PATHOLOGY AND LABORATORY MEDICINE

RESEARCH DAY 2017

MARCH 30, 2017

Program Guide

Schulich MEDICINE & DENTISTRY

Western

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. We have set a record with 71 research presentations this year. This is certainly an amazing achievement for our small department. As you listen to the platform presentations and view the posters, you realize our truly multi-disciplinary approach to studying health and disease.

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, Jina Kum, Vy Ngo, Terry Robbins, Shannon Hubbert, 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,
Schulich Medicine & Dentistry, Western University
Chief, Department of Pathology and Laboratory Medicine,
London Health Sciences Centre and St. Joseph's Health Care

AGENDA

9:30 - 9:40 am	**Welcome and Opening Remarks** The Great Hall, Somerville House **Dr. Subrata Chakrabarti** Chair/Chief, Pathology and Laboratory Medicine, Schulich Medicine & Dentistry, Western University and London Health Sciences Centre
9:40 - 10:00 am	**Group Photo**
10:00 - 11:00 am	**Platform Presentations - Session 1**
11:00 - 11:30 am	**Nutritional Break**
11:30 - 12:30 pm	**Platform Presentations - Session 2**
12:30 - 12:45 pm	**CME Evaluations**
12:45 - 1:30 pm	**Lunch** The Great Hall, Somerville House
1:30 - 2:30 pm	**Poster Presentations - Session 1**
2:30 - 3:30 pm	**Poster Presentations - Session 2**
4:00 - 7:00 pm	**Awards Ceremony** Windermere Manor

Overarching Learning Objectives:

1. Discuss the findings from Pathology research conducted in the Department of Pathology and Laboratory Medicine at Western University and the London Health Sciences Centre with colleagues and translate knowledge into practice, teaching, and research.

2. Understand which research tools and expertise are available to facilitate basic and clinical science research at the Department of Pathology and Laboratory Medicine at Western University and the London Health Sciences Centre.

3. Address emerging evidence in the pathogenesis of acute and chronic diseases.

This event is an Accredited Group Learning Activity (Section 1) as defined by the Maintenance of Certification program of The Royal College of Physicians and Surgeons of Canada, approved by Continuing Professional Development, Schulich School of Medicine & Dentistry, Western University (2.5 hours). Each participant should claim only those hours of credit that he/she actually spent participating in the education program.

Session 1
10:00 - 11:00 am

Time	Last Name	First Name	Title
10:00 am	Cecchini	Matthew	The Utility of CDX2 Loss as a Prognostic Marker in Stage II Colon Cancer
10:15 am	Good	Hayley	The Effect of Non-steroidal Anti-inflammatory Drugs on the Initiation of Dclk1+ Tuft Cell-derived Colitis-associated Cancer
10:30 am	Goebel	Emily	Insufficient and Scant Endometrial Samples: Determining Clinicopathological Outcomes and Consistency in Reporting
10:45 am	Kim	Yohan	Metastatic Efficiency is Dependent on Cell Volume Loss from Microparticle Release During Cancer Cell Extravasation

Session 2
11:30 - 12:30 pm

Time	Last Name	First Name	Title
11:30 am	Krstic	Milica	The Transcriptional Regulator TBX3 Promotes Progression from Non-invasive to Invasive Breast Cancer
11:45 am	Kubica	Matthew	Diagnostic Rates Before and After Implementation of The Paris System for Reporting Urinary Cytology
12:00 pm	Ngo	Vy	The Interaction Between p21 and Nrf2 Regulates Oxidative Stress in Cancer
12:15 pm	Langdon	Kristopher	Adult-onset Progressive Dementia and Myoclonic Epilepsy with Polyglucosan Bodies

The Utility of CDX2 Loss as a Prognostic Marker in Stage II Colon Cancer

Matthew J. Cecchini[1], *Joanna C. Walsh*[1], *Jeremy Parfitt*[1], *Subrata Chakrabarti*[1], *Rohann Correa*[2], *Mary J. MacKenzie*[3], *David K. Driman*[1]

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Introduction: Colorectal cancer (CRC) is the second commonest cause of cancer death in Canadians. The treatment for CRC is largely surgical with resection of the primary tumor, and treatment with adjuvant chemotherapy in specific cases, usually stage III and IV. In patients with stage II cancer, there is no clear benefit for chemotherapy but it is still commonly used for patients with perceived high risk. A recent landmark paper identified that cancers with loss of CDX2 expression had a significantly worse prognosis and this could be utilized to identify patients that would benefit from chemotherapy.

Methods: To validate these studies for clinical practice we obtained archival cases of stage II colon cancer that did not receive adjuvant chemotherapy. The pathology of all cases was reviewed and three blocks were selected for CDX2 immunohistochemistry. CDX2 expression was scored based on previously published criteria and using semi-quantitative measures of CDX2 expression.

Results: We studied 122 cases. CDX2 expression was diffusely lost in 11% and focally lost in up to 30% of cases. Further, we identified significant variation in CDX2 expression in a given tissue section in more than half of the cases. We did not identify a difference in survival based on CDX2 expression. Further, we assessed CDX2 expression in cases with metastatic disease and found the expression of CDX2 to be maintained in the majority of cases.

Discussion: Our results with whole slide immunohistochemistry are distinct from previous studies and may be due to the fact that these studies were largely based upon tissue microarrays in which only small parts of the tumor were assessed for CDX2 expression. This raises doubt about the use of CDX2 as a prognostic marker in clinical practice. We are currently working to identify other markers that may be more reliable predictors of survival in CRC.

Keywords: Colon cancer, CDX2, chemotherapy

The effect of non-steroidal anti-inflammatory drugs on the initiation of Dclk1+ tuft cell-derived colitis-associated cancer

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Introduction: Chronic inflammation, such as that in inflammatory bowel disease (IBD), is a major risk factor for the development of colorectal cancer (CRC). Doublecortin-like kinase-1 (Dclk1) is a marker of tuft cells, a rare and ill-defined mature cell type of the colon. Our previous work demonstrated that Dclk1+ tuft cells are long-lived and quiescent even with a mutation of the tumour suppressor APC. Yet under inflammatory conditions, these cells become powerful cancer-initiating cells. However, the mechanism by which inflammation leads to tumour-initiation from the Dclk1+ tuft cell is not known. Interestingly, Dclk1+ tuft cells express high levels of both cyclo-oxygenase (COX)-1 and -2, the enzyme target of non-steroidal anti-inflammatory drugs (NSAIDs), namely Aspirin and celecoxib, which have been shown to be chemopreventative in initiation of colon cancer. Therefore, we hypothesize that inhibition of the COX pathway with the use of these NSAIDs will inhibit Dclk1+ cell-derived tumour formation.

Methods: To test this hypothesis, Dclk1CreERT2/APCflox/flox mice were administered tamoxifen to induce an APC mutation in Dclk1-expressing cells. Mice were then exposed to the colitis-inducing agent dextran sodium sulfate (DSS), followed by daily treatment with Aspirin and/or celecoxib for the remainder of the experiment. Approximately 16 weeks post-tamoxifen, colonic tumour number and size were analyzed to determine the effect of these COX-inhibiting NSAIDs on tumour growth and initiation, respectively.

Results: Our results show that Aspirin treatment leads to a decreased number of Dclk1+ cell-derived tumours, whereas treatment with celecoxib results in an increased number of tumours. There was no significant difference in tumour size between treatment groups.

Conclusions: These findings suggest a role for COX-mediated inflammation in the mechanism underlying tumour initiation from Dclk1+ tuft cells. Our results provide insight into the mechanism and use of COX-inhibiting NSAIDs (i.e. Aspirin, celecoxib) in the chemoprevention and/or promotion of CRC in patients with IBD.

Keywords: DCLK1, colitis, colorectal cancer, NSAIDs, cyclo-oxygenase, gastroenterology

Insufficient and Scant Endometrial Samples: Determining Clinicopathological Outcomes and Consistency in Reporting

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Introduction: There are no defined pathologic criteria for reporting endometrial samples (ems) with limited tissue as either scant or insufficient and no consensus on the clinical follow up of patients with these samples. The purpose of this study was to compare clinicopathological outcomes and determine reporting consistency for scant and insufficient ems.

Methods: 1) Retrospective chart review of all patients with insufficient or scant ems from 2010-13 at our center to determine repeat sampling and final pathological diagnosis; 2) survey of gynecologists using an anonymous questionnaire about their practice for managing patients with these samples; 3) review of 99 ems previously reported as scant or insufficient in which four reviewers blindly and separately reassigned cases as scant, insufficient or diagnostic. Agreement was determined across reviewers.

Results: 1149 patients had insufficient (49%) or scant (51%) ems with no significant difference in rate of repeat biopsy (33% vs. 31%, p-value 0.33). Subsequent surgery was slightly higher following insufficient (16%) compared to scant ems (11%), p-value 0.02. Final diagnosis of uterine malignancy was higher in patients with previous insufficient ems than with scant (19% and 9% respectively) but not statistically significant. In the survey, 4/5 gynecologists reported managing patients with insufficient or scant ems similarly. In the case review, there was complete consensus across raters in 57% of cases (Fleiss Kappa 0.4891).

Conclusions: There was moderate agreement between reviewers when reporting limited tissue ems. Similar repeat ems rates between scant and insufficient samples suggest that our clinicians choose similar management for both of these terminologies, in keeping with the questionnaire results. As such, distinction between insufficient and scant ems may not be necessary in pathological reporting. Given the malignancy outcomes, both insufficient and scant ems merit repeat sampling in the appropriate context.

Keywords: gynecologic pathology; endometrial sampling; insufficient endometrial biopsy; clinicopathological outcomes; quality assurance

Metastatic Efficiency is Dependent on Cell Volume Loss from Microparticle Release During Cancer Cell Extravasation

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Introduction: Metastasis is the main cause of mortality and morbidity in cancer patients. Tumor cells from primary tumor enter the bloodstream (intravasation) and cross the vessel wall (extravasation) to form secondary colonies. Thus, targeting extravasation is a good candidate for halting cancer metastasis. Surprisingly, cancer cell microparticle release during extravasation led to significant cell volume loss affecting metastatic colony formation rates. Since induction of necroptosis (a programmed necrosis) resulted in a significant increase of microparticle release, we hypothesize that inducing cancer cell necroptosis leads to cell volume reduction, inhibition of cell extravasation and metastasis.

Methods: Invasive human breast/prostate cancer cell lines were cultured and injected into the chorioallantoic membrane (CAM) of chicken embryos. We performed intravital imaging of cancer cell microparticle release and extravasation. To quantitate microparticles released from cancer cells, we used a nanoscale flow cytometer to analyze plasmas from the CAMs or conditioned media.

