transplantation.

Keywords: TLR adaptors, mTOR, heart transplantation, RNAi, T cell exhaustion

Poster Abstract #2

Cul3 and βTrCP in the Nrf2/Keap1 pathway

Cynthia Yeung[1], Martin Duennwald[1]

[1]Department of Pathology and Laboratory Medicine, Western University

Introduction: The oxidative stress response plays a key role in the pathogenesis of cancer and other human diseases. Nrf2, a transcription factor, and Keap1, a key Nrf2 regulating protein within the oxidative stress response pathway, have been relatively well characterized. However, the roles of other molecules in the Keap1/Nrf2 pathway, such as Cul3 and βTrCP, and their mutations, remain mostly unexplored. Cul3 associates with Keap1 and helps target Nrf2 for degradation. βTrCP is the substrate recognition subunit of a ubiquitin ligase complex that promotes the destruction of specific substrates. The goal of the present research project was to study the mechanisms underlying the interactions between, and dysregulation of, Cul3 and βTrCP in the Keap1-Nrf2 oxidative stress pathway to identify potential therapeutic targets for cancer and neurodegenerative diseases.

Methods: Yeast models expressing Cul3 and βTrCP were established. Toxicity (growth) assays and split-ubiquitin interaction assays were employed to study the molecular, genetic, and cellular aspects of the Nrf2 interactions with Cul3 and βTrCP in yeast.

Expected Results: The expected findings of my study are (a) elucidation of the mechanisms by which interactions with Cul3, βTrCP, Nrf2, and Keap1 occur, and (b) identification of small molecules and other proteins that interact with Cul3 and βTrCP and modulate their interactions with Nrf2 and Keap1.

Significance: Knowledge of the roles of Cul3 and βTrCP not only contribute to the basic understanding of the Nrf2/Keap1 pathway, but also give insight into potential therapeutic targets for many diseases, including cancer.

Keywords: Cancer, neurodegenerative disease, oxidative stress, Keap1, Nrf2, Cul3, βTrCP, yeast model, ubiquitin proteasome system, protein misfolding

Poster Abstract #3

Long noncoding RNA ANRIL regulates VEGF mediated angiogenesis in diabetic complications

Anu A. Thomas[1], Biao Feng[1], Subrata Chakrabarti[1]

[1]*Department of Pathology and Laboratory Medicine, Western University*

Introduction: Noncoding RNAs (ncRNAs) such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are emerging as key players in various diseases. The lncRNA, ANRIL is associated with coronary artery disease. ANRIL is known to recruit polycomb repression complex 2 (PRC2) and regulates histone acetylator, p300 expression. We have demonstrated that both p300 and PRC2 components are increased in diabetic complications such as diabetic retinopathy through miR200b and that miR200b negatively regulates VEGF. In this study, we investigated potential regulatory role of ANRIL in VEGF-mediated angiogenesis in these complciations and mechanism of such regulation through miR200b and p300.

Materials and Methods: We examined human retinal endothelial cells (HRECs) following incubation in normal (5mM/L,NG) and high glucose (25mM/L,HG) for various durations. Cells in the aforesaid conditions were further transfected with siRNA for ANRIL or p300. Further experiments were conducted following miR200b mimic and antagomir transfection. We examined expression of various transcripts and performed tube formation assays for angiogenesis. In vivo study included assessment of retinal tissues from ANRIL knockout (KO) and wild-type mice with or without streptozotocin-induced diabetes.

Results: ANRIL expression was elevated after 48h exposure to HG, accompanied by upregulation of VEGF, p300 and reduced levels of miR200b. Glucose induced VEGF upregulation as well as increased tube formations were prevented following ANRIL knockdown. Retinal VEGF expressions were augmented in the wild-type diabetic animals compared to controls in association with increased p300 and reduced miR200b expression. Such changes were prevented in the ANRIL KO mice with diabetes.

Conclusion: Data from this study showed that glucose-induced upregulation of ANRIL is causally related to increased production of angiogenesis-related factors in the HRECs and possibly in the retinal tissues affected in DR. Identification of such mechanisms may have potential implications in the development of RNA based therapies in DR.

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

Poster Abstract #4

Mitochondrial-targeted calpastatin reduces hypoxia-reoxygenation injury in cardiomyocytes in vitro

Adam Tenn[1], Dong Zheng[1,2], Tianqing Peng[1,2]

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Introduction: Treatments for myocardial infarction also lead to further damage to the heart as a result of ischemia-reperfusion (I/R) injury. I/R injury has been shown to increase calpains-1 and -2 in the mitochondria, which is associated with mitochondrial dysfunction and cell injury. Furthermore, we found that mitochondrial targeted calpastatin (mt-CAST), a calpain inhibitor, overexpression attenuated the cardiac inflammatory response and myocardial dysfunction in a mouse model of sepsis. In the present study, we investigated the effect of mt-CAST overexpression on hypoxia-reoxygenation (H/R) injury in vitro. We hypothesize that inhibition of mitochondrial calpain activity will decrease cardiomyocyte apoptosis, ROS levels, and preserve ATP synthase activity.

Methods: Prior to the experiment, cells were transfected with an adenoviral vector containing either mt-CAST or hemagluttanin (HA). To mimic ischemia-reperfusion injury, cultured H9C2 rat cardiomyocyte cells were seated and placed into hypoxic conditions for 24 hours. The cells were then reoxygenated and incubated for another 24 hours. Caspase-3 and DNA fragmentation assays were performed to measure cell death. Mitochondria were isolated to measure the levels of ROS and several proteins including ATP5A1, calpains-1 and -2 and CAST.

Results: H/R lead to increased caspase-3 and DNA fragmentation in cardiomyocytes. Elevated mitochondrial calpains were also observed H/R conditions. In cells that were transfected with mt-CAST, CAST in the mitochondria was elevated. These cells displayed reduced caspase-3 activity and DNA fragmentation post H/R compared to Ad-HA controls.

Conclusions: Our study demonstrates the cytoprotective effect of mt-CAST in H/R injury. Adenoviral transfection of mt-CAST resulted in a decrease in cell death following H/R. In the future, mitochondrial ROS and ATP5A1 levels will be measured.

Keywords: hypoxia-reoxygenation, ischemia-reperfusion, cardiomyocytes, reactive oxygen species, calpastatin, calpain

Poster Abstract #5

Identification of a nuclear localization signal (NLS) within the PH domain of RGNEF

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Introduction: Rho Guanine Nucleotide Exchange factor (RGNEF), encoded by the gene ARHGEF28, is a 190kDA protein. RGNEF destabilizes NEFL, which encodes for a protein long implicated in ALS pathology. As well, histological study of the anterior horn of ALS tissue has shown RGNEF within pathological neuronal cytoplasmic inclusions. Our lab has previous reported an ALS-specific mutation in ARHGEF28. Recently we were surprised to identify a nuclear localization signal (NLS) within the Pleckstrin Homology (PH) domain, a domain known for membrane localization. RGNEF has previously been shown in the nucleus, though until now no NLS has been described.

Materials and Methods: A PH Domain (1083-1201) deleted construct (RGNEF-ΔPH), was created. RGNEF-ΔPH or a full length construct (RGNEF) were transfected in HEK293T cells using Lipofectamine 2000 (Invitrogen). Levels of nuclear localization were determined by two methods: cells were stained by immunocytochemistry and imaged by confocal microscopy; or cells were fractionated and levels of protein were determined by Western blot. In addition, cNLS Mapper, an online software, was used to identify a putative NLS and I-Tasser was used to determine 3-D configuration of the PH, including the location of the NLS.

Results: RGNEF-ΔPH shows reduced levels of localization to the nucleus compared to our full length construct. Cells transfected with RGNEF showed localization to the nucleus, while RGNEF-ΔPH showed significantly less expression in the nucleus when examined by confocal microscopy and fractionation experiments. Analysis by cNLS Mapper revealed a bipartite NLS within the PH domain. Modeling of the domain indicates that the basic residues of the putative NLS are located on the exterior of the domain.

Discussion and Conclusions: This is the first study to identify a putative NLS located in ALS. Modeling software confirms that this region is accessible for interaction with the Importin αβ Pathway. Further work is being done in our lab to mutate those basic residues of the putative NLS in order to confirm its function in nuclear transport.

Keywords: Amyotrophic Lateral Sclerosis (ALS); Nuclear Localization Signal (NLS); Subcellular Localization; Pleckstrin Homology (PH) Domain; Rho Guanine Nucleotide Exchange Factor (RGNEF)

Poster Abstract #6

Up-regulation of junctophilin-2 prevents ER stress and apoptosis in hypoxia/reoxygenation-stimulated H9c2 cells

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Introduction: Ischemic heart disease is the leading cause of death and reperfusion which can restore blood flow is the primary therapy strategy. Reperfusion initiates further damage to cardiomyocytes. So the ischemia-reperfusion (I/R) injury together is now recognized as the important combination determining the final myocardial infarction size and degree. It is believed that intracellular Ca2+ mishandling during ischemia-reperfusion plays a key role in cell death. Junctophilin-2 (JPH2) is a junctional membrane-binding structural protein. It mechanically maintains the fixed distance between T-tubule and sarcoplasmic reticulum(SR), allowing the proper Ca2+ induced Ca2+ release for stable excitation-contraction coupling. JPH2 decreases in diseased hearts. We hypothesize that over-expression of Junctophilin-2 can protect the cardiomyocytes from ischemia-reperfusion-induced apoptosis.

Methods: To mimic the ischemia-reperfusion, we subjected H9c2 cells to hypoxia and reoxygenation (H/R) by using the GENbag anaer. Adenoviral vector containing JPH2 was used to overexpress JPH2. Apoptosis was determined by measuring caspase-3 activity and DNA fragmentation. JPH2 protein levels, ER stress, and calpain activity were determined by western blot.

Results: In H/R stimulated H9c2 cells, the JPH2 protein level declined while apoptosis and ER stress were induced. Infection with Ad-JPH2 up-regulated JPH2 protein levels and prevented apoptosis and ER stress in H/R-stimulated H9c2 cells. JPH2 overexpression also inhibited calpain activity in H/R stimulated H9c2 cells. Mechanistically, H/R stimulation increased Ca2+ leak from SR/ER and this increased leakage of Ca2+ might result from ryanodine receptor-2 (RyR2) disability. Up-regulation of JPH2 inhibited Ca2+ leak from SR/ER via RyR2.

Conclusions and Future Directions: We have provided evidence that over-expression of JPH2 protected the H9c2 cells from ER stress and apoptosis after H/R stimulation. Our study suggests that JPH2 may be a therapeutic target for treating ischemia-reperfusion injury.

Keywords: *apoptosis, Ca2+ leak, ER stress, H/R injury junctophilin-2.*

Poster Abstract #7

Role of Wnt Pathway in regulating infantile hemangioma stem cell phenotype

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Introduction: Infantile hemangiomas are the most common vascular tumours of infancy, occurring predominantly in the head and neck region. Although benign, some may impinge on vital organs and cause life-threatening complications. Hemangiomas exhibit a unique lifecycle characterized early by proliferation of vascular endothelial cells followed by maturation of vessels and eventual regression into adipose tissue. In previous studies, we have shown that hemangiomas are derived from stem cells. Although the mechanisms regulating hemangioma stem cell phenotype are not known, a potential candidate may be the Wnt pathway which is evolutionarily conserved and plays a key role during development. As stem cell derivatives, hemangiomas phenotypically possess stem cell-specific transcription factors SOX2, OCT4 and Nanog which are downregulated upon differentiation. In this study, we aimed to determine the role of Wnt/β-catenin on the expression of stem cell-specific transcription factors.

Methods: To achieve our goal, we modulated the Wnt/β-catenin pathway in primary human hemangioma-derived stem cells. We then used sensitive and specific quantitative PCR to determine the expression of stem cell factors.

Results: Our results show that activation of the Wnt/β-catenin pathway increases the expression of all three stem cell specific transcription factors.

Discussion: These findings show that β-catenin transcription activity is required for this function as degrading β-catenin or inhibiting its binding to DNA prevented agonist-induced expression of stem cell factors. We are building on these exciting findings with the hope of developing treatments for accelerated differentiation of hemangioma stem cells.

Keywords: infantile hemangioma, stem cells, endothelial cells, β-catenin, Wnt pathway

Poster Abstract #8

Effects of olive oil as a nutritional intervention on β-cell development in offspring of diabetic rats

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Introduction: Maternal Type 1 Diabetes (T1DM) results in the generation of a pro-inflammatory environment in utero impairing fetal development and increasing the offspring's predisposition to Type 2 Diabetes (T2DM) in adulthood. Previously, our laboratory has demonstrated that the addition of olive oil enriched in oleic acid during gestation can improve the intrauterine environment by activating the peroxisome proliferator-activated receptors (PPARs). The present study is aimed at deciphering the effect of this treatment on the expression of PPAR targets and their role in β-cell differentiation and glucose metabolism. We hypothesize that the activation of the PPARs through olive oil intervention leads to enhanced β-cell development.

Methods: To test our hypothesis, pancreata of day 2 and 4-month old offspring of diabetic rats fed diets supplemented with olive oil during pregnancy were obtained. RNA extractions and subsequent qPCR analysis was performed to measure expression levels of the PPAR targets. We used Pdx-1, Glut2 and insulin expression as readouts of β-cell development. Once rat tissue data is fully analyzed, we will investigate the mechanism of oleic acid effects using rat insulinoma cells (InsE-1).

Results: Presently, we have established a STZ-induced maternal T1DM model and have modified the RNA extraction protocol for pancreas tissue to obtain 20 extractions for both time points (n=5 for each group: control + standard diet, control + olive oil, diabetic + standard diet, diabetic + olive oil). cDNA preparation has been optimized and 4 primer sets obtained for qPCR analysis.

Conclusions: Tissues from our in vivo studies have been harvested and processed. We expect that treatment with olive oil and oleic acid will increase the expression of PPAR targets. These findings may provide the opportunity to explore the development of cost-effective therapeutic strategies for the management of maternal T1DM in pregnancy and the prevention of metabolic disease in their offspring.

Keywords: Pregnancy, maternal diabetes, β-cells, olive oil, oleic acid, PPARs, Pdx-1, Glut2, Insulin, maternal diet

Poster Abstract #9

Pediatric focal active colitis. A retrospective review.

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Background: Focal active colitis (FAC) is a histopathologic diagnosis of uncertain clinical significance in individual patients. In adult cases, infection accounts for approx. 50%, Crohn's disease (CD) for 0-13%, and 20-30% are idiopathic (likely prep related). One previous study of 29 cases of pediatric FAC (no sodium phosphate bowel prep) showed 28% CD. Histologic features to distinguish between idiopathic FAC and inflammatory bowel disease (IBD) related FAC have not been determined. This study reviewed a larger cohort of pediatric patients to determine what proportion had IBD, and whether there was an amount or pattern of inflammation that predicted IBD.

Methods: One hundred patients aged ≤18 years with FAC were identified (2002-2015). Patients with a prior diagnosis of IBD or chronic colitis in the index biopsies were excluded. Original slides were assessed for a number of inflammatory criteria. Clinical data including presenting symptoms, medication history and final diagnoses were recorded. Data were analysed using Pearson correlations and Fisher's exact chi-square analyses.

Results: Sixty-eight biopsy sets from 68 patients were reviewed. Sixteen patients (24%) had a final diagnosis of IBD. When cases with terminal ileal inflammation and / or granulomas were excluded 6 of 54 remaining patients had a final diagnosis of IBD (11%). One patient with focal surface active inflammation only (non-aphthous) had a final diagnosis of CD. Of 17 patients with either focal surface active inflammation only or a single inflamed crypt, 3 (18%) had CD.
A final diagnosis of IBD was significantly associated with the presence of crypt abscesses in patients with and without terminal ileal inflammation (Fisher's exact=5.67, p=0.027 and Fisher's exact=7.99, p=0.025) and the presence of one or more elevated serum inflammatory markers (Fisher's exact=11.44, p=0.001 and Fisher's exact=15.02, p=0.001). It was significantly associated with TI inflammation (Fisher's exact=20.27, p<0.001). There were no significant relationships between IBD and amount of colonic inflammation, presence of aphthous lesions, upper GI inflammation, family history of IBD, presenting symptom or use of medication (all ps>0.05).

Discussion: In keeping with the previous pediatric study, this study demonstrated a 24% rate of IBD in pediatric patients with FAC; however, when patients with associated terminal ileal inflammation and / or granulomas were excluded, the rate was 11%, similar to the reported rates in adults. When TI inflammation is present in association with FAC there is a high probability of IBD (10/14 cases, 71%). In all patients, the presence of crypt abscesses or increased serum inflammatory markers is associated with a higher likelihood of IBD. In pediatric patients whose biopsies show FAC without terminal ileal inflammation, the clinical implications appear to be the same as those in the adult population and do not warrant more aggressive follow up.

Poster Abstract #10

Analysis of c-Kit and insulin receptor signaling interplay in regulating β-cell proliferation and function

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Introduction: The receptor tyrosine kinase c-Kit maintains normal glucose levels and regulates intracellular pathways responsible for β-cell function, proliferation, and survival in mice. In addition, c-Kit activity can also increase the gene and protein levels of insulin receptor (IR) in mouse islets. We hypothesized that the interplay between the two receptors on β-cells enhances insulin secretion and β-cell mass, yet consistent over-expression of c-Kit on β-cells and subsequent IR over-activation may induce insulin resistance under long-term stress.

Methods: To determine the interplay between the two receptors, we examined: 1) aged β-cell specific c-Kit overexpression transgenic [c-KitβTg] mice; and 2) a mouse model formed by crossbreeding the c-KitβTg line with inducible β-cell specific insulin receptor knockout mice [c-KitβTg;MIP-CreER;IRKO].

Results: Aged (~60 weeks of age) male c-KitβTg mice show increased glucose intolerance and impaired insulin secretion compared to age-matched WT mice, indicating defective glucose metabolism after 40 weeks of age. c-KitβTg islets demonstrated high β-cell mass and proliferation, and correlated with increased MAPK/ERK signaling. However, reduced syntaxin 1A was detected in aged c-KitβTg islets, suggesting possible dysfunctions in insulin granule exocytosis. Initial examination of the c-KitβTg;MIP-CreER;IRKO mouse model (16 weeks post-tamoxifen-induced IRKO) revealed higher glucose levels 15 minutes after glucose challenge compared to c-KitβTg littermates. Morphological analyses demonstrated lower β-cell mass and proliferation rate in c-KitβTg;MIP-CreER;IRKO mice compared to c-KitβTg littermates, suggesting that c-Kit -enhanced β-cell proliferation may partially depend on IR action.

Conclusions: Future experiments will focus on in vitro cell culture (INS-1 cell line and c-KitβTg isolated islets with or without c-Kit and/or IR knockdown) to verify the activity of receptor cross-talk, and continue in vivo studies using the established c-KitβTg;MIP-CreER;IRKO mouse model to identify the cellular mechanisms by which c-Kit interplays with IR to regulate the function of β-cells.

Keywords: Diabetes mellitus, β-cell function, INS-1 cell line, tamoxifen – induced temporal knockout, Insulin receptor, c-Kit transgenic overexpression

Poster Abstract #11

Nrf2 interactions with p21, prothymosin alpha, and Keap1 in cancer

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Introduction: Reactive oxygen species (ROS) cause DNA damage and oxidative stress, which can initiate and promote carcinogenesis. NF-E2-related factor 2 (Nrf2) is a transcriptional master regulator of the antioxidant response required for maintaining cellular redox homeostasis. Similar to its protection of normal cells from ROS, constitutively expressed Nrf2 protects cancer cells from chemotherapeutics to facilitate tumour development and chemoresistance (Padmanabhan et al., 2006). The present study uses a yeast model to study the expression of Nfr2 and its protein modulators, p21, prothymosin alpha (PTα), and Keap1, to elucidate their mechanisms of interaction and further clarify their role in oxidative stress and cancer.

Methods: Spotting assays for growth assess the toxicity of Nrf2, p21, PTα, and Keap1 in yeast. The split-ubiquitin system will determine how p21, PTα, and Keap1 compete for the interaction with Nrf2. Cancer-associated mutations and truncations are introduced and their effects on protein interactions are examined. Our findings will be confirmed in mammalian cells by Nfr2 transcription reporter assays.

Results: The expression of wild-type Nrf2 is toxic in yeast. p21 is minimally toxic, while PTα is mildly toxic. Compared to an Nrf2 empty vector control, co-expression of Nrf2 with p21 antagonizes Nrf2 toxicity to allow improved growth. In contrast, co-expression of Nrf2 with PTα exacerbates toxicity compared to Nrf2 alone.

Discussion: Preliminary results suggest that the interaction between p21 and PTα with Nrf2 alters its level of toxicity in the cell. By attempting to understand the cellular and molecular mechanisms of Nrf2 and how its toxicity can be rescued through protein-protein interactions, we can provide a basis for the development of therapeutic drugs targeting Nrf2 overexpression and toxicity in cancer and other diseases related to oxidative damage.

Keywords: Nrf2, p21, PTα, Keap1, ROS, oxidative stress, cancer, yeast model

Poster Abstract #12

Double-stranded RNA mediates microvascular endothelial cell death through toll-like receptor 3 following cardiac allograft transplantation

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Introduction: The field of programmed cell death (PCD) has recently been expanded to include novel forms of regulated necrosis (RN) and apoptotic cell death, which have been implicated in various disease models. One modality of cell death, mediated via toll-like receptor 3 (TLR3), has recently been implicated in renal ischemia reperfusion injury (IRI) but has yet to be studied in a cardiac transplantation model.

Hypothesis: I hypothesize that TLR3 activation is a key mediator of cell death following cardiac allograft transplantation and is detrimental to graft survival. Inhibition of this pro-inflammatory receptor, using a double-stranded RNA (dsRNA)/TLR3 blocker, will result in less graft infiltrate by recipient immune cells and prolong allograft survival.

Materials and Methods: Murine microvascular endothelial cells (MVEC) were treated with polyinosinic:polycytidylic acid (PIC), a synthetic analog of double-stranded RNA, to induce TLR3-mediated cell death. Necrotic cell death was then measured using SYTOX Green nucleic acid staining. Various reactive oxygen species (ROS) inhibitors, N-acetyl-cysteine (NAC) and butylated hydroxyanisole (BHA), were added to determine the link between ROS production and necrotic cell death by TLR3 activation. MVEC will undergo hypoxic conditions and TLR3 expression as well as RNA levels will be measured. Finally, an in vivo mouse model of cardiac allograft transplantation will be used and we will examine cell death following ischemia reperfusion and dsRNA/TLR3 blocker perfusion.

Results: Following treatment with PIC, apoptotic cell death is higher in MVEC compared with untreated MVEC in non-proliferative conditions. Early data suggests that this cell death is independent of ROS production and the necroptotic pathway (a form of PCD).

Discussion and Conclusions: Our data suggests that cardiac cells exhibit death kinetics independent of ROS production but related to caspase activation. Developing our understanding of this pathway will be beneficial in determining appropriate treatments that will prolong graft and therefore patient survival.

Keywords: Toll-like receptor 3, Double-stranded RNA, Apoptosis, Ischemia reperfusion injury, Transplant model

Poster Abstract #13

Selective upregulation of kallikreins in mucoepidermoid carcinoma

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Introduction: As a rare salivary gland disease with complex pathophysiology, mucoepidermoid carcinoma (MEC) continues to challenge clinicians with respect to diagnosis and disease course prediction. Although prognosis of this salivary gland malignancy is generally favourable, high-grade tumours show a significantly reduced five-year survival rate. Misdiagnosis can delay imperative treatment. Recent studies have found altered expression of human tissue kallikreins (KLKs), a serine protease family, in various cancers. This suggests that they could be useful biomarkers for MEC. Our preliminary studies on KLK mRNA expression indicate selective modulation of various KLKs in MECs. The objective of my current study is to characterize KLK protein expression in MEC specimens compared to normal salivary gland tissue, and to determine KLK localization. I hypothesize that protein expression and mRNA levels will be highly correlated.

Methods: To test my hypothesis, I performed single label fluorescence immunohistochemistry. I then assessed fluorescence intensity as a proxy for protein levels using ImageJ.

Results: My results show that kallikrein protein expression and mRNA levels are not highly correlated. Additionally, no significant differences in fluorescence intensity are found between normal salivary gland tissue and MEC specimens.

Discussion: My results suggest that KLKs may not be involved in MEC tumour progression and may not be suitable biomarkers for MEC. However, future studies should analyze protein activity levels and circulating serum protein levels as it is possible that KLKs are being released while tissue KLK levels remain constant.

Keywords: Mucoepidermoid carcinoma, kallikreins, immunohistochemistry, salivary gland tumours, prognostic markers

Poster Abstract #14

Role of DNA methylation in adipogenic differentiation potential of marrow progenitor cells

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Introduction: Diabetes mellitus is a highly prevalent disease characterized by hyperglycemia, resulting in micro- and macro-vascular organ dysfunctions. Our laboratory has shown that the vascular dysfunction and inadequate vascular repair in diabetes entails both angiogenic and vasculogenic impairments. Specifically, we have shown that diabetes causes depletion of regenerative stem cells in the bone marrow. This depletion is associated with altered cellular composition of the marrow with enhanced adipogenesis and impaired osteoblastogenesis. I hypothesize that hyperglycemia skews lineage commitment in the bone marrow cells towards adipogenesis, which alters the stem cell niche and causes depletion of regenerative vascular stem cells.

Methods: Bone-marrow derived precursor cells (bm-MPCs) were cultured in the presence of adipogenic media with or without high levels of glucose mimicking diabetes and various epigenetic modifiers. I then investigated the mechanisms involved in glucose-induced programming of stem/progenitor cells that skews cell lineage commitment.