Results: Our results show that an increase in circulating cancer cell microparticle release significantly reduces extravasation rates of cancer cells and metastatic colony formation rates. Although pro-apoptotic cancer cells released elevated amounts of microparticles that resulted in reduced extravasation rates, extravasating cancer cells showed the absence of caspase-3 activity on microparticle release. Pro-necroptotic cancer cells showed an increase in cancer cell microparticle release with cell volume reduction and a decrease in cancer cell extravasation rates. Inhibition of intravascular cancer cell necroptosis improved extravasation rates remarkably and reduced microparticle release drastically.

Discussion: Our findings recapitulated that a reduction in cell volume by releasing microparticles facilitates extravasation, at the cost of reduced efficiency in forming secondary colonies. Although the pro-apoptotic process of cancer cells can stimulate more microparticle release, results from the inhibition of necroptosis and the pro-necroptotic process implicates that necroptosis is a more important regulator of cancer metastasis.

Keywords: Metastasis, extravasation, extracellular vesicle, microparticle, necroptosis, apoptosis.

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

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

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Introduction: In cell lines derived from the same breast cancer patient at different phases of progression, we have shown that TBX3 is abundant in the invasive 21MT-1 cells, and minimally expressed in the non-invasive, DCIS-like 21NT cells. There are two isoforms of TBX3, with slightly different DNA binding domains.

Methods: TBX3 isoforms were overexpressed in 21NT cells. Functional changes and gene expression changes were assessed. Cells were injected into the chick embryo vasculature. Cells were implanted into nude mice. In vitro angiogenesis assays were performed. Patient samples of early breast cancer cases (186) were examined for TBX3 expression by immunohistochemistry.

Results: Overexpression of TBX3iso1 or TBX3iso2 in non-invasive 21NT cells resulted in increased survival, growth and invasiveness in vitro, with increased extravasation and invadopodia formation in the chick embryo. Through genome-wide ChIP-array studies coupled to RNA-Seq we have mined the direct transcriptional targets of both isoforms. Our results indicate that both TBX3 isoforms promote invasiveness through altered expression of EMT-related genes, including the direct up-regulation of EMT-inducing transcription factor SLUG. SLUG expression is required for TBX3-induced migration and invasion of breast cancer cells. Assessing TBX3 levels in early stage breast cancer by immunohistochemistry revealed that expression was high in low-grade lesions, suggesting TBX3 involvement in progression through the low-grade DCIS molecular pathway. We will continue to validate markers from our genomic studies by immunohistochemistry to assess whether they are correlated with increased risk for developing invasive cancer.
Interestingly, only TBX3iso1 overexpressing cells exhibited significant tumorigenic potential in nude mice. We have performed data mining and supporting functional studies that suggest this is likely due to the promotion of angiogenesis and secretion of cancer-associated cytokines upon TBX3iso1 overexpression.

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, SLUG, breast cancer, ductal carcinoma in situ (DCIS), invasive mammary carcinoma (IMC), epithelial-mesenchymal transition (EMT)

Diagnostic rates before and after implementation of The Paris System for Reporting Urinary Cytology

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Background: Urine cytology is an efficient method of diagnosing clinically occult urothelial carcinoma; however, until recently there were no standardized criteria for reporting urine cytology and over-reporting of "atypical" cases posed challenges for clinical management. In 2016, The Paris System for Reporting Urinary Cytology (TPS) was developed as a means of standardizing urine cytology reporting through the use of set morphological criteria and diagnostic categories. TPS was implemented by the cytopathology division at London Health Sciences Centre (LHSC) in September of 2016.

Methods: We compared urine cytology results from two time periods: before (September-December 2015) and after (September-December 2016) the implementation of TPS at LHSC. Statistical comparisons in rates of diagnostic categories were performed using chi-square analyses ($P < 0.05$ significant).

Results: 2576 urine cytology specimens were submitted during the study periods and the results are summarized as follows:

	Sept – Dec 2015	Sept – Dec 2016	p-value
Total Cases	1317	1259	-
Negative for HGUC*	978 (74%)	1123 (89%)	0.002
Atypical Urothelial Cells (AUC)	288 (22%)	115 (9%)	< 0.001
Suspicious for HGUC	29 (2.2%)	4 (0.3%)	< 0.001
Positive for HGUC	22 (1.7%)	17 (1.4%)	0.51

*HGUC, high grade urothelial carcinoma

Discussion: Implementation of TPS at our institution has resulted in an increase in the proportion of negative cases reported, with a corresponding significant reduction in the number of atypical and suspicious cases. The proportion of positive cases did not differ between periods. It is likely that cases previously called atypical are now being reported as negative. Our results suggest that implementing TPS may provide clearer diagnostic direction for clinicians.

Keywords: urine cytology, Paris system, urothelial carcinoma, atypical urothelial cells

The interaction between p21 and Nrf2 regulates oxidative stress in cancer

Vy Ngo *and Martin Duennwald*

Department of Pathology and Laboratory Medicine, Western University

Introduction: Nrf2 (NF-E2-related factor 2) is the master transcriptional regulator of the antioxidant response required for cellular redox homeostasis. Constitutive Nrf2 activation, however, facilitates tumorigenesis and chemoresistance by protecting cancer cells from reactive oxygen species and chemotherapeutics. p21 is a poorly studied activator of Nrf2 with strong links to cancer. We present a novel approach to studying the mechanisms and functional outcomes of p21-Nrf2 interaction in baker's yeast (S. cerevisiae). Aberrant interactions between p21 and Nrf2 alter the oxidative stress response, leading to Nrf2 hyperactivation in cancer.

Methods: Yeast spotting assays will assess genetic protein interactions. The split-ubiquitin system will examine the physical binding patterns of wild-type and cancer-associated mutants of p21 and Nrf2. These findings will be validated in cultured mammalian cells. Nrf2 transcription activity will be determined by reporter assays and transcriptional profiling. The objective is to determine how p21 regulates Nrf2's activity as a transcription factor through Nrf2 localization, aggregation, and stability.

Results: Yeast spotting assays reveal a toxic growth phenotype for Nrf2 expressed in yeast, while p21 is only mildly toxic. When p21 is co-expressed with Nrf2, Nrf2 toxicity is rescued. Results from the split-ubiquitin system suggests that a strong physical interaction occurs. Interestingly, when the HeLa mammalian cell line is transfected with cancer-associated mutants of Nrf2, distinct protein aggregates are formed.

Discussion: p21 alters Nrf2 expression. The novel protein aggregates formed by cancer-associated mutants of Nrf2 serves as an interesting link between protein misfolding and cancer. This study will contribute to our understanding of the cellular mechanisms of p21 and Nrf2 interaction and allow for the classification of somatic mutations based on functional outcome. Regulation of p21 and Nrf2 activity has great therapeutic potential for the development of drugs and healthcare strategies that target protein quality control mechanisms and combat tumour development and chemoresistance.

Keywords: Nrf2, p21, oxidative stress, cancer, protein misfolding

Adult-onset Progressive Dementia and Myoclonic Epilepsy with Polyglucosan Bodies

***Kristopher D Langdon**, Arunee Singsnaeh, G Bryan Young and Robert R Hammond*

Department of Pathology and Laboratory Medicine, Western University

This 65 year-old left hand dominant male was referred for progressive cognitive decline with a working diagnosis of cortical basal degeneration versus Alzheimer's disease. The Patient also had a 10-12 year history of spontaneous myoclonic jerks partially controlled with Valproic Acid. There were no reported sensory or bladder changes and no episodes of status epilepticus. Neuropsychological assessment was consistent with generalized cognitive impairment that suggested a widespread dementing illness with a MoCA of 8/30 which had deteriorated from 14/30 in the year prior. Other exam findings demonstrated difficulty with upward gaze, apraxia and a wide-based and unsteady gait. Electroencephalographic studies revealed dysrhythmia Grade IV, generalized spikes, polyspike and wave discharges, several of which were associated with myoclonic jerks, consistent with generalized epilepsy. MRI revealed generalized cerebral and cerebellar atrophy with ventriculomegaly. Post-mortem examination failed to demonstrate significant neurofibrillary degenerative changes. Of note however, there were abundant polyglucosan bodies. These were most prominent within cerebellum, hippocampal CA4, cerebral white matter and subpial regions. Results from electron and confocal microscopy will be discussed as this pertains to neuronal localization as well as a comparison with age-matched controls and a case of childhood Lafora body disease.

Keywords: neurodegeneration; epilepsy; confocal imaging; polyglucosan bodies; Lafora disease

Session 1
1:30 - 2:30 pm

#	Last Name	First Name	Title
1	Goebel	Emily	Intradepartmental Consultations in Surgical Pathology: Review of a Standardized Process and Factors Influencing Consultation Rates and Practices
2	Cox	Jacqueline	Human Tissue Kallikreins (KLKs) in Polymorphous Low Grade Adenocarcinoma (PLGA)
3	Aref-Eshghi	Erfan	Identification of a DNA Methylation Signature of PTEN Loss in Prostate Cancer
4	Aref-Eshghi	Erfan	Glucose- and Aging-induced Heterogeneous Alterations of DNA Methylation in Endothelial cells
5	Armstrong	James	The Effects of BMP4 on the Cellular Phenotype of Human Tenon's Capsule Fibroblasts
6	Di Gregorio	Sonja	Aging as a Major Modifier of Polyglutamine Aggregation and Toxicity
7	Pillon	Brittany	Youth Suicide Age and Sex Related Trends in Ontario First Nations Communities: A Comparison
8	Harnett	Amber	Characterization of 5-Lipooxygenase Expressing Tuft Cells in Colitis-associated Cancer Using a Novel Mouse Model
9	Aldhafeeri	Hamad	In Vivo/Vitro CRISPR Screen for the Identification of Kinases that Regulate Prostate Cancer Metastasis

#	Last Name	First Name	Title
10	Gomes	Janice	Development of a Microparticle-based Tool as an Indicator of the Effectiveness of Dialysis Treatment
11	Hu	Jonathan	Effects of Shroom3 CRISPR/Cas9 Knockout on Nephron Duct Morphogenesis in Xenopus laevis
12	Chan	Enid	Evaluating the Comparison Between Intra- and Inter-host Evolutionary Rate Patterns Within Different Viruses
13	Harriman	Ariel	The Effectiveness of ProExC as a Surrogate Marker of High Risk Human Papillomavirus Infection in Oral Epithelial Dysplasia: A Pilot Study
14	Sharpley	Stephanie	The Profile of a Pathologists' Assistant (PA) in North America
15	Hubbert	Shannon	The Profile of a Forensic Pathologists' Assistant
16	Robbins	Terry	Suitability of RNA Isolated from Archived Formalin-Fixed, Paraffin-Embedded Oral Dysplastic Lesions for Diagnosis of an HPV Etiology
17	Hong	Guangliang	Administration of Nicotinamide Riboside Reduces Lung Injury and Improves the Survival in Sepsis
18	Chen	Jennifer	Dose Determination of Passive Exercise in ICU Patients with Sepsis