Results: My results to date indicate that adipogenic differentiation in the presence of high levels of glucose significantly induces DNA methyltransferase (DNMT) 1 and 3A, but not 3B. Interestingly, addition of a DNMT inhibitor, 5-Aza-2'-Deoxycytidine (Aza), normalized DNMT3A mRNA levels and prevented adipogenic differentiation in a dose-dependent manner. In contrast, inhibitor of histone deacetylase had no significant effect on the induction of key adipogenic transcription factors.

Conclusion: My findings suggest that modifications to DNA methylation affect the differentiation of bone-marrow derived precursor cells to adipocytes, whereas histone acetylation does not show significant changes. I will build on these studies to understand the mechanisms involved in the dysfunction of reparative stem cells in diabetes. The study may provide an effective avenue to reverse stem cell dysfunction and restore endogenous repair of the vasculature.

Keywords: Progenitor cells, adipogenesis, DNA methylation, epigenetics, hyperglycemia

Poster Abstract #15

Comparative utility of distal and mid esophageal biopsies in the diagnosis of eosinophilic esophagitis: a retrospective analysis

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Objective: Eosinophilic esophagitis (EE) is an increasingly common diagnostic entity. Characterized clinically by food impaction, dysphagia, and reflux-like symptoms, its differentiation from reflux esophagitis depends largely on the basis of endoscopic appearance and intraepithelial eosinophil count. Most biopsy guidelines suggest that biopsies be taken from the mid and distal esophagus in suspected EE cases. While there are accepted histological criteria for the diagnosis of EE, there is little data on whether this diagnosis can reliably be made on the basis of the eosinophil count from a single biopsy site or whether distal biopsies alone are useful. This study aims to evaluate the comparative utility of mid and distal esophageal biopsies in the diagnosis of EE.

Methods: Specimens were retrieved from a single institution spanning a three-year period. The specimen database was queried for all cases that included esophageal biopsies. Those cases with a mid and distal biopsy specimen taken from adult patients were flagged for possible inclusion. Clinical diagnoses were used to identify cases of EE, and pathology reports were screened for mention of increased intraepithelial eosinophils. Histological sections from cases of EE were manually reevaluated for the presence of eosinophils, and the maximum eosinophil count per high power field was recorded.

Results: Biopsies from 126 cases of EE were evaluated. Maximum eosinophil counts in the distal esophagus exceeded those in the mid esophagus in a majority of cases. The mean number of eosinophils in the distal and mid esophageal biopsies was 54 and 47 per high power field, respectively.

Discussion: In cases of EE, average eosinophil counts are higher in the distal esophagus. Our data suggest that a biopsy from the distal esophagus alone may provide sufficient information for the diagnosis of EE.

Keywords: eosinophilic esophagitis, distal, mid, biopsy

Poster Abstract #16

Pathologic resolution based on treatment of Grade II/III arteritis in post renal transplant biopsies

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Introduction: Grade II/III Acute vascular rejection (AVR) according to the Banff Criteria is classified by the presence of intimal arteritis (v) in kidney transplant biopsies. Intimal arteritis is determined histologically and consists of v1, v2, v3. The significance of isolated intimal arteritis (v1) in comparison to intimal arteritis with a background tubulointerstitial inflammation (t-i-v), is not well understood. We looked at whether treatment would resolve isolated v1 compared to (t2-3, i2-3, v1), v2 and v3 in patients who had subsequent for-cause transplant initial biopsies.

Methods: We retrospectively reviewed for-cause renal allograft biopsies at our center (UH) between 2005 and 2013. Those who had a biopsy positive vasculitis and a subsequent biopsy post-treatment were analyzed. Biopsies were classified as those with isolated v1, (t2-3, i2-3, v1), v2 or v3 arteritis. We looked at those who were treated in contrast to those who weren't based on their initial biopsy findings. Appropriate statistics were carried out for each pathological classification of arteritis and whether or not they received treatment.

Results: In total, 89 recipient allograft kidneys were reviewed. The average time between transplant and biopsy date was 323.6 days. Fourty-eight biopsies were diagnosed with isolated v1 arteritis, 46 (96%) of which received treatment and two patients (4%) did not. Of the 46 patients that received treatment, 39 (85%) had pathologic resolution of v1 arteritis on subsequent biopsy. The 2 patients that did not receive treatment had a 50% resolution rate. Of the 29 biopsies with v1 arteritis and tubulointerstitial inflammation (t2-3, i2-3, 1) all received treatment, with pathologic resolution of t-i-v in 23 patients (79%). Nine initial biopsies showed v2 intimal arteritis, only 3 (33%) of which had pathological resolution. Only three biopsies showed v3 intimal arteritis, with resolution in 1 (33%) of patients. Of the 89 total biopsies, 87 (98%) received treatment, with 66 (76%) resolving the intimal arteritis. The 4 patients with no treatment all had v1 with tubulointerstitial inflammation, and this was resolved in 2 (50%) patients. As expected, as intimal arteritis worsened, so did the prognosis.

Conclusion: Isolated v1 and v1 with tubulointerstitial inflammation (t2-3, i2-3, v1) fall under the same Banff Criteria Classification of Grade IIa AVR (Acute Vascular Rejection). Isolated v1 responds significantly better to treatment than t2-3, i2-3, v1 and v2/v3 (85% vs. 79%, 33% respectively) on repeat biopsy post-treatment. There was pathologic arteritis in one of two (50%) untreated isolated v1, bringing into question the need for treatment in this group. Further studies looking at treatment options and duration need to be conducted in a larger multi-hospital study.

Keywords: *Transplant, Intimal arteritis, tubulointerstitial inflammation, kidney allograft, Acute vascular rejection.*

Poster Abstract #17

Microparticle release during cancer extravasation: a novel anti-metastasis target

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Introduction: For metastasis to occur, disseminated tumor cells must leave the bloodstream and enter into the adjacent stromal tissue by a process called extravasation. However, less than 1% of extravasating tumor cells proceed to form a metastatic colony. Intravital imaging studies show extravasating metastatic cancer cells consistently release microparticles resulting in cell volume loss after extravasating into the adjacent tissue. Substantial literature shows that microparticle release is a key feature of necroptosis. We hypothesize that necroptosis, programed necrosis, is responsible for cancer cell microparticle release resulting in cell volume reduction.

Methods: To test my hypothesis, we quantitated the number of microparticles released by metastatic cancer cells undergoing extravasation in chicken embryo plasmas by nanoscale flow cytometry. We also measured cancer cell volume change due to extravsation by confocal intravital microscopy. Next, we measured extravasation rates of cancer cells and subsequent metastatic colony formation rates. Finally we measured amounts of microparticle release and extravasation rates of dimethyl fumarate (DMF, necroptosis inducer)/necrostatin-1 (Nec-1, necroptosis inhibitor)/vehicle treated cancer cells.

Results: Our results show that cancer cells released the most microparticles and lost the most cell volume in Day 18 animals. Those cancer cells in Day 18 animals exhibited the lowest extravasation rates and metastatic colony formation rates. DMF treated cancer cells also showed the most microparticle release and the lowest extravasation rates whereas Nec-1 treated cancer cells showed the lowest microparticle release but highest extravasation rates.

Discussion: Our findings show that an increase in microparticle release is correlated with a decrease in their metastatic efficiency due to excessive cell volume loss. Also an increase in microparticle release past a threshold due to necroptosis inversely affects cancer cell extravasation rates. Our results suggest necroptosis may be a novel therapeutic target to inhibit metastatic spread at the key step of cancer cell extravasation.

Keywords: *Extracellular vesicles, extravasation, intravasation, oncosomes, anti-metastasis therapies, necroptosis, intravital imaging, metastatic cancer, microparticle biogenesis*

Poster Abstract #18

Expression of kallikrein-related peptidases (KLKs) in adenoid cystic carcinomas

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Purpose: 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, prognosis and surveillance of tumors. However, the role of KLKs in salivary tumors has not been extensively studied. The purpose of this study was to determine whether dysregulated gene expression of KLKs occurs in adenoid cystic carcinomas (ACC), and whether it can be used as a tumor biomarker to guide therapeutic management of cancer patients.

Methods: Formalin fixed paraffin embedded (FFPE) tissue specimens were obtained from the Oral Pathology archives of Western University and London Health Sciences Centre. Messenger RNA 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 (Mann Whitney U-value, $p < 0.05$).

Conclusion: The 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. Previous research in laboratory has shown a downregulation of KLK 1, 11, 14 and an upregulation of KLK8 in mucoepidermoid carcinoma. Lastly, we previously found higher protein levels of KLK14 in ACC compared to normal salivary tissue using immunohistochemistry. Further research will focus on the possibility of a negative feedback loop whereby KLK protein levels and/or protein function decreases gene expression.

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

Poster Abstract #19

The effect of glucocorticosteroids on CRTh2^{+} Th2 cells

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Introduction: Glucocorticosteroid (GC) treatment has been the mainstay of asthma therapy for over 50 years. Despite its effectiveness, a subset of severe asthmatics display resistance to GC treatment. Our lab has shown that asthmatics taking the highest dose of inhaled steroids had the highest percentage of circulating Th2 cells. When we treated Th2 cells with the GC dexamethasone, there was an increase in CRTh2 surface expression. Here, we hypothesis that GC increase crth2 expression at the transcriptional level.

Methods: We identified CCRF-CEM cells as a model of Th2 cells. We treated CCRF-CEM cells with dexamethasone ($0.1\mu M$, $1\mu M$, and $10\mu M$ for 18 hours) with or without activation (PMA 20 ng; Ionomycin $1\mu M$). mRNA was isolated and the levels CRTh2 and GC responsive genes (i.e. GR, FKBP5,FKBP4) were determined using qRT-PCR.

Results: We showed that dexamethasone treatment of resting Th2 cells (18hrs) increased the level of CRTh2 mRNA as well as GR and FKBP5. However, we did not see an increase in CRTh2 expression in activated Th2 cells.

Conclusions: Our results suggest that GC treatment of resting Th2 cells elevates the level of CRTh2 expression at the transcriptional level. This increase was not observed in activated Th2 cells, which may due to reduction and/or interference of glucocorticoid receptor activity. Increased CRTh2 expression on Th2 cells may render cells more responsive to PGD2 activation, a pathway which inhibits apoptosis, and so GC treatment may actually help in sustaining the Th2 high severe asthma phenotype.

Keywords: Glucocorticosteroids, CRTh2, Th2, asthma, resistance

Poster Abstract #20

Nrf2-Keap1 antioxidant pathway activation in urothelial carcinoma

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Introduction: The transcription factor, Nrf2, and its repressor, Keap1, constitute an important protective mechanism against cellular damage and disease. In response to oxidative stress, Keap1 undergoes a conformational change, allowing Nrf2 to induce the expression of numerous cytoprotective genes. Despite its protective role, recent studies have demonstrated that mutations in the Nrf2 pathway lead to excess Nrf2 activation, which confers chemoresistance and a survival advantage to malignant cells. To date, aberrant Nrf2 activation has been demonstrated in only a small number of cancers. Through analysis of the cancer genome atlas database, we have identified that urothelial carcinoma also has a high prevalence of mutations in the Nrf2-Keap1 pathway genes. We aim to determine the prevalence of aberrant Nrf2 activation in this cancer.

Methods: After constructing tissue microarrays, 200 urothelial carcinoma biopsy samples will be assessed for Nrf2 activation by immunohistochemistry. The degree of Nrf2 activation will be correlated with clinical outcomes.

Expected Results: We hypothesize that aberrant Nrf2 activation will correlate with poorer clinical outcomes and increased chemoresistance in urothelial carcinoma. This study will be critical in contributing to our understanding of the prevalence of Nrf2-Keap1 pathway abnormalities in cancer. It also sets the stage for future studies to evaluate the use of Nrf2-Keap1 mutational analysis as a biomarker to determine prognosis and treatment outcomes.

Keywords: Nrf2, Keap1, cancer, urothelial carcinoma, chemoresistance

Poster Abstract #21

Long non-coding RNA Malat-1 regulates inflammatory cytokines in chronic diabetic complications

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Introduction: Only about 2% of the human genome codes for proteins; whereas, nearly the entire genome is transcribed. Metastasis associated lung adenoma transcript 1 (Malat-1) is an 8.7kb lncRNA molecule which is made up of a single exon, and is highly conserved. Furthermore, its baseline levels of transcription are high. Malat-1 has shown to be of importance in cancer metastasis; However, Malat-1 has not been studied in the context of diabetic complications. We have previously shown glucose induced upregulation of Malat-1 in endothelial cells in association with increased inflammatory markers. Here, we examined role of Malat-1 in the pathogenesis of chronic diabetic complications affecting the heart and kidneys. We hypothesize that Malat-1 regulates inflammatory molecules in chronic diabetic complications.