#	Last Name	First Name	Title
19	AlGhefari	Huda	A Primary Epithelioid Sarcoma of the Clivus, Case Report
20	Wallis	Julie	Quality Management for the Autopsy Service - London Health Sciences Centre
21	Bhatty	Dhwanil	The Effect of Ischemic Pre-conditioning on the Severity of Microvascular Dysfunction from Abdominal Ischemia-Reperfusion Injury
22	Chang	Winston	Corticosteroid-Induced CRTh2 Expression in TH2 Cells: A Role in Persistent Asthma
23	Biswas	Saumik	Role of Long Non-coding RNA MALAT1 in the Pathogenesis of Diabetic Retinopathy
24	Gan	Ingrid	Mitochondrial Permeability can Regulate Endothelial Cell Necroptosis and Promote Cardiac Allograft Rejection
25	Nadias	Claudinne	Leukocyte Common Antigen (CD45) Expression in Carotid Atherosclerotic Plaques
26	Asadi	Farzad	A Novel Theory on Mechanisms of Glucagon Secretion from Pancreatic Alpha Cells
27	Filek	Richard	A Clinico-Pathological Study of the Structural and Functional Changes to the Retina and Optic Nerve following anti-VEGF Treatments for Diabetic Macular Edema

Session 2
2:30 - 3:30 pm

#	Last Name	First Name	Title
28	Aref-Eshghi	Erfan	Genome-wide DNA Methylation Testing in Patients with Developmental Delay and Intellectual Disabilities
29	Veitch	Matthew	Effect of Mechanical Strain on TGFβ-induced Collagen Expression in Human Trabecular Meshwork Cells
30	Morrison	Matthew	Kallikrein-related Peptidases are Dysregulated in Pleomorphic Adenoma
31	Tai	Felicia	Investigating the Role of SLUG in TBX3-induced Epithelial-Mesenchymal Transition of Breast Cancer Cells
32	McLean	Lachlan	Oral Epithelial Dysplasia: Evaluation of Serial Biopsies using S100A7
33	Vijeyakumaran	Meerah	Effect of Estrogen and Glucocorticoid Signaling on Th2 cells – Implications for Severe Asthma
34	Wu	Derek	Characterization of Growth Hormone Secretagogue Receptor 1a for cardiac imaging
35	Ruicci	Kara	HRAS G12V Predicts for Innate Resistance to PI3Kα Inhibition in Head and Neck Squamous Cell Cancer
36	Sullivan	Rebecca	Expression of the Growth Hormone Secretagogue Receptor and Ghrelin in Human Heart Failure

#	Last Name	First Name	Title
37	Inkaran	Jeyanth	Salivary Gland Neoplasia – Culturing a Potential Model Cancer Cell Line Out of A-253 Cells.
38	Padda	Ranjit	Aberrant Polysialylation of Proteins Potentially leads to Prostate Cancer Metastases
39	Kum	Jina	Transforming Growth Factor-β1 Pathway Regulates Differentiation of Bone Marrow-derived Progenitor Cells
40	Kanagalingam	Tharsan	The Effect of Glucocorticosteroid Treatment on Th2 cells
41	Montwill	Natalie	Defining the cellular origin of infantile hemangioma
42	Poon	Andrew	Evolutionary arms race in cancer: Murine melanoma as a model for tumor heterogeneity
43	Schroeder	Peyton	The Relation of Injury Severity for Child Occupants under the Age of Six and the Time of Day of Motor Vehicle Collisions
44	Trelford	Charles	The Validation of a 3D Bioartificial Tissue of the Tenon's Capsule
45	Plantinga	Paul	Histological Elucidation of Tumour Deposits in Colorectal Adenocarcinoma

#	Last Name	First Name	Title
46	Lall	Karan	Deciphering the Role of the UBL Domain in Parkin Degradation
47	Thomas	Anu	ANRIL: a regulator of VEGF in Diabetic Retinopathy
48	Shun	Edward	The Impact of miR-128 in Cold Ischemia-Reperfusion Injury in Cultured Rat Cardiomyoblasts
49	Shin	Alice	Role of Doublecortin-like Kinase 1 (Dclk1) Positive Tuft Cells in Colitis-associated Colorectal Cancer
50	Kahramanoglu	Zeynep	Carboplatin Paradoxically Increases Angiogenic Factors in Ovarian Cancer Cells
51	Alharbi	Hajed	Immunological Impact of CLI095 on Dendritic Cell Maturation and Hypoxia-re-oxygenation Induced Inflammation Injury
52	Oakie	Amanda	The Examination of Prolonged High-fat Diet on Islet Function in Adult MIP-βIRKO Mice
53	Kerkhof	Jennifer	Clinical Validation of a NGS Pipeline that Outperforms Sanger Sequencing and MLPA Analysis
54	Rogala	Ben	Investigating antigen presenting cell-phenotype of granular cell tumors
55	Zheng	Dong	Cardiac-specific Over-expression of Tcap Reduces Doxorubicin-induced Cardiotoxicity in Mice

#	Last Name	First Name	Title
56	Shah	Meera	Histogenesis and Immunohistochemical Profiles of Granular Cell Tumours
57	Teng	Xiaomei	Endotheliocyte-specific Deletion of Capns1 Reduces Diabetic Cardiomyopathy in Mice by Improving Angiogenesis
58	Sanwal	Rajiv	Characterization of 5-Lipoxygenase expressing Epithelial Cells in Colitis-associated Colorectal Cancer
59	Shah	Sundip	Analysis of CD123 and Retinoblastoma (Rb) protein immunohistochemistry in Blastic Plasmacytoid Dendritic Cell Neoplasm
60	Teitelbaum	Daniel	Alteration of Mitochondrial Sirtuin Expression in Endothelial Cells Under High Glucose Conditions
61	Shi	Thomas	A Case Report of Sudden Death from Intracardiac Leiomyomatosis
62	Nesen	Cornelius	Detecting virus compartmentalization within hosts from genetic sequence variation
63	Bushra	Maham	The Effects of Repeated Exposure to Whole Body Vibration on Murine Intervertebral Disc and Knee Joint Health

Intradepartmental Consultations in Surgical Pathology: Review of a Standardized Process and Factors Influencing Consultation Rates and Practices

***Emily A. Goebel**, Helen Ettler, Joanna C. Walsh*

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Introduction: Intradepartmental consultations (ICs) are important for quality assurance (QA) and ensuring diagnostic accuracy in surgical pathology. Our department has instituted a formal process for documentation of ICs and pathologists are encouraged to use an IC form; however, informal ICs also take place. This study reviews IC data and factors that influence formal (written) and informal (verbal) IC rates.

Methods: Formal IC records from January to December 2015 were reviewed and percentage of consultations provided and requested by each pathologist were determined and correlated with years of experience and office location. Pathologists were invited to complete an anonymous survey about their IC practice.

Results: Twenty-four pathologists (96%) completed the survey. Formal IC was requested on 5% of total surgical pathology cases for 2015. Ninety-two percent sometimes requested informal, usually undocumented ICs. Formal ICs were documented in the final report always (76%) or most of the time (24%). Mean formal:informal IC rates were 79:21% (pathologist estimates). With increased years of experience, the ratio of requested to provided ICs tended to decrease. Perceived level of expertise of a colleague was the highest ranked reason for colleague selection for both formal (100%) and informal (83%) IC. Sixteen percent of respondents chose good/friendly relationship with a colleague as their first reason for selecting a colleague for informal IC. Office proximity was a higher ranked factor for informal IC. A poor relationship with a colleague was a potential deterrent to formal IC for 32% and informal IC for 65%. Publication of IC rates led 27% to increase their formal IC rate.

Conclusion: Written documentation of IC aids in QA and determination of IC metrics; however, informal, undocumented ICs still occur. Reasons for IC and choice of consulting pathologist are multifactorial with interpersonal relationships and office proximity having a greater impact on informal IC practice.

Keywords: intradepartmental consultation, quality assurance, surgical pathology

Human Tissue Kallikreins (KLKs) in Polymorphous Low Grade Adenocarcinoma (PLGA)

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Introduction: Polymorphous low grade adenocarcinoma (PLGA) is the second most common malignant salivary gland tumor of the minor salivary glands. Human tissue kallikreins (KLKs) are a family of highly conserved serine proteases expressed by various tissues throughout the body. KLKs have become powerful tumor markers for the diagnosis of the cancer patient (e.g. PSA (KLK3)). The literature demonstrates a link between KLKs and salivary gland neoplasms. The purpose of this study is to determine levels of KLK mRNA in tissue samples of formalin fixed paraffin embedded polymorphous low grade adenocarcinoma (PLGA). Secondly, we wish to determine if KLK expression is limited to tumor cells alone.

Methods: Nineteen cases of PLGA were reviewed (1987-2013). A diagnosis of PLGA was confirmed, demographic data was collected, and formalin fixed paraffin-embedded PLGA and normal salivary gland tissue samples were obtained. RNA isolation was achieved, followed by conversion to complementary DNA via reverse transcription. Synthesized DNA primers were added to target kallikrein DNA and through PCR, the quantitative level of expression of KLKs 1-15 was recorded. Samples exhibiting high and low KLK expression were selected for immunohistochemistry staining, using a standard protocol.

Results: Preliminary PCR data reveals an increase in the mean KLK (1-15) mRNA expression in all of the PLGA tissue samples, as compared with normal salivary gland tissue. KLK1, KLK4, KLK10, KLK12, and KLK15 showed statistically significance (Mann Whitney U test, $p<0.05$). Immunohistochemistry results demonstrate tumor specific staining. Notably, all samples demonstrating high KLK mRNA expression showed equivalent or increased staining grade scores relative to the low KLK mRNA expression samples, with respect to a specific KLK.

Discussion/Conclusion: KLK mRNA is increased in tissue samples of polymorphous low grade adenocarcinoma. Furthermore, the tumor cells stain positively and specifically for kallikreins.

Keywords: Salivary gland neoplasm, polymorphous low-grade adenocarcinoma, human tissue kallikreins

Identification of a DNA methylation signature of PTEN loss in prostate cancer

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Introduction: Loss of function mutations in Phosphatase and Tensin homolog (PTEN) are associated with a poor prognosis in prostate cancer; however, molecular consequences of loss of PTEN are not well understood. Using a genome-wide approach, we aimed to describe the DNA methylation profile of human prostate cancer tissues, and to identify the specific “epi-signature” associated with loss of PTEN gene expression.

Methods: The PTEN status of macrodisected FFPE tissues (30 tumors and 16 normal; prostatectomy and matched needle biopsy) was assessed by immunohistochemistry and FISH analysis. Genome-wide methylation analysis was conducted using Illumina infinium methylation 450k arrays. A moderated T-test and a multivariable linear model analysis compared PTEN loss with genome-wide DNA methylation. Hierarchical clustering and gene enrichment analysis were conducted, and validated algorithms were used to identify regional methylation changes.

Results: Comparison of the normal and tumor tissues identified over 250 abnormally methylated regions including promoter hypermethylation of tumor suppressor genes such as APC and the Protocadherin cluster. The level of PTEN loss was associated with 28 defectively methylated regions including hypomethylation of the PTK6 promoter (associated with cancer progression), and genes involved in the regulation of cell growth, differentiation and senescence including AGPAT4, FGFR2, ISL2, and DIP2C.

Conclusion: We identified a DNA methylation epi-signature of PTEN loss in human prostate cancer involving genes and pathways responsible for cancer progression. Follow up study of a larger clinically-defined prostate cancer cohort is warranted to confirm these findings and assess clinical prognostic utility of these findings.

Keywords: DNA Methylation, Prostate Cancer, PTEN

Glucose- and aging-induced heterogeneous alterations of DNA methylation in endothelial cells

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Introduction: Endothelial damage due to glucose toxicity and subsequent epigenetic changes are characteristic features of chronic diabetic complications. Interestingly, cellular phenotypic changes in diabetes are similar to those in aging, but are accelerated in diabetes. We investigated DNA methylation associated with aging and high glucose exposure in various endothelial cells(ECs).

Methods: Human Umbilical Vein ECs(HUVECs, immature large vessel EC) and Human Retinal microvascular ECs(HRECs, mature microvascular EC) were grown. They were incubated with either 5mM glucose(NG, mimicking euglycemia) or 25mM glucose(HG, mimicking hyperglycemia) for 2 or 7-days. Following DNA extraction, genome-wide methylation assays wer conducted using Illumina-Infinium-Methylation-EPIC-Bead Chip array. The data were divided into 4 groups (NG-2days, NG-7days, HG-2days, HG-7days) for each cell type. Using a linear mixed effects model, incorporating the cell type as random effect, we searched for CpG sites with methylation levels correlating with the eight conditions.

Results: Hierarchical clustering of the cells using the top 7,020 CpG sites($p<5.76e-8$) revealed three clusters: HUVECs NG-2 and 7days, HG-2days; HUVECs HG-7days and HRECs NG-2days; HRECs, NG-7days, HG-2 and 7days. Among the 108 genomic regions that identified to contain a minimum of three adjacent probes ($p<0.001$) were the hypermethylation of the promoters of metalloproteinase inhibitors(BATF3), Fibronectin, vascular disease associated genes(SLC26A11, SMAD6), cell cycle regulations(CCND2), and hypomethlated genes involved in cell death and apoptosis(LRDD, ATAD5), TGF-beta signalling pathway(LDLRAD4), glucose metabolism and insulin regulations(IGBP1).

Conclusion: Data from this study demonstrated that both during physiologic aging and high glucose exposure associated endothelial DNA methylation patterns vary between an immature macrovascular cell and a mature microvascular cell, being more pronounced in the former. Furthermore, although aging caused similar changes in methylation, it was exaggerated following high glucose exposure.

Keywords: Aging, Diabetes, Methylation, Endothelial cells

The Effects of BMP4 on the Cellular Phenotype of Human Tenon's Capsule Fibroblasts

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Introduction: Glaucoma filtering surgery has a substantial failure rate. The challenges of this surgery are largely based on anticipating and modulating the post-surgical healing response in Tenon's capsule. Novel methods of reducing scar tissue development are in high demand. Recent evidence has shown that, in the skin, dermal myofibroblasts can differentiate into adipocytes. It was shown that this reprogramming is contingent on BMP4 signalling. In the present work, we wish to assess whether the same methods can be leveraged to stimulate Tenon's capsule fibroblasts to undergo the same transformation.

Methods: Primary human Tenon's fibroblasts (pHTCFs) were cultured in DMEM/F12 media supplemented with 10% FBS (Fisher Scientific). Upon confluency, four experimental groups were created. The first group was exposed to BMP4 for 48 hours and then cultured for four days in adipocyte induction media. The second group was not exposed to BMP4 but was cultured for four days in adipocyte induction media. The third group was exposed to BMP4 for 48 hours and then cultured in standard growth media. The final group received no BMP4 and was cultured in standard growth media. Cells will be fixed in paraformaldehyde 6, 7, 8, 9 and 10 days after BMP4 induction. Oil Red O dye will be used to determine the presence of adipocytes, immunostaining for α-SMA will be used to identify myofibroblasts.

Results: Pending.

Discussion: This work has the potential to identify Tenon's capsule fibroblasts and/or myofibroblasts as a plastic cell type. One that could be manipulated to treat the excessive formation of scar tissue. If Tenon's capsule fibroblasts can be induced to differentiate into adipocytes as has been shown in skin, this mechanism may warrant further evaluation as a novel anti-fibrotic therapy. Such a therapy would be non-cytotoxic and safer than current alternatives.

Keywords: Glaucoma filtering surgery, Tenon's Capsule, myofibroblast differentiation, scaring, wound healing

Aging as a major modifier of polyglutamine aggregation and toxicity

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Introduction: Huntinton's disease (HD) is an inherited neurodegenerative disorder characterized by neurodegeneration in the striatum in an age dependent manner. HD is caused by an expansion of the CAG repeat in exon 1 of the huntingtin (htt) protein encoding a polyglutamine (polyQ) region. The expanded polyQ region leads to protein misfolding and aggregate formation yet the role of these aggregates in HD remains unknown. Generally, protein misfolding is greatly exacerbated upon aging, which is the greatest risk factor for the development of neurodegenerative disorders. The relationship between aging, protein misfolding, and aggregation is poorly understood. Here we aim to use budding yeast as model to study protein misfolding in aging.

Methods: Chronologically aged yeast cells expressing polyQ proteins have been used to investigate the role of aging in protein misfolding and its toxicity. Resultant changes in localization and aggregation have been documented by fluorescent microscopy. PolyQ aggregation has been evaluated biochemically by SDD AGE and filter trap assays. Additionally, a variety of cellular quality control proteins (e.g. molecular chaperones and heat shock proteins) have been co-expressed with htt polyQ in aged yeast cells to gain a greater understanding of protein quality control mechanisms involved in age-dependent polyQ toxicity. Furthermore, htt polyQ expressed in Neuro 2A (N2A) cells serve to validate our findings in mammalian neuron-like cells.

Results: Aging exacerbates polyQ toxicity in the absence of molecular chaperones. Toxicity is also increased in the presence of yeast prions in the aging paradigm. Remarkably, polyQ aggregates begin to breakdown as the cells age and the loss of such aggregates precedes cell death.

Conclusions: PolyQ aggregation and toxicity are modulated upon aging in yeast. PolyQ toxicity is dependent upon cellular protein quality control mechanisms, which are conserved from yeast to humans. Additionally, loss of aggregation exacerbates the toxicity associated with protein misfolding and may thus play a role in the enhanced cell death in age-dependent neurodegenerative diseases.

Keywords: Huntington's Disease, neurodegeneration, protein misfolding, aging

Youth suicide age and sex related trends in Ontario First Nations communities: A comparison

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Introduction: The suicide epidemic within First Nations communities in Ontario, Canada has recently been a major topic of media interest; however, literature regarding the age/sex trends associated with suicide within these communities is scarce. The purpose of this project was to identify these age/sex trends, specifically within the youth populations of First Nations communities in Ontario, and compare with those documented in the available literature. It is thought that by identifying and understanding these trends, as well as other risk factors that the youth in these specific communities are facing, preventions strategies can better target and address the needs of youth in these communities.

Methods: A scoping review following Arksey and O'Malley's framework was conducted to explore the current literature. Four databases were searched using predefined terms with a publication date restriction of 2000-2016. This yielded 302 results which were then screened for duplication and relevance yielding in 29 results. Of these, after reviewing the abstracts, 16 papers were then selected for full-text evaluation. The age and sex trends of youth that have committed suicided in Ontario were then identified. These trends were compared to those identified in a file review of 151 coroner's case files held at the Office of the Chief Coroner for Ontario from 2012-2015.

Results: Trends identified within the First Nations youth population differ from those demonstrated within the general population. In youth 10-19 years of age within the First Nations community, females are more likely to commit suicide than their male counterparts. This is opposite the general population. Average age at suicide also differs between the sexes.

Conclusions: Based on the results of this comparative review, it is recommended that further research is conducted to identify the trends and risk factors associated with suicide in Ontario's First Nations communities – specifically in youth. By better understanding these trends and risk factors, prevention strategies can be tailored to meet the specific needs of individuals within these communities.

Keywords: suicide, adolescents, youth, age, sex, gender, First Nations, Ontario

Characterization of 5-Lipooxygenase Expressing Tuft Cells in Colitis-associated Cancer Using a Novel Mouse Model

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Introduction: Our lab previously demonstrated that tuft cells, a rare and ill-defined cell type in the gut, serve as the cellular origin of colitis-associated cancer (CAC) identifying them as a potential target in cancer therapy. However, isolating a marker unrelated to the central nervous system (CNS) has proved challenging until now. We recently identified arachidonate 5-lipooxygenase (5-LO) as an alternative specific marker of tuft cells. Therefore, we aim to generate and characterize a novel transgenic mouse model to assess the capacity of APC mutated 5-LO expressing cells to give rise to CAC. We hypothesize that 5-LO is a superior marker of tuft cells within the gut and under the setting of colitis, loss of APC will render 5-LO expressing tuft cells susceptible to tumorigenesis.

Methods: To test this hypothesis we generated a novel transgenic mouse 5-LO-GFP-DTR-CreERT2 (5-LO-GDC) that allows us to ablate 5-LO with DT, conduct genetic fate mapping studies when crossed to R26Tomato mice, and assess the capacity of APC mutated 5-LO expressing cells to give rise to CAC when crossed to APCf/f mice. Therefore, to characterize the expression of tuft cells tamoxifen or DT was administered and tissue specific expression of GFP/Tomato was determined via immunofluorescence (IF).

Results: IF confirmed GFP expression within tuft cells, whereas no cre-mediated Tomato expression was observed, indicating that cre-mediated APC loss was not induced. Additionally, administration of DT did not significantly change the number of GFP+ cells per crypt villus axis in the intestine or colon.

Conclusions: These findings suggest that 5-LO is a marker of tuft cells. However, non-significant Tomato expression and DT ablation indicates our model needs refinement before this novel mouse can be used for studying CAC.