Methods: We examined Malat- 1 knockout (Malat-1 KO) and littermate controls (wild type), with or without streptozotocin induced diabetes after one and two months of follow-up. Transcript and protein analyses were carried out with RT-PCR and Mouse Tissue ELISAs respectively.

Results: All diabetic animals showed hyperglycemia, glucosuria and reduced body weight gain. Examination of cardiac and renal tissues demonstrated increased Malat-1 RNA expression in the heart and kidneys in the wild type diabetic animals in association with augmented production of downstream inflammatory molecules eg. interleukin-6, tumour necrosis factor α and interleukin-1 β. Protein expression paralleled these findings. Elevation of inflammatory markers was significantly decreased in the Malat-1 KO diabetic animals compared to the wildtype diabetic animals.

Conclusions: This is the first study to examine effects of Malat-1 in a diabetic animal model. The data generated from this study will provide direct evidence as to the importance of Malat-1 in the pathogenesis of chronic diabetic complications involving the heart and the kidney.

Keywords: Malat-1, Long Coding RNA, Diabetic Complications, Inflammatory Cytokines, Animal Model.

Poster Abstract #22

Implementation of a peer to peer learning initiative in Anatomical Pathology residency training

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Background and Objectives: Peer learning is the acquisition of knowledge through interactive teaching among individuals with equal levels of training. The participants are not professional teachers but through the process are able to enhance the learning of the group and solidify their own understanding of important concepts. It is not a substitute for formal teaching, but it is an important addition to the repertoire of learning activities.

Methods: We have organized peer learning rounds for the past 1.5 years. These rounds organized and attended by residents occur 2-3 days per week at the end of the day. Each resident contributes cases from their daily sign out so that all areas of Pathology have representation. The cases typically have important teaching points to share with the group. We have evaluated the success of the rounds through the distribution of a survey to the residents that have participated.

Results: Since its creation, we have seen over 761 cases from all areas of Pathology, presented by residents of all levels of training. We have scanned over 150 representative slides from the rounds that are stored in our own cataloged data base. There is consensus from participants that these rounds have provided a venue for continual exposure to diverse topics, discussion of complex subjects and to maintain camaraderie and communication between residents.

Conclusions: Peer to peer teaching is an effective adjunctive teaching modality that provides additional exposure to core pathology teaching concepts in an open and safe learning environment.

Keywords: Medical education, peer to peer learning, Anatomical Pathology, Residents

Poster Abstract #23

Ontario growth standards for infants: A retrospective autopsy study

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Introduction: Statistics Canada predicts on average 747 infant deaths per year in Ontario based on birth and infant death databases from 1993 to 2008. A postmortem examination is required in a significant number of these deaths to determine the cause and manner of death. In these cases, the pathologist compares the autopsy findings to standardized population parameters using body and organ measurement charts. Although a number of resources are available, many are outdated and have significant limitations.

Objectives: To create Ontario population-specific organ and body measurement mean charts for children under one year of age; to develop growth graphs for each age group, and to explore the relation between the body weight and cause of death.

Methods: Data were collected from 1260 Coroner's files from the archives of the Office of the Chief Coroner for Ontario which investigated the deaths of children under the age of one year for the period of 2000-2010. Recorded data included various quantitative and qualitative characteristics pertaining to body measurements and organ weights and associated relevant pathological findings. To assist in creation of a new standard resource, a survey of Ontario pathologists and literature review were undertaken.

Results: The survey identified 20 reference sources most frequently used by Ontario pathologists in the course of pediatric autopsies. The survey confirmed these references to be outdated and limited in scope. In our study, the difference in body weights was significant when correlated for age and gender. For male and female gender, body weight difference was significant between cases of sudden unexplained death and control groups for some of the age groups.

Conclusions: Guidelines are proposed to assist in creating a new Ontario population autopsy body and organ measurement resource. To date, data on organ and body measurements have been analyzed; however, no significant trends have been identified.

Keywords: Autopsy; Infants; Growth Standards; Body Measurements; Organ Weights.

Poster Abstract #24

The role of RGNEF in cellular scaffolding in ALS

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Introduction: ALS is a motor-neuron disease, characterized by muscular spasticity and wasting as well as difficulties breathing, speaking and swallowing. We will focus our interdisciplinary research on a newly discovered protein called rho guanine nucleotide exchange factor (RGNEF). RGNEF is a 191kDa, RNA binding protein encoded by the ARHGEF28 gene (Volkening et al., ALS, 2010).

Methods: I will co-express previously identified candidates with wild-type RGNEF and several mutant constructs in yeast and monitor resultant localization and toxicity to further explore these interactions. Two mutations in the gene encoding for RGNEF have been identified in ALS patients that are predicted to result in a severely truncated version of the protein. Constructs representative of these mutations will also be produced for expression in yeast and mammalian cell lines. I will validate results from my yeast studies in mammalian cell models and in ALS patient samples. In addition, we will evaluate how RGNEF modulates cellular scaffolding through the use of various biochemical assays that evaluate cytoskeletal structure and function.

Results: Candidates identified by our Split-ubiquitin screens in yeast show that RGNEF interacts with several proteins involved in cell scaffolding and signaling (tubulin for example). The cellular and molecular mechanisms of these interactions are completely unexplored. Using our yeast model and cultured mammalian cells we aim to determine how RGNEF modulates cellular scaffolding and thus explore the role of RGNEF in modulating protein misfolding in ALS.

Discussion: Degeneration of the axonal cytoskeleton is a hallmark of ALS neuropathology. Several biomarkers including neurofilaments, tau, and tubulin have been identified as indicators of cytoskeletal damage that impairs axonal transport and integrity. These processes precede loss of function and cell death of motor neurons but are poorly understood (Abdelhak, 2015). Our research Aims to determine how the interactions between cell scaffolding proteins and RGNEF modulate ALS pathogenesis.

Keywords: ALS, RGNEF, Protein misfolding, protein quality control, Aging, Cell scaffolding, Yeast model

Poster Abstract #25

Bio-modulation of primary human tenon's capsule fibroblasts using a novel application of coated magnesium

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Introduction: Trabeculectomy is currently the predominant surgical method for the treatment of glaucoma, but has only a moderate success rate due to unpredictable and variable wound healing responses. A hallmark of wound healing is increased deposition of extracellular matrix by fibroblasts which become activated following injury. The present adjunctive use of antimetabolite drugs inhibits this process, but cannot be precisely dosed and adds risks. Magnesium is also implicated as having the ability to negatively modulate wound healing, but its rapid corrosion in solution in its untreated form prevents its use. The aims of this study are to regulate corrosion through the use of biocompatible coatings and examine the ability of coated magnesium to modulate human Tenon's capsule fibroblast activity.

Methods: Primary cultures of human Tenon's capsule fibroblasts (HTCFs) were established from tissue specimens obtained during ophthalmic surgery. Characterization was performed through immunostaining. Cells were subsequently grown on three types of coated magnesium: hydroxyapatite, dicalcium phosphate dihydrate (DCPD), and DCPD with stearic acid, as well as a glass control. Cellular assays were performed, including cell metabolism (MTT), proliferation (BrdU), cytotoxicity (LDH), and apoptosis (ELISA).

Results: Compared to glass cultures, cells grown on two out of three coated magnesium discs showed decreased cell metabolism and proliferation, while the last, hydroxyapatite, showed an increase. None of the samples showed an increase in cytotoxicity. It is expected that apoptosis will not be significantly increased compared to control.

Discussion: Two of the coated magnesiums, DCPD and DCPD-SA, were effective in negative modulation of HTCF activity. This information may be useful for developing a method for modulating wound healing in trabeculectomy in a controlled, predictable, and directed manner.

Keywords: Open-angle glaucoma, trabeculectomy, fibrosis, fibroblasts, magnesium, Tenon's capsule, primary culture, coated metals

Poster Abstract #26

Understanding MIC-1: A new diagnostic biomarker in prostate cancer

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Introduction: Prostate cancer (PCa) is one of the leading causes of cancer-related deaths in men globally. Emerging evidence has revealed an association between PCa and a new divergent cytokine, MIC-1. However, the effects of MIC-1 on T-cells, the association of elevated MIC-1 serum levels with tolerogenic dendritic cells (Tol-DCs) and regulatory T-cells (Treg) levels, and the use of serum MIC-1 levels as a prognostic biomarker to detect PCa recurrence have not been elucidated. Therefore, we hypothesize that elevated MIC-1 expression can result in immunosuppression and that MIC-1 serum levels can be used as a biomarker for PCa recurrence.

Methods: To test these hypotheses, we will first use an in vitro and animal PCa model to study whether high or low MIC-1 levels have any effects on T-cell proliferation, cytotoxicity, apoptosis, tumour-antigen recall response, Texhaustion, and Treg levels. MIC-1 levels will be regulated through the introduction of siRNA's and cDNA's into TRAMP-C2 cell lines via transfection methods. As for the PCa patient samples, peripheral blood mononuclear cells (PBMC's) will be isolated and stained with Tol-DC and Treg fluorescent antibodies to analyze the immune phenotype. Next, RNA will be extracted from PBMC's and used for q-RT-PCR to detect the expression of immunosuppressive genes. While, finally, ELISA will be used to measure MIC-1 concentrations in the serum of PCa patients.

Results: The work towards this project is underway. Current efforts involve finding an optimal transfection setting using siRNA to knockdown the MIC-1 gene expression. Further work involves utilizing a PCa animal model to study whether high or low MIC-1 levels have any effect on T-cells. Lastly, serum and RNA were isolated from 16 PCa patients.

Conclusions: The findings from our study will provide valuable knowledge about MIC-1's role in PCa and new insights into developing more clinically applicable diagnostic and prognostic PCa markers.

Keywords: MIC-1, cancer, prostate, immunosuppression, T-cells.

Poster Abstract #27

Utilization of Immunohistochemistry and Special Stains at Autopsy

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Introduction: Postmortem decomposition with resultant tissue autolysis hinders microscopic analysis due to loss of architectural and cellular details. The purpose of this project was to explore the utility that immunohistochemistry (IHC) and special stains could offer diagnostically in autopsy tissue evaluation once autolysis had occurred. This information can be valuable for establishing specific pathological diagnoses for tissues sampled for microscopy from decomposed bodies.

Methods: Cardiac, pulmonary and skin tissues with suspected pathology (myocardial infarct, asthma, congestive heart failure and healing bruise) were collected from hospital autopsies. The tissues were subjected to autolysis for 0, 24, 48, 72-hour, 1- and 2-week intervals after the initial autopsy with further formalin fixation. Tissues were stained with either IHC (CK7, CK20, CKAE1/ AE3, TTF-1, S100, CD68) or special stains (Perls Prussian Blue, PASD, Masson trichrome, myeloperoxidase). Slides were blindly evaluated by two independent observers, with stain intensity graded on a scale from 0 to 3.

Results: All evaluated stains maintained their staining characteristics over the studied postmortem intervals with only some of them demonstrated slight degrading in intensity (PASD, myeloperoxidase, TTF-1, CK7 and CKAE1/ AE3). For all stains, the intensity remained constant within the first 48 hours of autolysis. Cytokeratins demonstrated increase in intensity of background staining somewhat limiting the assessment. None of the stains showed false positive results.

Conclusions: Our study further supports the use of IHC and special stains on tissues subjected to autolysis. Further studies are warranted to evaluate their utility on a wider spectrum of autopsy tissues and longer postmortem intervals.

Keywords: Pathology, autopsy, immunohistochemistry, special stains, decomposition, autolysis

Poster Abstract #28

In vivo CRISPR screen for the identification of kinases that regulate prostate cancer metastasis

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Introduction: To date there are no known genes that are responsible for metastasis because each disease site has individual qualities regarding metastatic spread. Our focus is on prostate cancer metastasis and we have developed a novel high-throughput means of performing in vivo screens for regulators of prostate cancer metastasis. We propose to use a focused CRISPR library screen that will "knock out" all human kinases to determine which ones are responsible for prostate cancer metastasis.

Methods: We will use PC-3M-LN4 and DU145 metastatic prostate cancer cell lines and inject 1× 105 cells into embryonic Day 9 chicken embryos (N>8/cell line). At 7 days post-injection, we count the number of cancer colonies using fluorescence stereoscopy. We will enumerate stellate-shaped colonies and non-stellate colonies. For the CRISPR kinase library, we are using a lentiviral format which will be used to infect the prostate cancer cell lines.

Results: Our initial results showed that DU145 tends to form more colonies (170.1 ±13.5 colonies/embryo) than the PC-3M-LN4 (24.4±4.5). Moreover, 90% of DU145 colonies exhibited a stellate morphology compared to 35% for PC-3M-LN4 colonies.

Discussion: DU145 appears to be an ideal cell line for the transduction of the CRISPR library because of the larger number of micrometastases with a high percentage of these being of the stellate morphology. If this cell line results in 100% stellate colonies then out CRISPR screen will attempt to isolate those that exhibit a clumped morphology, representing kinases that inhibit invasion. Putative genes will be then identified by genome sequencing of the isolated clumped colonies and the sgRNA will be determined which is specific for the kinase of interest.

Keywords: prostate cancer, DU145, metastasis, CRISPR, knocking out, kinases

Poster Abstract #29

Development of a single chain antibody for detection of Escherichia coli O157.

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Introduction: Antibody engineering has grown exponentially as more therapeutic antibodies are developed and become commercially available. Together with advances in microbial cloning and expression, it has made it possible to synthesize efficient and specific recombinant antibodies such as single chain variable fragments (scFv). Although most of the research and development focuses on therapeutic antibodies, scFv represent an alternative to conventional diagnostic reagents for pathogen detection. Thus, in this study we used a humanized scFv targeting the 5T4 antigen as a template to engineer a scFv antibody against the E. coli O157 antigen (scFvO157).

Methods: Hybridoma cells producing an anti-E. coli O157 monoclonal antibody were used to obtain the genetic sequences of the variable regions of the heavy (VH) and light (VL) chains. Informatics tools such as multiple sequence alignment and 3D-modeling were used to aligned both chains with the humanized scFv5T4 sequence. Then, the complementarity determining regions (CDRs) were grafted into the humanized backbone. The construct was complemented with a TEV cleavage site and a biotin tag at the N- and C-terminals respectively, before it was cloned and finally expressed in E. coli BL21(DE3). Different conditions for IPTG induction and protein extraction were evaluated, including native and denaturing extraction procedures. Finally, the scFvO157 was cleaved and purified for further characterization.

Results: By using a combination of native and denaturing conditions, we have been able to obtained a 27kDa protein, which corresponds to the approximate molecular weight of the scFvO157. Compared to the scFv5T4 template, most of it has been detected in the insoluble fraction, suggesting that the grafted CDRs might have affected the structural stability of the scFvO157.

Conclusions: Engineering a new scFv from a previous functional template is feasible as long as physicochemical properties contributing to the proper folding and stability are taken into consideration during the initial design.

Keywords: antibody engineering, E. coli O157, recombinant antibodies, scFv, single chain variable region.

Poster Abstract #30

Inducible beta-cell specific β1-integrin knockout affects islet architecture, beta-cell survival and function

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Introduction: It has been shown that β1-integrin is essential for pancreatic beta-cell development and maintenance throughout life in rodents and human fetal islets. However, the effects of a temporarily controlled β1-integrin knockout (β1KO) specific to pancreatic beta-cells of mice in vivo remains to be determined.

Materials and Methods: We have generated C57BL/6 mice with CreER recombinase specific to the mouse insulin promoter (MIP), allowing us to induce a β1KO upon injection of tamoxifen. Mice at 3-4 weeks of age received 4mg of tamoxifen per 20g bodyweight via intraperitoneal injection for 3 consecutive days and sacrificed at 8 or 16 weeks respectively for adult studies, and 25-35 weeks for aged studies. Metabolic studies were conducted by intraperitoneal injection to examine glucose tolerance, insulin tolerance and glucose stimulated insulin secretion. Immunofluorescence staining was used to examine beta-cell mass, islet size and islet density. Pancreatic islets were isolated and protein levels were determined by western blot.

Results: The protein level of β1-integrin in male β1KO mouse islets was reduced (~60%) at 8 weeks post induction compared to littermate controls. Both male and female β1KO mice had significantly impaired glucose tolerance, and male β1KO mice had significantly impaired glucose stimulated insulin secretion ($p < 0.05$). Beta-cell mass was significantly reduced in β1KO mice, along with a significant reduction in small islets (females only) and medium and large islets (males only) ($p < 0.05$). Islet density was also significantly reduced in males, but significance was not reached in female mice. Male mice also had a significant reduction in cyclin D1 and c-PARP protein expression, as well as a reduction in p-FAK, p-ERK and p-AKT ($p < 0.05$). Female protein has yet to be analyzed. Aged β1KO mice maintained impaired glucose tolerance.

Conclusion: Our research shows that β1-integrin is an important regulator of pancreatic beta-cell mass, function, and survival in adult mice.

Keywords: Diabetes, beta-cell, β1-integrin, CreER, mouse insulin promoter, insulin, glucose metabolism

Poster Abstract #31

Molecular testing from cytology samples turning promise into practice

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Lung cancer is the most common cause of cancer related deaths in Canadians and is often diagnosed at an advanced stage. A subset of patients with lung adenocarcinoma will have mutations in EGFR or fusions in ALK that are amenable to targeted therapy. To determine eligibility for these therapies, molecular testing must be performed often on cytology FNA samples to determine the status of EGFR and ALK. Given the limited tissue from these samples it is imperative that a robust and validated pathway is available to optimize the sample quality and limit the need of re-biopsy and false negatives. To validate the use of cytology specimens at our center we had 4 specific objectives; (1) determine the minimum tumor cellularity required to identify mutations in EGFR, (2) validate molecular testing on Cytolyt fixed samples, (3) develop methodology to isolate DNA from Pap and Diff-Quik stained slides (4) validate the use of ALK antibodies in Cytolyt fixed samples.

We have utilized a lung adenocarcinoma line (H1975), which carries the L858R and T790M mutations along with a fibroblast cell line to simulate normal stroma as a model system. Using dilutions of H1975 we can determine the minimum tumor cellularity required to detect EGFR mutations. We performed FNA biopsies of resected lung adenocarcinomas at the time of frozen section to generate cytology samples fixed in Cytolyt for molecular testing. Using the Pinpoint DNA extraction kit we are currently isolating DNA from Diff-Quik and Pap stained slides to expand our capabilities for molecular testing and provide new sources of DNA. To validate the use of ALK antibodies we obtained fresh colonic tissue and fixed this in Cytolyt or formalin and performed immunohistochemistry to detect ALK expression in ganglion cells. Taken together this will validate molecular testing on cytology samples and expand our institutional capabilities.

Keywords: *Cytology, EGFR, ALK, next generation sequencing, ion torrent, immunohistochemistry*

Poster Abstract #32

Etiology of motor vehicle collision fatalities in southern Ontario

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Introduction: Factors such as alcohol use, speed, restraint use, weather conditions, and driving experience have been identified as potential risks for fatalities in motor vehicle collisions. Furthermore, past literature shows a discrepancy in motor vehicle collision-related deaths between urban and rural locations. We hypothesize that southern Ontario motor vehicle collisions involve causation factors and injury patterns previously suggested to be unique to urban or rural roadways.

Methods: Southern Ontario motor vehicle collision cases were analyzed with cases from other urban and rural regions throughout Canada. Data was collected and analyzed from a database of fatal motor vehicle collisions compiled by the Motor Vehicle Safety Research Team based at Western University, and through associated motor vehicle safety research team databases in other provinces. Toxicological data was collected from reports contained within the Office of the Chief Coroner of Ontario.

Results: Significant differences in injury severity and incidence of motor vehicle collision fatalities were seen between urban and rural locales. Less fatalities occurred on urban roadways, and higher odds for spinal and thoracic injuries were found for rural collisions. Fatal frontal, rollover, and side impact collisions occurred with a higher odds on rural roadways. Significant contributing factors for all collisions included young age (14-29), male gender, presence of unskilled drivers, and nighttime hours adjusted to the log binomial model.

Discussion: A combination of higher posted speed limits, deleterious driving behaviour, and increased proximity from medical trauma centers may account for the increased incidence of fatalities demonstrated for Canadian rural roadways. Increased police surveillance, traffic law enforcement, and access to medical centres should aim to address the high number of fatal motor vehicle collision cases that occur on rural roadways.

Keywords: motor vehicle collisions, traumatic injuries, population health, forensic biomechanics, road safety, collision analysis

Poster Abstract #33

Mitochondrial dependent necroptosis mediates microvascular endothelial cell survival following cardiac allograft transplantation

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Introduction: Tissue injury post cardiac allograft transplantation is associated with various forms of programmed cell death (PCD). One form of PCD, known as necroptosis, has been implicated to cause mitochondrial mediated cell death by inducing mitochondrial permeability pore (mPTP) opening. On the other hand, contradictory results in past studies suggest that mitochondria may be dispensable in the necroptotic pathway. This has yet been studied in a heart transplantation model. In this study, we aim to demonstrate the contribution of mitochondria in cardiac cell death in vitro and eventually on an in vivo transplant model.

Methods: Murine microvascular endothelial cells (MVECs) were cultured and treated with tumor necrosis factor alpha (TNFα) and caspase-8 inhibitor (IETD) to induce necroptotic cell death. We will use various inhibitors that target mPTP and other upstream mitochondrial regulators. The pathway will later be studied in an in vivo mouse heterotopic heart transplantation model.

Results: TNFα and IETD induced necroptotic death is inhibited by necroptotic inhibitor necrostatin-1. Drug inhibitors that target mPTP and other mitochondrial regulators brought down cell death to near baseline levels.

Discussion: Our data shows that mitochondria may be an important mechanistic mediator in myocardial necroptosis, indicating that targeting the mitochondria may be beneficial to prevent regulated necrosis. Findings from this study may help us gain insight on establishing possible target therapy to improve graft health and survival.

Keywords: Necroptosis, mitochondria, endothelial cells, cardiac allograft injury

Poster Abstract #34

Cortical ependymoma presenting with long-term refractory epilepsy: case report and review

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Abstract: Majority of ependymomas are infratentorial and intraventricular. Supratentorial ependymomas located purely in the cortex, are extremely rare with a handful of cases being reported. We report a 34 year-old female with right insular cortical ependymoma presenting with long-term refractory epilepsy.

Clinical Presentation: This is a 34-year-old female who was diagnosed with epilepsy since 8-years-old. Her most common stereotypic seizure is feeling of buzzing in the left side of her face associated with occasional left arm and face numbness. Occasionally, she would have a different type of seizure which includes auditory hallucinations, nonspecific cephalgic sensation.

An extensive workup on previous occasions including an MRI which demonstrated DNET in the right temporal region and this was resected in 1990. However, she continued to have epilepsy and scalp EEG in the fall of 2007 as well as in the fall of 2009, demonstrated involvement of her right temporal and possibly right insular region. She underwent depth electrode insertion in July 2011. Although she had many stereotypic seizures, the recording did not show the origin of her seizure. Subsequently, she was readmitted again in July 2014 with coverage that added the insula into the context. Her seizures were captured and the habitual seizures correlated with a clear electrographic onset in the posterior insula and parietal operculum with spread to the temporal lobe neocortex and mesial structures. Her MRI demonstrated an area resembling cortical dysplasia in the right insula as a likely source of her seizures as it corresponded to the focus of the electrographic onset in her seizures. The posterior insular cortex was surgically resected and it showed a firm white yellowish lesion that is different from the surrounding normal brain tissue. Pathological examination reveals tumor with cells arranged in aggregates punctuated by nuclear free areas around vessels reminiscent of perivascular pseudorosettes. Tumor cells show diffuse immunolabeling with GFAP, and 'cytoplasmic' and 'dot-like' EMA expression consistent with ependymoma in the cortex.

Conclusion: In this article, we report a rare case of young patient with supratentorial cortical ependymoma. Cortical ependymoma should be considered as a differential diagnosis of a supratentorial cortical mass in a young patient, especially in the setting of longterm epilepsy. The clinicopathological features of supratentorial cortical ependymomas would be discussed in this review.

Poster Abstract #35

Choriocarcinoma arising within Crohn's related neoplastic lesions: report of a rare post-partum pitfall!

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Introduction: Choriocarcinoma can be classified as gestational or non gestational. The former is associated with pregnancy related lesions, such as hydatidiform moles. Rarely, choriocarcinoma may be non-gestational, following dedifferentiation events in somatic cells. Primary non gestational choriocarcinoma in the colon is rare, while its association with inflammatory bowel disease is exceedingly uncommon. To our knowledge, this is the first report of choriocarcinoma arising within Crohn's related neoplastic lesions in the post-partum setting.

Case report: A 26 year old woman presented 1 month post-partum with abdominal pain. Her medical history was significant for severe, uncontrolled Crohn's disease. She had a normal pregnancy, without complications. Abdominal CT imaging demonstrated a perianal abscess. Three weeks later, she developed increasing abdominal pain, nausea and vomiting; she was admitted for small bowel obstruction. Further investigations revealed an elevated serum b-HCG and two liver lesions. Percutaneous biopsies of the liver lesions showed poorly differentiated adenocarcinoma with features suggestive of gastrointestinal origin. Ten days later, the patient underwent a subtotal colectomy for acute bowel perforation. Pathologic assessment of the colectomy specimen showed two separate colonic tumors (cecal and sigmoid) and typical background features of Crohn's disease. Histologically, the cecal tumor was a poorly differentiated adenocarcinoma with syncytiotrophoblastic differentiation, arising within a Crohn's related dysplastic lesion (DALM). The sigmoid tumor was an adenocarcinoma with focal choriocarcinomatous differentiation, also arising within a DALM lesion. The choriocarcinoma component in the sigmoid cancer showed classic biphasic morphology, with syntiotrophoblastic and cytotrophoblastic cells and hemorrhage. Three weeks post-operatively, the patient died after developing wide spread metastases. She received comfort measures, but no chemotherapy in the post-operative period, because her level of function was too poor. Her final serologic investigations were as follows: b-HCG 39 (units?), 374 IU/L, AFP 1.8 ug/L, CEA 31.2 ug/L, CA-125 426.6 U/mL.