Keywords: 5-Lipoxygenase, tuft cells, colitis, CRC, transgenics

In Vivo/Vitro 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 regulating metastatic spread. Our focus is on prostate cancer metastasis and we have developed a novel high-throughput means of performing in in vitro 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, if any, are responsible for prostate cancer metastasis.

Methods: For the in vivo screen, we generated a metastatic prostate cell line (DU145) that forms morphologically homogenous micrometastases with a stellate phenotype. This was done by isolating stellate micrometastases from the chick model after 7 days post-injection. For the in vitro screen, we are plating benign prostatic cells (BPH) in Matrigel. After 7 days of incubation, individual cells will clonally expand to larger spheroid colonies that do not appear to have any invasive characteristics.

Results: In vivo model, our results showed that sublines of DU145 form a higher percentage of stellate morphologies (98%) compared to the parental cell lines (93%). In vitro model, 1.0×103 BPH cells/well have the highest plating efficiency (450±3)(45.3%) in Matrigel. Cas9 was transduced into BPH cells by a lentivirus, and its expression was validated by western blot and immunofluorescence.

Discussion: The in vitro CRISPR screen with BPH cell lines will attempt to isolate those that exhibit a stellate morphology, representing kinases that inhibit invasion, a potential tumor suppressor. Putative genes will be then identified by genome sequencing of the isolated "hit" colonies.

Keywords: prostate cancer, BPH, metastasis, CRISPR-Cas9, kinases

Development of a microparticle-based tool as an indicator of the effectiveness of dialysis treatment

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Introduction: Chronic Kidney Disease (CKD) is a debilitating disease that affects ~2.6 million Canadians. Although several stages of CKD exist, End Stage Renal Disease (ESRD) is the most detrimental and requires renal replacement therapy (kidney transplant or dialysis). Dialysis is unfortunately accompanied by various iatrogenic consequences such as cardiovascular complications, and results in endothelial dysfunction. Endothelial dysfunction arises from endothelial inflammation leading to release of microparticles. These extracellular vesicles contain antigen markers of the donor cells and can be easily detected within patient plasma. They represent a novel approach to creating a non-invasive diagnostic tool. We hypothesize that a microparticle based assay has the potential to indicate the severity of disease and overall impact of dialysis treatment on patient health.

Methods: Microparticles were assessed and enumerated by Nanoscale Flow Cytometry by staining with endothelial (CD31, CD62e, CD62p), leukocyte (CD62l, CD45), platelet (CD41a) and erythrocyte (CD235a) fluorophore-conjugated antibodies. By building a standard operating procedure for microparticle analysis, we will ensure consistent validation and quality of data generated from dialysis patients. This method will accurately determine microparticle levels released by specific cell types studied. Additionally, we will use cultured cell lines and conditioned media as positive controls for specificity of antibodies used. Finally, we will use patient plasma that was serially collected (prior, during, and post) from dialysis patients (n=15) to quantify and assess microparticles.

Results: We expect differences in microparticle levels between the patient samples collected prior, during, and post dialysis treatment. Due to endothelial dysfunction, we expect an increase in microparticles, in post-dialysis plasma.

Conclusion: Blood-borne microparticles have the potential to act as a tool to monitor the endothelial dysfunction within hemodialysis patients. This assay could be further used to assess the effectiveness of clinical trials that are being done to improve dialysis treatment.

Keywords: Microparticles, dialysis, Chronic Kidney Disease, endothelial dysfunction

POSTER ABSTRACT #11

Effects of Shroom3 CRISPR/Cas9 Knockout on Nephron Duct Morphogenesis in Xenopus laevis

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Introduction: Kidney diseases such as Chronic Kidney Disease and Polycystic Kidney Disease affect millions of people worldwide. These diseases are related to kidney damage and reduced kidney function. Genome wide association studies have associated Chronic Kidney Disease patients with the shroom family member 3 gene (Shroom3), a key developmental molecule that contributes to cell morphogenesis. The gene is responsible for producing defective kidney podocytes, and is expressed in kidney nephron tubules. In this study, pronephric kidney tubule disruptions due to loss of Shroom3 in Xenopus laevis frogs will be investigated.

Methods: Shroom3 knockout Xenopus laevis embryos will be produced using the CRISPR/Cas9 gene editing system. Shroom3 disruption will be confirmed by a T7 Endonuclease assay and by direct sequencing of the target region. Embryos will be fixated at stage 33-37 and prepared by in situ hybridization. A fluorescent lectin probe will be used to visualize the kidney tubules. The pronephric ducts of Shroom3 knockout embryos will be compared with wildtype embryos, across multiple time points, to identify and characterize potential defects in ductal morphogenesis.

Results: The expected findings are that the loss of Shroom3 in Xenopus laevis pronephric kidneys will exhibit disruptions in nephron duct morphogenesis, and that loss of Shroom3 in the ducts will show shortened tubules.

Discussion: The outcome of this study will provide experimental evidence to support a mechanism of Shroom3 contribution to kidney damage and decreased kidney function. The experimental observation can be extrapolated to mammalian kidneys and provide insight on the effects of variant Shroom3 genes in kidney diseases.

Keywords: Shroom3, Xenopus laevis, kidney disease, nephron morphogenesis, ductal defects

Evaluating the Comparison Between Intra- and Inter-host Evolutionary Rate Patterns Within Different Viruses

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Introduction: In HIV, rates of evolution within a host tend to be greater than the rates among hosts. Currently, the favoured explanation for this trend is that archived HIV variants from an early stage of infection in the previous host tend to establish the infection in the next host. Here we apply phylogenetic methods to quantify within- and between- host evolutionary rates in other human viruses to assess whether this is pattern is unique to HIV or retroviruses.

Methods: In this study, within and between host evolution rates are compared within a variety of viruses, including HIV-1, HIV-2, SIV, Dengue Virus, and HCV, to determine if there are differences in these two parameters. For each virus, longitudinal sequence data with known sequence dates was collected from public databases. DNA sequences were aligned using MUSCLE. We used TempEst to determine the presence of a temporal signal, a measure of how strongly sequences differed over time. Aligned sequences will be processed by BEAST to estimate rates of evolution.

Results: In the collected data, the number of virus sequences per patient ranged from 17 to 834, with an average length of 809 bases. On average, sequences from each patient were sampled at 5 different timepoints, with approximately 28 sequences per timepoint. Each dataset was assessed for temporal signal by evaluating the linearity of root-to-tip divergence over time. Datasets with a negative correlation were discarded. Based on preliminary analysis between 4 patients infected with a virus, HCV demonstrated the highest within-host evolution rate of 2.1×10–5/day whereas HIV-2 demonstrated the lowest rate (1.45×10–6/day).

Conclusions: The next steps involve running BEAST analysis and taking those results for comparison of within host evolution and between host evolution. Datasets will be tested under different model settings (e.g., molecular clock models) to prevent inaccurate rate estimation due to model misspecification.

Keywords: virus evolution, molecular clock, HIV

The effectiveness of ProExC as a surrogate marker of high risk human papillomavirus infection in oral epithelial dysplasia: a pilot study

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Introduction: p16 immunohistochemistry is the most commonly used surrogate marker for detecting biologically significant high risk (HR) human papilloma virus (HPV) infection. Though highly sensitive, p16 does not have optimal specificity and recent research has shown that it may have a poor positive predictive value in the identification of HR HPV positive lesions of the oral cavity. ProExC expression has been investigated as a surrogate marker for HPV detection in the uterine cervix; however, the relationship between ProExC expression and HPV associated oral epithelial dysplasias (OED) has not been investigated. We hypothesized that ProExC will stain a subset of OED that are diffusely p16 positive, show histopathological features consistent with HPV infection and have detectable HPV 16 E6 oncogene mRNA.
Methods: 12 consecutive cases of oral epithelial dysplasia with histologic features suggestive of HR HPV infection and 3 cases of epithelial dysplasia without histologic features suggestive of HR HPV infection were stained with p16 and ProExC by immunohistochemistry. All cases were examined for HR-HPV E6 mRNA expression in order to confirm the presence of HPV.

Results: 11 cases of severe dysplasia/carcinoma in-situ (CIS) and 1 case of moderate dysplasia with histologic features of HR HPV infection were diffusely positive for p16. Eleven of 12 (92%) of p16 positive cases were positive for ProExC. All cases of oral dysplasia without histologic features of HPV infection were negative for p16 and for ProExC. HR HPV E6 mRNA was detected in all p16 positive cases of epithelial dysplasia with histologic features suggestive of HPV infection.

Conclusion: ProExC showed similar performance to p16 in identifying cases of severe dysplasia/CIS likely to be associated with HR HPV infection; however, may fail to identify cases of HPV associated moderate or mild dysplasia.

Keywords: HPV, HPV 16 E6 oncogene, oral epithelial dysplasia, squamous cell carcinoma, immunohistochemistry, ProExC, p16

The Profile of a Pathologists' Assistant (PA) in North America

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Introduction: The purpose of this study is to provide a comprehensive report of the demographics and practice characteristics of PA's who work in the surgical gross room at various institutions across North America. Eligible participants will be those who satisfy the definition of a PA set by the American Association of Pathologists' Assistants (AAPA), which can be found on the AAPA website: http://www.pathassist.org/?page=AboutUs_WhatIsAPA. This study aims to promote the PA profession and provide further insight for future students. More precise regulation of the profession has been proposed for the coming years, and it is important for current and future PA's to be aware of the present situation and understand the effects of impending changes. To our knowledge, a study this comprehensive has not been conducted before. A former study in the United States by Grzybicki et al. (2001) looked at certain features of the PA profession, however our study examines more parameters and covers a larger territory.

Methods: Information from participants will be collected via an anonymous survey that has been designed through a database called Qualtrics. Members of an institution's Pathology department, who meet the eligibility requirements, will be contacted via email and provided with a link to the survey. Each question will be analyzed individually by quantifying the percentage of participants that select, or provide, a certain a response. Gryzbicki et al. previously sent out 515 surveys which resulted in a response rate of 66.8%. As we are recruiting individuals across North America, we aim to distribute at least 600 surveys, but we anticipate the same ratio of surveys sent out to responses received.

Results: Preliminary data obtained from PA's affiliated with the Master of Clinical Science - Pathologists' Assistant Program at Western University reveals two trends: PA's in current practice have joined the profession through a myriad of different avenues and have been practicing in the field for a wide range of durations – from less than 1 year to more than 25 years. Data collection is still ongoing and more responses are required to further establish statistically significant results.

Conclusion: This study will serve to benefit the Pathologists' Assistant community and those aspiring to join the profession or further understand the role PA's play in healthcare.