Conclusions: Herein, we report an extremely rare case of synchronous primary colonic adenocarcinoma with choriocarcinomatous de-differentiation, arising in a patient with Crohn's disease. This diagnostically challenging case illustrates the need for careful clinical and pathological analysis in order to avoid extemporaneous conclusions for unusual carcinomas presenting in unique and complex clinical scenarios.

Keywords: inflammatory bowel disease, DALM, non-gestational choriocarcinoma

Poster Abstract #36

TGFβ regulates collagen deposition by modulating PTEN expression and activity in trabecular meshwork cells

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Introduction: Glaucoma, which affects nearly 70 million people worldwide, is caused by the fibrosis of the trabecular meshwork tissue (TM). Fibrosis of the TM prevents normal drainage of aqueous humor, leading to increased intraocular pressure, optic nerve damage and blindness. Fibrosis of the TM is mainly caused by the increased levels of active transforming growth factor-β (TGFβ) in the aqueous humor of glaucoma patients. We aim to decipher the role of Phosphatase and Tensin Homolog (PTEN) in the fibrosis of TM, since PTEN is a major regulator of extracellular matrix (ECM) deposition and TGFβ inhibits its expression in other cell lines.

Methods: Human donor TM cells were cultured with 5ng/ml of TGFβ2 for 12, 24, or 48 hrs. Protein and mRNA were extracted to study the expression of PTEN and collagen. Phosphorylation of PTEN was examined to analyze its activity. Effects of PTEN overexpression or inhibition of PTEN activity on ECM deposition were also examined.

Results: TGFβ2 treatment significantly increased PTEN (2.15 fold, $p<0.05$) and collagen mRNA (4.1 fold, $p<0.0001$) in human TM cells at 24 hrs (N=3). TGFβ2 also significantly increased PTEN protein expression (2.05 fold, $p<0.001$) and, as expected, increased expression of collagen protein. However, phosphorylation of PTEN also increased significantly (1.76 fold, $p<0.05$), indicating that its activity is inhibited. This further correlated with increased phosphorylation of AKT. Inhibition of PTEN activity using small molecule inhibitor VO-OHpic increased collagen deposition, while overexpression of PTEN decreased collagen deposition induced by TGFβ2 in TM cells.

Conclusions: Our previous study has shown that deletion of the PTEN gene induced excess ECM deposition by dermal fibroblasts. Inhibition of PTEN activity by phosphorylation is thus a novel mechanism by which TGFβ2 induces collagen deposition in TM cells. Regulation of PTEN activity could serve as a therapeutic target with high potential to prevent excess ECM deposition in the TM of glaucoma patients.

Keywords: *Glaucoma, trabecular meshwork, PTEN, excess ECM deposition, fibrosis, aqueous outflow resistance, TGFβ2*

Poster Abstract #37

Effect of glucocorticoids on CRTh2 protein expression in Th2 cells in allergic asthma

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Introduction: Chemoattractant receptor-homologous molecule expressed on T-helper 2 cells (CRTh2) activation contributes to excessive inflammation seen in allergic asthma. To reduce the symptoms, asthmatic patients are typically prescribed glucocorticoids. However, our laboratory has recently shown that glucocorticoid treatment of human T-helper 2 (Th2) cells caused increased CRTh2 mRNA expression. The present study is aimed at validating these unexpected results. We hypothesize that glucocorticoid treatment increases CRTh2 expression at the protein level.

Methods: Immunostaining with immunoperoxidase, diaminobenzidine and antibody for CRTh2 will be used on control tissues as well as a Th2 cell line (CRM). Once optimized, we will examine the number of CRTh2-expressing cells following dexamethasone treatment, a glucocorticoid analogue. The staining will be quantified by counting number of positive cells.

Results: Presently, we have optimized immunoperoxidase staining for CRTh2 on formalin-fixed tissues. We found positive signal in various control tissues: nasal polyp, antral polylp and squamous cell carcinoma. We will now use this method to test the impact of dexamethasone treatment on CRTh2 expression by the Th2 cell line.

Conclusion: We expect that glucocorticoid treatment will increase the number of cells expressing CRTh2, a marker of Th2 cells. These findings may lead to the discovery of a negative side effect of glucocorticoid treatment in allergic asthma, which is mediated by Th2 cells.

Keywords: *Th2 cells, CRTh2, glucocorticoids, dexamethasone, asthma, immunoperoxidase-staining*

Poster Abstract #38

Preliminary evidence suggests human tissue kallikreins are dysregulated in pleomorphic adenoma - a benign salivary gland tumor

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Introduction: Pleomorphic adenoma (PA) is the most common salivary gland tumour. It is a benign slowly growing, firm single nodular mass with symptoms of varying duration. PA presents across a wide age range, but is most commonly seen in the 4th to 7th decades with a slight female predilection. Malignant transformation of PA occurs in as many as 5% of cases. Insight into the pathogenesis of PA may gained by analyzing biomarkers such as human tissue kallikreins (KLKs). To better understand the pathologic and physiologic functions of KLKs in PA, we will investigate the expression patterns of KLK1−15 at the mRNA level and protein level and compare with normal salivary controls. We hypothesize that various KLKs will be co-expressed and dysregulated.

Methods: Fresh PA tissue specimens (N=17) and normal salivary gland controls were obtained. The samples were subjected to quantitative real-time reverse transcription polymerase chain reaction (RT- qPCR) experiments to detect the mRNA levels of KLK1−15. Statistical analyses were carried out using Wilcoxon signed rank test with the level of significance set at $P<.05$. To corroborate our findings, we will assess the protein level expression of KLK1−15 by immunohistochemistry.

Results: Preliminary findings revealed expression of mRNA for KLK1−15 in all samples, with a statistically significant decrease in KLK1, 12, and 13 mRNA levels in PA tissues relative to control tissues.

Conclusions: KLK1, 12, and 13 mRNA levels in PA tissues are statistically significantly decreased relative to those in normal salivary gland control tissues. Immunohistochemistry experiments are currently underway to corroborate these findings.

Keywords: Pleomorphic adenoma; salivary gland tumours; human tissue kallikreins; quantitative real-time reverse transcription polymerase chain reaction; immunohistochemical analyses

Poster Abstract #39

Expression of the growth hormone secretagogue receptor 1a and ghrelin in human heart failure

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Introduction: Heart failure (HF) occurs when the heart is unable to provide adequate blood flow and oxygen to organs across the body. Current detection of HF uses biomarkers in the circulation, and not in cardiac tissue. Our group is focusing on characterizing the growth hormone secretagogue receptor-1a (GHSR-1a) and its ligand ghrelin as a cardiac specific biomarker due to their presence in the cardiomyocytes of the heart. We have developed a fluorescent analog of ghrelin, Cy5ghrelin(1-18), which specifically binds GHSR-1a in cardiac tissue, and detects changes in cardiomyocyte differentiation. I will be focusing on characterizing GHSR-1a and ghrelin in human cardiac tissue and correlating expression to HF severity.

Methods: Cardiac tissue samples from 2 patient cohorts, including transplant patients and surgery patients, have been obtained from the cardiac surgery unit of London Health Sciences Center. We will use Cy5-ghrelin(1-18) to examine GHSR-1a levels and fluorescent antibodies to determine levels of ghrelin and natriuretic peptide type B, a gold standard biomarker of HF. Masson's trichrome stain will be used to test fibrotic tissue in severe HF patient samples. We will use quantitative fluorescence microscopy to examine levels of ghrelin, GHS-R1a and BNP in both patient cohorts. We will then correlate these levels with the clinical data.

Results: Previous work in our lab has shown elevated levels of GHSR-1a in the right atrium and left ventricle of both transplant patients and surgery patients with end stage HF. Preliminary results indicate an increase of GHSR-1a and BNP in end stage HF patients.

Conclusions: Establishing GHS-R1a as a cardiac specific biomarker can greatly impact the diagnosis of HF and help with personalized medicine. This biomarker can be used in conjunction with a panel of biomarkers to help understand the progression of HF in patients.

Keywords: Growth hormone Secretagogue Receptor 1a, Ghrelin, Heart Failure, Fluorescence Microscopy, Immunohistochemistry

Poster Abstract #40

Clinical utilization of genome-wide methylation testing in pediatric patients

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The complexity of pediatric developmental disorders is underscored by genetic and environmental factors, which can be facilitated by epigenetic mechanisms. DNA methylation is an epigenetic mechanism essential to many biological processes, including transcriptional regulation, establishment of chromatin states, imprinting, X-chromosome inactivation, development and tissue specification, genomic stability and silencing of the repetitive elements. Accordingly, DNA methylation defects have been shown to play a key role in many pediatric and adult onset disorders, including imprinting diseases and carcinogenesis. While DNA methylation defects can occur in pediatric patients with specific epi/genetic syndromes, there is little known about genome-wide DNA methylation changes in patients with developmental and intellectual disabilities (DD/ID). Our laboratory has performed genome wide DNA methylation screening in ~1000 patients with a wide range of disorders associated with DD/ID, including imprinting disorders, Fragile X syndrome and chromatin remodeling genetic syndromes, to clinically validate the use of genome wide DNA methylation screening and define epi-signatures of various DD/ID conditions. Methods: DNA methylation of the peripheral blood specimens were assessed using Illumina HumanMethylation450 BeadChip and methylation data analysis was performed using Partek Genomic Suite software. Results: We have validated this approach for sensitive detection of imprinting disorders, including Angelman syndrome, Prader-Willi syndrome, Beckwith-Wiedemann syndrome and Silver-Russell syndrome and of Fragile X syndrome. We discovered novel epigenetic signatures associated with genetic conditions including ATRX Syndrome, Floating-Harbor syndrome and DNMT1 neuropathy dementia syndrome. We have clinically validated the genome wide DNA methylation test for imprinting syndrome disorders, Fragile X, and identified epi-signatures of multiple genetic conditions.

Keywords: DNA methylation, epigenetics, developmental delay and intellectual disability

Poster Abstract #41

Educational value and lessons learned for videos of grossing gynecological pathology specimens

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Introduction: The purpose of this project is to develop educational videos for grossing gynecological specimens for pathologists' assistant (PA) students, evaluate their educational value, and share our lessons learned.

Methods: Educational grossing videos of 10 different gynecological specimen types were created. Six PA students with no previous grossing experience were randomly divided into two equal groups. One group viewed the placenta video first, then read the grossing template; the other group read the template first, then viewed the video. Both groups completed a questionnaire to assess the learning utility and experience, flow, strengths and challenges of the two tools.

Results: The combination of the video and template was rated as the most useful for teaching grossing technique by 4/6 participants with 2/6 rating the video alone as the most useful. All participants agreed that videos should be used to teach specimen grossing. Comments were: the video was a visual and auditory aid with step-by-step easy to follow instructions and helpful tips. Suggestions were: improvement of the video's technical quality and inclusion of more abnormal findings. Challenges with video development were: 1) technical issues (lighting, background noise, camera stability, field of view); 2) large file storage issues; and 3) file security given the sensitive material. There were minimal resources and cost.

Discussion/Conclusion: The video for grossing gynecological specimens was rated as a useful tool for teaching grossing technique. The videos are designed to complement the grossing templates that have been implemented in the department, and this is reflected in the questionnaire feedback. Although there were some challenges, these could be addressed and will inform our future development of educational videos for our PA students and other learners.

Keywords: specimen grossing, gynecology, videography, video learning

Poster Abstract #42

The effect of selective inhibition of cholinergic pedunculopontine nucleus neurons on attention as measured through the 5-Choice Serial Reaction Time Task in rats

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Introduction: Cholinergic cell group 5 (Ch5) is a part of the brainstem's ascending reticular activating system (ARAS) and has been implicated in attention. Bilateral lesions of Ch5 are lethal and previous animal lesion studies attempting to decipher the role of Ch5 in attention have had limitations. These limitations included incomplete inactivation of Ch5 neurons and compensation by neighboring nuclei. My study attempts to improve upon previous lesion study findings and clearly uncover the role of Ch5 neurons using Designer Receptors Exclusively Activated by a Designer Drug (DREADDs) to reversibly and temporarily inhibit Ch5 neurons during a behavioral attention task in rats.

Methods: I will use transgenic Long-Evans rats to specifically express the DREADDs in Ch5 neurons and temporarily inhibit those neurons for 1-2 hours upon injection of the designer drug, Clozapine-N-Oxide (CNO). I will then test attentional behavior during this 1-2 hour period through the 5-Choice Serial Reaction Time Task (5-CSRTT) after intraperitoneal injections of either CNO or vehicle to discern the role of Ch5 in attention.