Keywords: survey, surgical pathology, gross room, hospital, healthcare

The Profile of a Forensic Pathologists' Assistant

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Pathologists' assistants (PA) are health professionals who work under the supervision of a pathologist in the gross room and the autopsy suite. They undergo extensive training, both academically and practically to aid in rendering a pathologic diagnosis and cause of death. However, it is the sole responsibility of the pathologist to deliver the interpretation of any and all findings. This profession is not well understood by the general public and little is known about the demographics of the individuals that make up the PA population. To our knowledge, there are no comprehensive studies providing the demographics and practice characteristics of North American PAs working at forensic units. In order to promote this profession and provide insight into statistics and work style of PAs, we have developed an anonymous online survey that eligible participants can voluntarily complete. Consent forms, study and contact information are sent to pathology departments across North America. If they agree to allow their colleagues to take part, they will forward the information and link to potential participants who can then access the survey. The database we are utilizing, Qualtrics, has data analysis capabilities to evaluate the compiled responses and quantify the results from multiple-choice questions as percentages. Open-ended questions are manually organized into common themes that are further elucidated through charts. We are currently in the early stages of data collection, therefore we have no statistically significant results to report at this time. The conclusions from this study will be able to provide additional insight to future students and the broader community as to the population and job description of PAs.

Keywords: Pathology, pathologists' assistant, PA, forensic, post-mortem, autopsy

Suitability of RNA Isolated from Archived Formalin-Fixed, Paraffin-Embedded Oral Dysplastic Lesions for Diagnosis of an HPV Etiology

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Introduction: HPV-related head and neck cancers have had an increased rate incidence over the past few decades, particularly those affecting the oropharynx. Of high-grade oral dysplastic lesions, 18 % are thought to be associated with high-risk (HR) HPV. In the oropharynx, evidence of HR HPV infection is prognostically significant and impacts patient management; however, little is known about the natural history of HPV associated oral disease. Further knowledge of the spectrum of HPV associated oral disease may aid in future clinical decision making. When HPV DNA integrates into the host genome, there is an increased expression of E6/7 viral oncogenes in clinically significant lesions. Detection of E6/7 mRNA by RT-qPCR can identify HPV-related lesions with a high sensitivity. Before it is apparent the lesions may be HPV-related, tissue has already been already formalin-fixed and paraffin-embedded (FFPE), resulting in degradation of RNA. We sought to determine the quality of RNA in archived specimens, and if HPV-16 E6 expression could be detected in these specimens.

Methods: RNA was extracted from 1 mm full thickness punches harvested from 20 archived FFPE squamous cell carcinomas (SCCs). This included tissues with fixation times of 24 hours or 6+ days from 2015 and 2006. RNA was extracted in the same manner from 14 archived FFPE moderate to severe dysplasias. RNA was reverse transcribed to cDNA for RT-qPCR with housekeeping genes and HPV-16 E6 as amplification targets.

Results: RNA extraction of SCCs yielded a mean 2.90 µg RNA (range 0.81 – 4.85 µg). RNA yield appears independent from archival and fixation times as no statistical differences were witnessed between these groups. All RNA isolated from SCCs yielded β-actin amplified products by RT-qPCR. Of the selected dysplasias 11/14 were positive for HPV-16 E6 expression by RT-qPCR with primer 1, and 12/14 positive with primer 2. This was largely concordant with our other detection methods of p16 and ProExC IHC.

Conclusions: Quality RNA for RT-qPCR testing can be isolated from FFPE oral cavity tissues, including decade old samples, regardless of fixation time. This RNA can be utilized to detect HPV-16 E6 expression with apparent certainty for both current and historical cases.

Keywords: HPV, dysplasia, RT-qPCR, E6 oncoprotein, squamous cell carcinoma, immunohistochemistry, oral, FFPE, ProExC, p16

Administration of nicotinamide riboside reduces lung injury and improves the survival in sepsis

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Introduction: Sepsis and subsequent multiple organ failure remain the major cause of morbidity and mortality in intensive care units. However, there is no cure available to correct this life-threatening condition. Activation of Sirt1 reduces multiple-organ injury in sepsis. Since NAD+ is required for Sirt1 activation and it is limited during sepsis, boosting NAD+ may be a new therapeutic strategy to reduce septic organ injury. Nicotinamide riboside (NR) can boost intracellular NAD+ levels. However, the effects of NR in sepsis remain to be determined. We hypothesize that NR prevents organ injury in sepsis by increasing intracellular NAD+ and Sirt1 activation.

Methods: Endotoxemia and sepsis were induced in mice by lipopolysaccharide (LPS, 4 mg/kg, i.p.) and feces-injection-in-peritoneum (3.75g/kg, i.p.), respectively. NR (300 mg/kg, i.p.) was given 30 min before LPS or feces injection. Apoptosis (caspase-3 activity), myeloperoxidase activities, TNF-α expression and pulmonary microvascular permeability were analyzed. Macrophages were challenged by LPS (100 ng/ml) and microvascular endothelial cells were incubated with a conditioned medium from LPS-stimulated macrophages.

Results: Administration of NR elevated NAD+ levels, reduced inflammation as evidenced by decreased myeloperoxidase activities and TNF-α expression, inhibited apoptosis and attenuated pulmonary microvascular permeability in endotoxemic and septic mouse lungs, lending to an improvement of the survival in septic mice. These protective effects of NR were associated with decreased plasma HMGB1 in septic mice. In LPS-stimulated macrophages, NR increased NAD+ contents, and inhibited apoptosis and HMGB1 release. NR also prevented apoptosis in endothelial cells induced by a conditional medium from LPS-stimulated macrophages. However, inhibition of Sirt1 with EX527 abrogated these inhibitory effects of NR on apoptosis and HMGB1 release.

Conclusions: NR reduces lung injury and improves the survival in sepsis possibly by boosting NAD+/Sirt1 signaling and inhibiting HMGB1 release. Thus, NR may be a potentially useful drug to prevent organ injury in sepsis.

Keywords: Nicotinamide riboside, NAD+, Sirt1, lung injury, sepsis

Dose determination of passive exercise in ICU patients with sepsis

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Introduction: Sepsis is a common and deadly syndrome that accounts for 25-50% mortality in patients admitted to the Intensive Care Unit (ICU). The pathophysiology of sepsis is complex, with inflammation-induced endothelial cell injury leading to impaired microcirculation and organ perfusion playing a central role. Although our understanding of sepsis pathophysiology has advanced, specific therapies are still lacking. Passive exercise may be a potential therapy that reduces sepsis-associated endothelium damage by increasing blood flow via vasodilation. In this study, we will measure the effect of varying intensity of passive exercise on cerebral blood flow, global hemodynamics (blood pressure, stroke volume, cardiac output), and myocardial strain. We will also assess whether optimal dose of exercise varies between patients. We hypothesize that in septic patients, passive exercise will increase cerebral blood flow in a dose-dependent manner without changing global hemodynamics.

Methods: We will passively exercise 20 London Health Science Center (LHSC) ICU patients using an in-bed cycle ergometer. After collecting resting baseline data, we will increase cadence from 5 rotations per minute (RPM) to 55 RPM in 10 RPM intervals each lasting 5 minutes. During each interval, we will record cerebral blood flow and global hemodynamics using the transcranial doppler (TCD) and Finapres® NOVA, respectively. We will also record 2D echocardiogram images during the last 2 minutes of each interval, and use these images to determine myocardial strain.

Results: We expect that passive exercise will increase cerebral blood flow without changes in myocardial strain and global hemodynamics, and that the optimal dose of exercise will vary between individuals depending on their demographics, comorbidities, illness severity and level of life-support.

Conclusions: This study will establish whether passive exercise is effective for increasing organ perfusion without causing organ ischemia in septic patients, and determine if the optimal dose of passive exercise differs between patients.

Keywords: Sepsis, ICU, endothelium, organ perfusion, cerebral blood flow

A Primary Epithelioid Sarcoma of the Clivus, Case Report

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Introduction: Epithelioid sarcomas are rare mesenchymal neoplasms that occur usually in the extremities of young adults. We present a case of epithelioid sarcoma of the clivus in a 19-year-old male with subsequent extension into right petrous ridge and compressing the pons, resulting in bilateral sixth cranial nerve palsy. Although the location is rare, a previous case has been described arising from the clivus in a 5-year-old child.

Clinical Presentation: He first presented with headaches and diplopia in the spring of 2012. An MRI was done, showed an anterior skull based mass involving the clivus extending into sphenoid sinus anteriorly, involving the sella superiorly and extending posteriorly to compress the pons. This was resected in a 2 stage procedure with the first stage being a transcrusal approach and second being an endoscopic trans-sphenoidal approach. A subtotal resection was achieved followed by stereotactic radiation course. Unfortunately, on October 2014 with serial MRIs he was found to have a growing lesion on the right side, adjacent to the site of his previous resection abutting the medial aspect of the petrous temporal bone and causing compression of the right cerebellum. A second resection was done followed by focal radiation. Pathological examination for both resections reveals highly cellular neoplasm composed of sheets of small to medium sized epithelioid cells with distinct eosinophilic cytoplasm, pleomorphic nuclei and prominent nucleoli. There are numerous mitotic figures. Tumor cells are strongly and widely immunopositive for vimentin, EMA and cytokeratin (AE1-AE3, CKCAM5.2). There is no INI-1 immunolabelling for the majority of the tumor cells. The morphological appearance and immunohistochemistry profile corroborate a diagnosis of epithelioid sarcoma. Local recurrence was diagnosed in 2015 but patient declined further non-surgical interventions.

Conclusion: This report adds epithelioid sarcoma to the differential diagnosis of clival and skull base tumors, especially in younger patients.

Quality Management for the Autopsy Service – London Health Sciences Centre

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Introduction: In the last 5 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 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 administered to all of the autopsy pathology staff, including Pathologists, Pathologists' Assistants, Pathologists' Assistant students, Neuropathologists, Residents and Medical Laboratory Assistants (MLAs). Following the administration of the questionnaires, results were summarized and used to create a list of proposed solutions to issues raised by the respondents. This proposal was peer-evaluated, and then accepted recommendations were implemented. A follow- up survey is currently being conducted with staff to confirm that the recommendations are effective.

Results: Based on the results of the interviews with the structured questionnaire, a list of 48 recommendations was generated. A monthly implementation schedule was created so that all feasible recommendations were implemented between July and November 2016. A three month trial period was given before evaluations were sent to staff.

Discussion: This project is in its last phase. Quality management issues have been identified, solutions proposed, implemented and are now being evaluated. Based on the results of the follow-up survey, any adjustments to their implementation will be made. New documentation to support quality management in the Autopsy Suite at staff level has been created and is currently being made available on Organizing Medical Networked Information (OMNI). This project has been a collaborative and comprehensive effort to improve efficiency and safety within the autopsy service at LHSC.