Expected Results: I expect the animals to show reduced attentional capabilities after the CNO injections compared to vehicle injections, and this reduction should manifest as a decrease in accurate responses and an increase in omissions in the 5-CSRTT.

Significance: The results of my study will support the hypothesis that Ch5 is involved in attentional processes and potentially pave the way for research on its role in neurological diseases with attentional deficits like Parkinson's or Alzheimer's.

*Keywords: Attention, selective, sustained, Cholinergic Cell Group 5, DREADDs, 5-CSRTT, ARAS, PPT, LD*T

Poster Abstract #43

Structural and functional changes to the retina and optic nerve following anti-VEGF treatments in diabetic retinopathy

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Introduction: Diabetic Macular Edema (DME) is characterized by an abnormal accumulation of fluid in the macula, the central portion of retina, due to leakage from surrounding blood vessels. Anti-VEGFs are often the first line of treatment for diabetic retinopathy (DR) patients with DME. Over the past 10 years, there have been increased clinical reports of DR patients using anti-VEGFs developing signs of glaucoma and optic neuropathy. This will be the first long term study to evaluate and analyze the safety and efficacy of anti-VEGF drugs at different stages of DR with varying severity of DME. We hypothesize that increased exposure to anti-VEGF will result in increased retinal ganglion cell (RGC) death and morphological changes to the optic nerve.

Methods: Rat retinal cell cultures will be exposed to 0, 0.0625, 0.125, 0.25 and 0.5 mg/mL of rat anti-VEGF either for 24 or 48 hours. Cytotoxicity will be measured by LDH and ELISA and a MTT assay will be performed for cellular metabolic activity. The in vivo study consists of 20 control rats and 20 STZ-induced diabetic rats; each group of 5 will be treated with, 2 μL of rat IgG (0.125 mg/mL) (control), 2 μL 0.0625 mg/mL anti-VEGF, 2 μL 0.125 mg/mL (clinical dose) anti-VEGF, and 2 μL 0.25 mg/mL anti-VEGF respectively. Immunohistochemistry staining for VEGF and Thy1.1, a RGC marker, and TUNEL assay will be used to evaluate apoptotic cell death after 3 weeks of treatments. For the in vivo clinical pathological analysis, DR patients with underlying DME will undergo pre-injection and 6, 12 and 24 month follow-up tests using the visual fields, heidelberg retinal tomograph (HRT), optical coherence tomography (OCT) and OPTOS fluorescein angiography to monitor diabetic ischemia.

Results: To date, 36 patients with no history of prior anti-VEGF injections have been recruited into the study. From them, 16 patients have completed their 6 month testing and noticeable trends have emerged. The thickness of the macula has decreased (6 months -61um), the RNFL has decreased (6 months -12.67um), average cup to disk (C/D) ratio (average C/D 6 months +0.027) has increased and the cup volume has increased (6 months +0.004 mm3).

Conclusions: The preliminary results suggest that anti-VEGF treatment is detrimental to the optic nerve by causing damage to RGCs. The analysis of RGC cultures and STZ-induced diabetic rats will be performed to further test the hypothesis. The results suggest monitoring both the retina and optic nerve status in patients undergoing frequent injections.

Keywords: diabetic macular edema, diabetes, retina, optic nerve, anti-VEGF, safety, efficacy

Poster Abstract #44

Role of high glucose-induced matrix metalloproteinases in adipogenesis

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Introduction: Bone marrow-derived mesenchymal progenitor cells (MPCs) are multipotent cells capable of chondrogenic, osteogenic and adipogenic differentiation when induced. Previous studies from our laboratory have shown that diabetes enhances adipogenic differentiation in marrow MPCs while suppressing osteogenesis and this skewed differentiation leads to depletion of regenerative stem cells. It has been suggested that remodeling of the extracellular matrix plays an important role in adipogenesis. The aim of this study is to examine the potential role of matrix metalloproteinases in high glucose-induced adipogenesis.

Methods: MPCs derived from bone marrow mononuclear cells were seeded at a high density and treated with either Batimastat or CP 471474 (both pan inhibitors of matrix metalloproteinases; MMPs) in control media or adipogenic induction media. Expression levels of adipogenesis-specific genes were measured by RT-qPCR. Cells were also stained with Oil Red O to highlight adipocytes.

Results: Our results show that pan inhibitors of MMPs interfere with adipogenic transcription factor expression, even though terminal differentiation of cells was not altered. There were clear morphological changes suggesting that matrix remodelling may play a role in adipocyte function and lipid accumulation but not differentiation.

Discussion: Extracellular matrix remodelling has been suggested to play a role in adipogenic differentiation. Since our studies have shown enhanced adipogenesis in marrows of diabetic patients and experimental animal models, we attempted to understand the matrix factors controlling the differentiation of bone marrow MPCs into adipocytes. Our results show that MMPs are not involved in MPC differentiation but may regulate function through altered lipid accumulation.

Keywords: Progenitor cells, adipogenesis, matrix remodelling, matrix metalloproteinases, differentiation

Poster Abstract #45

Immunological impact of CLI095 on ischemia reperfusion

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Introduction: Ischemia reperfusion injury (IRI) is an unavoidable consequence in kidney transplantation. It is the major cause of reducing graft survival after transplantation. IRI is the result of inflammation and subsequent damage caused mainly by innate immune receptors called Toll Like receptors (TLRs). Damaged cells from ischemic injury release molecules including high-mobility group box 1(HMGB1) and heat shock proteins known as damage -associated molecular patterns (DAMPs) which can be recognized by TLRs to promote inflammation. Stimulation of different TLRs induces distinct patterns of gene expression, which not only leads to the activation of innate immunity but also led to the development of acquired immunity.

Hypothesis: In this study, we hypothesize that TLR4 signaling play an important role in renal IRI and targeting its pathway would have a significant impact in the prevention of IRI.

Method: Bone marrow derived dendritic cells were stimulated by lipopolysaccharide (LPS) with or without Cli095, a specific inhibitor of TLR4. Expression of TLR4, proinflammatory cytokines, and DCs maturation markers were then tested by Flow Cytometry, qRT-PCR, and ELISA. To mimic an in vivo IRI situation, we have generated a C57BL/6 mouse model by renal pedicle clamping for an hour followed by 24 h of clamp release. To see the effect of CLI095 in vivo, CLI095 were injected (i.p.) 1 or 18 hour before clamping. Kidneys and blood were collected, histological and biochemical analysis were done.

Result: We have showed that Cli095 able to reduce the expression of pro inflammatory cytokines (IL6 and TNFα) in response to LPS activation. In addition, DCs that pretreated with Cli095 showed low expression of maturation marker in compare to cells that only subjected to LPS.

Conclusion: Allograft damage and side effect of immunosuppression are two major problems in kidney transplant recipient. Thus, any therapy, which specifically targets TLR4, has potential application in the prevention of IRI and prolongation of renal allograft life time.

Keywords: Kidney, Ischemia Reperfusion injury, Toll like receptors, Innate immunity, CLI095

Poster Abstract #46

Quality management for the autopsy service – London Health Sciences Centre

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Introduction: In recent years, the Autopsy Service at the London Health Science Centre (LHSC) has faced increased case volumes. There is recognition that there is room for improvement in certain areas of quality management to increase efficiency to meet these caseload demands. The goals of this project are to create and implement a series of recommendations to improve the efficiency and experience of autopsy staff at LHSC.

Methods: A structured questionnaire was developed and then administered to all of the autopsy pathology staff, including Pathologists, Pathologists' Assistants (both current staff and students in the Master's of Clinical Sciences, Pathologists' Assistant program), Neuropathologists, Residents and Medical Laboratory Technicians (MLTs). Following the administration of the questionnaires, results were summarized and used to create a list of proposed solutions to issues discovered. This proposal will be peer-evaluated by a panel of autopsy staff and then pending acceptance will be implemented into practice. The goal is to introduce these new practices during my rotation in the Autopsy Suite (approximately summer of 2016), so that I can evaluate their efficacy first hand. A follow up interview will also be conducted with staff after an adequate trail/adjustment period has passed, to confirm that the recommendations are effective.

Results: A structured questionnaire was developed and used to interview all autopsy staff members. Based on the results of the interviews, a list of recommendations was created. There were 48 recommendations in total, which have been subdivided based on plausibility of implementation in the Autopsy Suite.

Discussion: The first phase of this project is complete. The second phase will involve determining how to implement the recommendations, implementing them, and then evaluating their efficacy. The list of recommendations from the questionnaire represents a direct approach to examine issues experienced by staff in the Autopsy Suite. The panel review of the implementation suggestions will confirm that the recommendations are well conceived and reasonable.

Keywords: quality management, efficiency, safety, supply management

Poster Abstract #47

Presence of glypican-1 on extracellular vesicles fails to discern pancreatic cancer from benign pancreatic disease

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Introduction: Pancreatic cancer is one of the deadliest cancers as disease-associated symptoms typically only appear during the later stages of the disease. No effective early-detection screening test for pancreatic cancer exists. Current biomarkers suffer from poor specificity to pancreatic cancer and are commonly elevated in benign pancreatic disease (BPD). A recent study suggests that the presence of the pan-specific cancer marker, Glypican-1 (GPC1), on extracellular vesicles (EVs) identifies pancreatic cancer with perfect specificity and sensitivity (Melo SA et al, Nature, 2015). Therefore, I hypothesize that a liquid biopsy enumerating GPC1-positive EVs will represent a blood test capable of discerning pancreatic cancer from BPD.

Methods: I have obtained plasma samples from patients with metastatic or resected pancreatic cancer and patients with BPD. Using nanoscale flow cytometry to analyze patient plasma, GPC1 EVs ranging from 100–1000nm in size have been enumerated and compared between patient groups. Because glycoprotein 2 (GP2) is an exocrine pancreas-specific marker, I have also tested the utility of a test enumerating EVs concurrently positive for GPC1 and GP2 (GPC1-GP2).

Results: GPC1 and GPC1-GP2 EV counts are highly inconsistent within patient groups and there is also no significant difference between groups. The lack of difference between metastatic and resected cancer groups reveals a lack of correlation of GPC1 EV levels with tumor burden. Interestingly, the co-occurrence of GPC1 and GP2 is uncommon among all patient groups. This implies that detected GPC1 EVs may not originate from the pancreas.

Conclusion: These findings indicate that a liquid biopsy enumerating GPC1 EVs cannot effectively distinguish between patients with pancreatic cancer and BPD. Contradictory to the previous findings regarding GPC1, GPC1 may not be useful in the early-detection of pancreatic cancer.

Keywords: Pancreatic cancer, BPD, biomarkers, flow cytometry, extracellular vesicles, GPC1, GP2

Poster Abstract #48

Role of inflammation on Dclk1+ tuft cell's initiation of colon cancer

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Introduction: The role of stem versus mature cells in the initiation and progression of cancer remains largely unexplored. In the gastrointestinal epithelium, the contribution of mature cells to intestinal tumor formation has recently been proposed to occur through dedifferentiation of cells upon chronic tissue injury. It has previously been shown that doublecortin-like kinase 1 (Dclk1), a marker of mature intestinal tuft cells, labels quiescent and long-lived cells within the colon that can also serve as facultative stem cells. In this study, we have attempted to characterize Dclk1+ tuft cells as colon cancer initiating cells with tissue injury. We hypothesize that quiescent, APC mutated Dclk1+ tuft cells serve as cancer-initiating cells after activation by inflammation-induced cellular dedifferentiation.

Materials and Methods: We employed Dclk1-CreERT; ROSA26-mTmG; APCf/f mice to examine the effects of infectious (Citrobacter) colitis on colonic tumor formation. The effect of the inflammatory cytokines IL-1B and IL-6 on transformation of APC mutated Dclk1+ cells were studied in vitro using "mini-gut" organoids derived from Dclk1-CreERT; ROSA26-mTmG; APCf/f mouse intestine.

Results: Using Dclk1-CreERT; ROSA26-mTmG; APCf/f mice, we determined that infection-induced colitis promotes the development of colonic tumors by activation of Dclk1+ cells. Our in vitro findings suggest that inflammatory mediators upregulated in colitis may alter Dclk1+ cells in vitro.

Discussion: Our data suggest that Dclk1+ tuft cells are activated by genetic modification and inflammation-induced tissue injury to initiate colon cancer. These data provide novel insight into the pathogenesis of colitis-associated colorectal cancer and identify potential targets for future therapy in clinical practice.

Keywords: colorectal cancer, DCLK1, tuft cell, inflammatory bowel disease, colitis, APC

Poster Abstract #49

Development of an educational module for grossing colorectal specimens

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The purpose of this project was to develop an education module to highlight the procedure for grossing colorectal cancer specimens. This module includes written instructions along with visual aids and links to synoptic reports. These visual aids include schematic drawings along with high quality photographs, compiled into a presentation using Photoshop and PowerPoint. The photographs provided help explain and illustrate various aspects of grossing such as radial margins and serosal assessment in colon cancer specimens. The module also covers the importance of total mesenteric excisions (TME), which is a type of surgery performed for rectal cancer. This educational module will present the procedure for the proper assessment of colorectal cancer specimens and act as a resource for anyone learning to gross this type of specimen, especially Pathologists' Assistant students and residents.