Keywords: quality management, efficiency, safety, supply management

The Effect of Ischemic Pre-conditioning on the Severity of Microvascular Dysfunction from Abdominal Ischemia-Reperfusion Injury

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Introduction: Ischemia is a frequent clinical problem occurring as a consequence of trauma, hemorrhage, vascular stenosis and embolism. While reestablishing the blood flow remains the primary treatment, reperfusion can paradoxically exacerbate the severity and size of injury. Previously, it has been demonstrated that ischemic preconditioning (PC) can offer protection to the tissues when subjected to prolonged ischemia. The purpose of this study was to test the effects of a novel conditioning protocol (ischemic conditioning) where ischemic organs are subjected to short bouts of reperfusion, followed by complete restoration of blood flow.

Hypothesis: We hypothesize that conditioning ischemic organs with a brief period of blood flow prior to complete reperfusion will reduce the full Ischemia-Reperfusion injury.

Methods: Male Wistar rats underwent 20 minutes of abdominal ischemia, induced by infra-hepatic aortic clamping followed by three different brief periods of reperfusion prior to complete restoration of blood flow. Following 2 hours of reperfusion, liver microvascular perfusion, leukocyte activation and hepatocellular death were assessed. Arterial blood samples were collected at baseline, after 20 min ischemia and at the conclusion of reperfusion for biochemical analysis, and myeloperoxidase levels.

Results: We expect to find significant improvement in hepatic perfusion, reduced inflammation and hepatocyte death in rats that underwent ischemic conditioning protocol prior to complete reperfusion.

Conclusion: If successful, the findings will have applications in many elective surgical procedures such organ transplantation and coronary artery bypass surgery and emergent interventions such as decompression following abdominal compartment syndrome. Additionally, we hope to elucidate endogenous mechanisms related to transient reperfusion that mediate the observed protective function following reperfusion.

Keywords: Ischemia reperfusion injury, conditioning, ischemia, reperfusion, inflammation, no-reflow

Corticosteroid-Induced CRTh2 Expression in T_H2 Cells: A Role in Persistent Asthma

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Introduction: Corticosteroids are a common asthma treatment, but are ineffective in severe asthma (SA). We have previously reported that corticosteroids upregulate CRTh2 expression and this may help sustain TH2 cells—potentially causing SA. Our collaborators have reported that simultaneous withdrawal of chronic corticosteroid and OVA challenge in an asthma mouse model resulted in a goblet cell rebound. This rebound featured goblet cell hyperplasia and IL-13 levels greater than allergen treated corticosteroid naïve mice. Although CD4+ T cells were the major player in this rebound, it is unclear if this in vivo finding is further evidence of corticosteroid-sustained TH2 cells. Furthermore, it is unknown whether corticosteroid treatments impact CRTh2 expression on other cell types in the asthmatic lung.

Hypothesis: We hypothesize that prolonged corticosteroid exposure in the asthma mouse model increases CRTh2 expression and TH2 cell numbers, suggesting a mechanism for the goblet cell rebound.

Methods: Lung biopsies from chronic corticosteroid-administered OVA asthma mice will be assessed for CRTh2, CD3 and CD4 using immunohistochemistry (IHC) and immunofluorescence staining. Staining will be quantified using cell counts and fluorescent intensity. CRTh2 IHC will also be used to assess expression on other cell types and lung regions. Lung biopsies from the chronic OVA challenge only, chronic corticosteroid treatment only, or saline control mice will also be assessed.

Expected Outcomes: We expect that: a) corticosteroid treatment will upregulate CRTh2 expression in vivo and b) CD4+ CRTh2+ (TH2) cells will increase in association with the goblet cell rebound.

Discussion: Our study will provide in vivo support to corticosteroid-induced CRTh2 expression and sustained TH2 cells in SA. These findings will also illustrate corticosteroid's impact on CRTh2 expression in other cells in the lung. The adverse role of corticosteroid in severe asthma will warrant future screenings for asthma severity and the development of alternative clinical solutions.

Keywords: Asthma, severe asthma, corticosteroid, TH2 cells, CRTh2

Role of Long Non-coding RNA MALAT1 in the Pathogenesis of Diabetic Retinopathy

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Introduction: Diabetic retinopathy (DR) is the leading cause of blindness in the working population in industrialized countries. Recent advances in genetics have identified that epigenetic alterations contribute to the development and progression of DR. Long non-coding RNAs (lncRNAs), involved in epigenetic alterations are aberrantly expressed in diabetic retinas; however, a majority of lncRNAs have not been comprehensively characterized. Based on preliminary evidence provided by our laboratory, we hypothesize that MALAT1 regulates inflammatory and angiogenic processes through specific epigenetic mechanisms in diabetic retinopathy.

Methods: To test this hypothesis, we first confirmed the upregulation of MALAT1 in human retinal microvascular endothelial cells (HRECs) following incubation in high glucose (25mM/L, HG) compared to normal glucose (5mM/L, NG). Total RNA was extracted using TRIzol and RT-qPCR was used to confirm the expression of MALAT1, TNF-α, and IL-6 transcripts. We also pre-treated HRECs with a global histone methylation inhibitor (DZNep) in NG and HG to analyze whether MALAT1 interacts with epigenetic mediator proteins. Next, our in vivo study assesed retinal tissues from MALAT1 knockout (KO) and wild-type (WT) mice with or without STZ-induced diabetes of two months duration.

Results: MALAT1, TNF-α and IL-6 expression levels were significantly upregulated following HG exposure. Further, using DZNep to inhibit the activity of Polycomb Repressive Complex 2 significantly down-regulated MALAT1 ($p<0.0001$) and EZH2 transcript level expressions ($p=0.0051$). However, IL-6 and TNF-α transcript expressions were not significantly down-regulated after DZNep treatment in HG compared to HG without DZNep treatment. In our in vivo model, retinal IL-6 and TNF-α expressions were increased in WT diabetic mice and were prevented in the MALAT1 KO diabetic mice.

Conclusions: These findings may uncover a novel mechanism of MALAT1 in DR progression and a potential therapeutic target for DR. Our study will also provide a foundation to propagate future studies of lncRNA mechanism(s) in DR.

Keywords: Epigenetics, diabetic retinopathy, long non-coding RNAs, methylation, acetylation, inflammation

Mitochondrial permeability can regulate endothelial cell necroptosis and promote cardiac allograft rejection

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Introduction: Transplant injury is invariably associated with programmed cell death (PCD) resulting in delayed graft function and organ rejection. Many forms of PCD have been described including apoptosis, pyroptosis, ferroptosis, and necroptosis. They are induced by various death receptors (DRs) including tumor necrosis factor receptor-1 (TNFR1), DR4/5, and Fas engagement. We were the first to describe receptor-interacting serine/threonine protein kinase 3 (RIPK3) mediated necroptosis in transplant injury, where tissue necrosis and graft rejection were attenuated in RIPK3 null kidney and heart allografts following transplantation. Until now, the effect of mitochondrial dysfunction in the necroptotic pathway remains controversial – it is suggested that mitochondrial dysfunction may promote necroptosis in some studies but not in others. Here, our goal was to determine if mitochondrial injury participates in cardiac cell necroptotic death and accelerates graft rejection.

Methods: In vitro, we induced necroptosis in murine microvascular endothelial cells (MVECs) with TNFα and caspase-8 inhibitor. Necrotic cell death was measured using Sytox Green nucleic acid staining and quantified with the Essen Bioscience Incucyte Zoom live cell imaging.
In vivo, cardiac grafts from wildtype C57BL/6 (H-2b) and mitochondrial permeability transition (MPT) deficient Ppif-/- (H-2b) mice were heterotopically transplanted intra-abdominally using allogeneic BALB/c recipient mice (H-2d) with or without rapamycin treatment.

Results: In cultured MVECs, we found that TNFα and caspase-8 inhibition triggered cells to undergo RIPK1- and RIPK3-dependent necroptosis and this cell death was attenuated by MPT inhibition. Ppif deficient cardiac allografts showed no difference in survival compared to wildtype C57BL/6 grafts without rapamycin treatment. However, with rapamycin treatment, Ppif deficient cardiac allografts had prolonged survival compared to wild type grafts (MSD=85 versus 31 days, $p<0.0001$).

Keywords: heart, transplantation, cell death, necro

Leukocyte Common Antigen (CD45) Expression in Carotid Atherosclerotic Plaques

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Introduction: Inflammation is an important component of the development of atherosclerosis and is often associated with vulnerable plaques, which are more likely to lead to poor clinical outcomes. The present study is aimed at analyzing carotid atherosclerotic plaques for expression of leukocyte common antigen (CD45), a surrogate marker of inflammation, and discovering CD45's correlative value to stroke risk and clinical imaging.

Methods: We will be analyzing a subset of carotid endarterectomy specimens obtained in the Canadian Atherosclerotic Imaging Network (CAIN2) study. These samples were processed, immunohistochemically stained for CD45, and scanned into digitized mosaic files. These images will be annotated for CD45 expression and quantitatively analyzed using Aperio ImageScope's Positive Pixel Count algorithm, a threshold-based colorimetric program. These data will then be used to create a 3D model of the tissue demarking areas of inflammation and examined by the CAIN group for correlation to clinical outcomes and imaging findings.

Results: Our expected findings are that a) CD45 expression, as a marker of inflammation in atherosclerotic plaques, will be predictive of stroke risk, and b) CD45 expression will correlate with imaging findings related to inflammation.

Discussion: Our study will provide information on CD45's value as an indicator of inflammation and resultant stroke risk. This study will also help refine methodology for quantifying inflammation in tissue and in clinical imaging with the goal of improving screening and treatment of atherosclerosis.

Keywords: atherosclerosis, CD45, inflammation, stroke, carotid artery

A novel theory on mechanisms of glucagon secretion from pancreatic alpha cells

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Introduction: Lowering blood glucagon can mitigate serious hyperglycemia even in severe hypo-insulinemic patients. Interestingly, glucagon secretion is under the influence of neural, hormonal and nutritional stimuli; however, the mechanisms that govern the response of pancreatic alpha cells to each of these stimuli have not been addressed. Here, we hypothesized that there are distinct secretory granule populations in the pancreatic alpha cells that selectively respond to each of neural, hormonal or nutritional stimuli.

Methods: Alpha TC1-6 cells were transfected with a fusion gene construct, FC-glucagon, to mark secretory granules. Cells were cultured in DMEM (control) and treated with gamma amino butyrate (GABA, 25 μM), insulin (100 pM) or palmitate (0.75 mM) for 48h. FC- glucagon in the cell extracts and media were purified using co-immunoprecipitation and measured by ELISA. The extracts were processed for LC-MS/MS to find the protein signature. In addition, FC-glucagon and granule marker (CgA) were followed by immunofluorescence microscopy.