Keywords: colorectal, pathology, cancer, grossing, teaching

Poster Abstract #50

MRI signaling to non-cancerous abnormalities that could mimic cancer in prostate

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Prostate Cancer (PCa) is the most common non-cutaneous cancer in North American men. Early detection increases the number of treatment options as well as improving survival rates. Trans-rectal ultrasound-guided biopsy is the current accepted standard for diagnosing PCa. However, there is an approximately 40% chance of over- or under-estimating the Gleason grade. Multi parametric MR imaging (mpMRI) has revealed promising results in mapping of PCa and the surrounding tissue. It has the potential to be used as a non-invasive procedure to predict the locations of PCa foci, and prognosis. The aim of the study is to test and examine non-cancerous pathology lesions and normal structures that could mimic cancer in mpMRI signals.

In this study, 17 radical prostatectomy specimens from LHSC were marked with 10 strand-shaped fiducial markers per specimen after fixation in formalin. The external fiducials (lamb kidney) were soaked in Magnevist while the internal fiducials (cotton embroidery floss) were soaked in Magnevist and blue dye. These fiducials were used as landmarks in histology processing and MRI. Ex vivo whole gland imaging was performed with T1- and T2-weighted 3T MRI protocols and then specimens were sliced at 4.4 mm intervals, which were then processed for whole-mount histology sections. Initial registration between fiducial markers on histology and MR images was performed. Based on this registration, we developed an interactive digital technique for deformable registration of in vivo to ex vivo MRI with digital histopathology images. Four radiologists individually identified and contoured potential lesions according to the Prostate Imaging Reporting and Data System (PI-RADS) without prior knowledge or exposure to the pathology report of each case. The relationship between MRI signals and non-cancerous abnormalities that could mimic PCa has not been tested previously in correlation with histopathology digital imaging.

Analysis of the radiology data showed prostatic intraepithelial neoplasia (PIN), atrophy and benign prostatic hyperplasia (BPH) as the main non-cancerous abnormalities responsible for cancer like-signals on mpMRI. This will help increase the accuracy of detecting PCa and play a major role in the diagnosis and classifying the confounders that mimic cancer in MR images.

Keywords: Prostate imaging, 3D prostate reconstruction, prostate MRI, prostate registration, in vivo MRI, prostate cancer

Poster Abstract #51

The regulation of miRNA-711 by HIF-1 and DNA methylation in cardiomyocytes during ischemia-reperfusion injury

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Introduction: Cardiac ischemic injury is a leading cause of morbidity and mortality worldwide with the only treatment being reperfusion of the tissue. Reperfusion has shown to cause further damage to cardiac tissue. Previous studies have demonstrated that ischemia-reperfusion (I/R) injury changes the micro RNA (miRNA) expression pattern of cardiac tissue. miR-711 has been reported to be up-regulated by 10 fold and further research identified miR-711 to have pro-apoptotic effects on cardiomyoctyes. The regulatory pathways of miR-711 are still unknown. In this study, we have attempted to determine potential regulators of miR-711 expression during I/R. We hypothesize that miR-711 expression is regulated by HIF-1 and DNA methylation.

Methods: To test this hypothesis, we cultured rat cardiomyoblast cell line (H9C2). To determine the involvement of DNA methylation during I/R injury we used 5-Azacytide (5AZ), a potent inhibitor of DNA methylation. To determine if HIF-1 is involved we inhibited HIF-1 expression with a silencer RNA. We then chemically induced ischemia by treating the H9C2 cells with Antimycin A (AA) in serum and glucose free media, then we induced reperfusion by exchanging the media with serum and glucose containing media. Finally, we measured the levels of miR-711.

Results: Our results show treatment of H9C2 cells with AA increases the expression of miR-711. Treatment with AA followed by reperfusion also increases miR-711 expression. One time data with AA and 5-AZ treatment suggests an increase in miR-711 expression, however these results are still inconclusive and will be confirmed after repeating the experiment. Results of HIF-1 inhibition on miR-711 expression are also in progress.

Conclusions: Our current results demonstrate that inducing I/R injury increases miR-711 in H9C2 cells. Identifying the regulatory pathway of miR-711 will open up the possibility of targeting this pathway with therapeutic interventions in order to improve cardiac tissue survival after being exposed to I/R injury.

Keywords: Ischemia-reperfusion injury, miR-711, HIF1, ischemic heart disease, cardiomyocytes, DNA methylation

Poster Abstract #52

Evaluating the utility of protein biomarker, S100A7, as a predictor for the transformation of oral dysplasia

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Introduction: Despite advancements in the treatment of oral cancer, the 5-year survival has been relatively unchanged, mostly because the diagnosis of oral cancer often occurs at advanced stages of disease. The progression to oral cancer often follows a stepwise progression through various severities of dysplasia. Histopathology is considered the 'gold standard' for diagnosing dysplasia and oral cancer, leading to subjectivity and variation in diagnosis. Recent work with the protein biomarker, S100A7, in oral dysplasia and squamous cell carcinoma has shown predictive value in the transformation from dysplasia to cancer.

Objective: To determine if there is a correlation between the expression of S100A7 and the histologic grade of oral dysplastic lesions using immunohistochemistry. Our hypothesis is that the expression of S100A7 will correlate with the histologic grade and can thus be used as a reliable prognostic marker for the severity of oral dysplasia.

Methods: 90 biopsies from 27 subjects from 2002-2015 have been obtained from the Western University Pathology Department database. These formalin fixed paraffin embedded samples are undergoing immunohistochemistry to identify the expression of S100A7 using a standard protocol. Expression will be measured both subjectively and objectively and correlation studies will be employed.

Results: Pending.

Discussion: The identification of a reliable, quantitative measure in the diagnosis of dysplasia and the ability to predict the likelihood of transformation to higher grades of dysplasia or malignancy will potentially lead to more individualized treatment and better patient outcomes.

Keywords: dysplasia, cancer, S100A7, transformation, squamous cell carcinoma

Poster Abstract #53

Mechanisms underlying chemotherapy-induced vascular proliferation in ovarian cancer

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Introduction: Ovarian cancer is the leading cause of death among gynaecological malignancies in Canada with over 1500 deaths in 2010. Current treatment consists of surgical debulking of the primary tumour followed by platinum-based carboplatin therapy. Despite an initial response to chemotherapy, a large proportion of patients undergo relapse leading to a significant clinical challenge. The mechanisms underlying carboplatin treatment failure are not fully understood. After observing significant vascular proliferation post-carboplatin treatment in ovarian serous adenocarcinoma patient samples, we hypothesize that platinum-based chemotherapy alters ovarian cancer cells and the tissue microenvironment leading to neovascularization, which in turn contributes to tumour growth.

Methods: To test this hypothesis, we challenged ovarian cancer cells and vascular endothelial cells in culture with carboplatin. To identify potential target factors involved in neovascularization, we profiled different angiogenic factors including matrix proteins, secreted pro-angiogenic factors, and chemokines using quantitative polymerase chain reaction. We will perform a similar screen in pre- and post-chemotherapy patient samples to support the clinical significance of our in vitro findings.

Results: After carboplatin treatment, there was a significant reduction in viability of ovarian cancer cells and vascular endothelial cells. In addition, our studies showed several genes are altered when ovarian cancer and endothelial cells are exposed to carboplatin including a few surprising genes such as leptin.

Discussion: The increase in leptin expression may be an exciting therapeutic target in ovarian cancer, as leptin signalling has been shown to be involved in tumour vascularization in breast cancer as well as its many downstream effects on other pro-angiogenic factors. We plan to modulate leptin as well as other potential pro-angiogenic factors of interest in an effort to investigate the functional significance after chemotherapy treatment in both cell types. Ultimately, our findings may lead to the identification of novel therapeutic targets for patients with ovarian cancer.

Keywords: Ovarian cancer, neovascularization, carboplatin, chemotherapy, relapse

Poster Abstract #54

Role of inflammation in transformation of Dclk1+ colon cancer initiating cells

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Introduction: Recently, it has been proposed that mature non-stem cells can contribute to colorectal tumor formation through dedifferentiation that can occur upon chronic tissue injury. Our previous work demonstrated that Doublecortin-like kinase 1 protein (Dclk1), a mature intestinal tuft cell marker, identifies a rare and ill-defined cell type in the gut that serves as a cellular origin for colitis-associated colorectal cancer. We have observed that quiescent APC mutated (APCf/f) Dclk1+ tuft cells express high levels of cyclo-oxygenase (COX). Thus, we postulate that inhibition of inflammation with corticosteroids or non-steroidal anti-inflammatory drugs (NSAIDs) that inhibition of COX will affect colonic tumor formation.

Methods: To test this hypothesis, small intestinal crypts were isolated from tamoxifen-treated Dclk1-CreERT2/TGFP/APCf/f mice and organoid "min-gut" cultures established. The organoids were then treated with either PBS (control), dexamethasone, or Aspirin and the effects on organoid growth assessed. The effects on the extent of Dclk1+ cell derived lineage tracing and organoid size were evaluated as an outcome measure.

Results: Treatment of organoids with dexamethasone or Aspirin led to a decreased number of Dclk1+ lineage-traced crypts in the culture. Changes in organoid morphology consistent with spheroid formation was observed less frequently in dexamethasone- or Aspirin-treated cultures.

Conclusions: Our findings suggest that inhibition of inflammation by corticosteroids or NSAIDs inhibits stem cell activity of Dclk1+ cells.

Keywords: gastroenterology, inflammatory bowel diseases, colorectal cancer, stem cells, inflammation

Poster Abstract #55

Mitotically active sclerosing stromal tumour of the ovary: Report of a case series

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Introduction: Sclerosing stromal tumour (SST) of the ovary is a rare neoplasm that typically occurs in the second and third decades of life. To date, all reported cases have behaved in a benign manner. In their usual form, these neoplasms exhibit scant, if any, mitotic activity.

Cases: We report a case series of six SSTs with increased mitotic activity (between 7 and 12 mitoses per 10 high power fields in the most mitotically active areas). Follow up is available in 4 of 6 cases (ranging from 3 weeks to 68 months) and one tumour recurred within the pelvis.

Discussion: We recommend that the term mitotically active SST is used for such neoplasms. Given that one of the tumours in our series exhibited recurrence in the pelvis, we suggest that mitotically active SSTs do not have an invariable benign behaviour but can, in occasional cases, exhibit local recurrence. Parallels can be drawn with the recently described mitotically active cellular fibroma, another benign ovarian stromal neoplasm which occasionally recurs locally, but which does not metastasize.

Keywords: ovary, sclerosing stromal tumour, mitotically active sclerosing stromal tumour, recurrent sclerosing stromal tumour

Poster Abstract #56

Expression levels of growth hormone secretagogue receptor 1a and ghrelin in early diabetes

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Introduction: Diabetic cardiomyopathy (DCM) is a highly prevalent cardiac disorder in the diabetic population and can lead to heart failure. Early DCM is subclinical and there is a limited array of tools available for early detection of DCM, thus limiting early initiation of treatment against heart failure. There has been increasing evidence supporting the cardioprotective role of ghrelin and its receptor, growth hormone secretagogue receptor 1a (GHS-R1a). Our laboratory has previously observed a decrease in cardiac GHS-R1a expression concomitant with reduced cardiac function in a streptozotocin(STZ)-induced mouse model of diabetes, identifying GHS-R1a and ghrelin levels as potential novel biomarkers of DCM. In this study, I aimed to determine if cardiac GHS-R1a and ghrelin levels change in diabetes using an STZ-induced diabetic mouse model. I hypothesize that cardiac expression levels of GHS-R1a and ghrelin are decreased in STZ-diabetic mice.

Methods: Heart tissues were sectioned from 8 control mice and 8 STZ-diabetic mice, on which immunohistochemistry was performed for ghrelin and histochemistry was performed for GHS-R1a. Samples were imaged under fluorescence microscopy, with mean fluorescent intensity reflecting ghrelin and GHS-R1a levels. A custom-written ImageJ script was then used to quantitate levels of ghrelin and GHS-R1a in each tissue sample.

Results: Levels of GHS-R1a and ghrelin were observed to be significantly lower in heart tissues of STZ-diabetic mice versus control mice.

Conclusions: These findings demonstrate that cardiac expression of GHS-R1a and ghrelin are decreased in diabetes. The results of my study are the first to indicate the potential usage of GHS-R1a and ghrelin as novel biomarkers of DCM, a step towards early detection and eventually treatment in preventing the progression to heart failure in diabetic patients.

Keywords: Diabetic cardiomyopathy, ghrelin, growth hormone secretagogue receptor 1a, biomarker, diabetes, heart failure

Notes

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