Results: There was a significant decrease in glucagon levels in response to insulin (57±5 pg/mg cell protein; p=0.03) and GABA (54±3; p=0.02), and a significant increase in response to palmitate (124± 0.67; p= 0.008) compared to the control (83±5). Venn diagram analysis showed 5, 5 and 25 unique proteins in GABA, insulin and palmitate groups, respectively. Interestingly, unique proteins in the GABA group (heat shock 70 kDa protein (HSP70)-1B, HSP70-p2, HSP70 -p 1-like, HSP70- p1A, and Interleukin-1 receptor-like 1) were annotated as “protein complex assembly”. In the insulin group, Retinoblastoma-associated protein was annotated as localization protein.

Conclusion: Here for the first time we are presenting a multi secretory granules model for glucagon secretion from the pancreatic alpha cells. We have identified distinct glucagon-associated protein signatures that are responsive to neural, hormonal or nutritional stimuli. Importantly, these unique proteins can be considered as potential therapeutic targets for diabetes.

Keywords: Diabetes, Glucagon, Secretory granule, Proteomics, Pancreatic alpha cells

A Clinico-Pathological Study of the Structural and Functional Changes to the Retina and Optic Nerve following anti-VEGF Treatments for Diabetic Macular Edema

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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. The purpose of this study is to evaluate and analyze the safety and efficacy of anti-VEGF drugs on the retina and optic nerve in patients with DME and varying levels of diabetic retinopathy (DR). We hypothesize that increased exposure to anti-VEGF will result in increased in-vitro retinal cell death and in-vivo morphological changes to the optic nerve.

Methods: DR patients with underlying DME underwent pre-injection, 6, 12 and 24 month follow-up tests using visual fields, Heidelberg retinal tomography, optical coherence tomography and OPTOS fluorescein angiography. In vitro, rat retinal cell cultures were exposed to 0, 0.0625, 0.125 (clinical dose), and 0.25 mg/mL of ranibizumab (Lucentis) for 48 and 72 hours. Cellular metabolic activity was measured by MTT assay, cytotoxicity by LDH, and apoptosis by cell death ELISA.

Results: A total of 30 patients were enrolled in the study. The average macular thickness decreased ($p<0.0001$) at 6 months, and 12 months compared to baseline. Average retinal nerve fiber layer thickness decreased ($P<0.0001$) by 12 months. Average cup to disk ratio and vertical cup to disk ratio increased ($p<0.0009$) by 12 months. Cup volume increased ($p<0.007$) at 6 months, and 12 months compared to baseline. In vitro, MTT showed a significant decrease ($p<0.003$) in cellular metabolic activity at the 0.125 mg/mL clinical dose and double the clinical dose compared to control at 48 and 72 hours. LDH showed a significant increase ($p<0.005$) in cytotoxicity at the clinical dose and double the clinical dose compared to control at 48 and 72 hours. ELISA showed a significant increase ($p<0.001$) in apoptosis at half the clinical dose, the clinical dose and double the clinical dose compared to control at 48 and 72 hours.

Conclusions: Clinically, anti-VEGF appears to have potentially detrimental effects on the optic nerve by decreasing retinal nerve fiber layer thickness, increasing cup/disk ratio and cup volume over time. In vitro, anti-VEGF treatment appears to decrease cellular metabolic activity, and increase cytotoxicity and apoptosis of retinal cells. The results provide a cautionary note to monitor both the retina and optic nerve status in patients undergoing frequent injections.

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

Genome-wide DNA methylation testing in patients with Developmental Delay and Intellectual Disabilities

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Introduction: Developmental Delay and Intellectual Disabilities (DD/ID) are caused by the interaction of genetic and environmental factors that is facilitated by epigenetic mechanisms. The most widely studied epigenetic mechanism is the DNA methylation, which is a covalent modification occurring primarily at cytosines within CpG dinucleotides. DNA methylation defects have been shown to play a key role in many pediatric and adult onset disorders, including imprinting diseases and carcinogenesis. However, there is little known about genome-wide DNA methylation changes in patients with DD/ID. Our laboratory has performed a genome-wide DNA methylation testing in 1,000 patients with a wide range of disorders associated with DD/ID to clinically validate the use of a genome-wide DNA methylation assay and define epi-signatures of various DD/ID conditions.

Methods: Briefly, the DNA methylation array was performed in peripheral blood using the Infinium HumanMethylation450 Beadship, and the data were analyzed using a custom algorithm by Partek Genomic Suite software. Methylation patterns of individual patients were compared to normal reference cohort of >300 individuals and were prioritized based on the statistical parameters including methylation difference, p-value, and F-value; and functional parameters including distance to the CpG islands and gene promoters, and the proximity to the known disease-causing genes.

Results: We validated this approach for sensitive detection of imprinting disorders including Angelman, Prader-Willi, Beckwith Wiedemann, and Russell-Silver syndrome, as well as Fragile X syndrome. We also discovered novel highly specific diagnostic epigenetic signatures in the peripheral blood of patients with Alpha Thalassemia/Mental Retardation syndrome X-Linked, Floating-Harbor syndrome, Autosomal Dominant Cerebellar Ataxia with Deafness and Narcolepsy (ADCA-DN) and Claes-Jensen X-linked Mental Retardation syndrome.

Conclusion: These findings demonstrate clinical utility of genome-wide DNA methylation testing in patients with a wide-ranging spectrum of epi/genetic conditions, and provide better understanding for the pathology of DD/ID syndromes, in which specific DNA methylation changes could lead directly to an aberrant expression of genes.

Keywords: Epigenetics, DNA methylation, Intellectual disability

Effect of Mechanical Strain on TGFβ-induced Collagen Expression in Human Trabecular Meshwork Cells

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Introduction: Dynamic remodeling of extracellular matrix (ECM) in the trabecular meshwork (TM) is vital for providing the appropriate resistance to aqueous humor outflow and maintaining the intraocular pressure (IOP) within physiological ranges. However, excess deposition of ECM in the TM of primary open-angle glaucoma (POAG) patients leads to increased resistance to aqueous outflow, causing increased IOP and associated optic nerve damage in these patients. Increased levels of active transforming growth factor-beta (TGF-β) in the aqueous humor is implicated in the excess deposition of ECM in the TM of POAG patients. Since mechanical strain induced by increased IOP is known to regulate ECM remodeling in the TM, we will investigate the role of mechanical strain in regulating ECM deposition induced by TGF-β in TM cells.

Methods: Human trabecular meshwork cells (HTMCs) will be cultured and subjected to one of four treatments: TGF-β2 (5ng/mL), stretch (15%, 1 cycle/sec, 48 hrs.), both TGF-β2 and stretch, and no treatment (control). After 48 hours, the cells will be lysed and the protein extracts will be used to detect collagen I (a measure for ECM deposition), phosphatase and tensin homolog (PTEN) (a major regulator of ECM deposition) and its phosphorylation by Western blot.

Results: TGF-β2 induced an increase in collagen I expression compared to controls; however, stretch inhibited the TGF-β2-induced increase in collagen I expression. Stretch alone did not cause any major change in collagen I expression compared to controls. PTEN expression and its phosphorylation showed changes similar to that of the collagen expression.

Conclusions: Inhibition of TGF-β2-induced collagen I expression by mechanical strain confirms that mechanical strain has a role in regulating the remodeling of ECM in the TM. Therefore, signaling mechanisms that allow mechanical strain to prevent the excess ECM deposition caused by TGF-β2 could serve as valuable therapeutic targets for treatment of glaucoma.

Keywords: Trabecular meshwork, extracellular matrix, collagen, TGF-β, mechanical strain, glaucoma, intraocular pressure

Kallikrein-related peptidases are dysregulated in pleomorphic adenoma

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Introduction: Pleomorphic adenoma (PA) is the most common benign salivary gland tumor. 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 5% of cases. Kallikrein-related peptidases (KLKs) have been identified as biomarkers in many human tumors and may influence tumor behavior. We investigated KLK1–15 in PA to determine an expression profile, and we hypothesize that KLKs are dysregulated in PA.

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 $P<.05$. Protein expression of KLK1, 12 and 13 were evaluated 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. Immunohistochemical stains showed decreased intensity for KLK1, 12 and 13.

Conclusions: KLK1, 12 and 13 mRNA levels in PA are statistically significantly decreased relative to those in normal salivary gland tissues.

Keywords: Pleomorphic adenoma; salivary gland tumors; kallikrein-related peptides; immunohistochemistry

Investigating the Role of SLUG in TBX3-induced Epithelial-Mesenchymal Transition of Breast Cancer Cells

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Introduction: Through genome-wide expression studies on breast cancer cell lines derived from a single patient at different phases of cancer progression (21T series), our laboratory has shown that levels of transcriptional regulator TBX3 are up-regulated in invasive 21MT-1 cells and minimally expressed in non-invasive 21NT cells. Overexpression of TBX3 leads to changes characteristic of epithelial-mesenchymal transition (EMT) in 21NT breast cancer cells. The direct transcriptional targets of TBX3 isoforms and mRNA transcript levels were examined through ChIP-array and RNA-Seq studies, respectively. TBX3 was demonstrated to directly up-regulate transcription of the SNAI2 gene, which encodes SLUG, a potent EMT-inducing transcription factor. We hypothesize that SLUG expression may have a role in the observed changes associated with TBX3 up-regulation.

Methods: SLUG levels were knocked down by shRNA-mediated lentiviral transduction in 21NT transfectant cells (21NT+ empty vector, 21NT+TBX3iso1, and 21NT+TBX3iso2). Phenotypic and functional assays were conducted to investigate growth, invasiveness, and migratory ability of the cells. Expression of mesenchymal/EMT markers and downstream targets of SLUG are currently being assessed by qPCR and Western Blot.

Results: With overexpression of TBX3iso1 or TBX3iso2 there was an increase in migration and invasion relative to the empty vector control. However, with SLUG knockdown the migration and invasion levels were reduced to baseline. Growth of 3D colonies in Matrigel was also marginally reduced with SLUG knockdown, despite high levels of TBX3 expression.

Discussion: SLUG expression was found to be required for TBX3-induced migration and invasion of breast cancer cells. Our investigation on the molecular mechanisms associated with epithelial-mesenchymal transition in breast cancer holds clinical potential for identifying possible therapeutic targets and biomarkers to prevent disease progression in patients diagnosed with breast cancer.

Keywords: TBX3, SLUG, breast cancer, epithelial-mesenchymal transition (EMT)

Oral Epithelial Dysplasia: evaluation of serial biopsies using S100A7

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Introduction: Diagnosis of oral cancer is often made at an advanced stage of disease, resulting in continued high mortality rates despite advancements in treatment. The progression of epithelial dysplasia to oral cancer often follows a stepwise progression. Histopathology is considered the 'gold standard' for diagnosing dysplasia and high risk lesions for progression to oral cancer, but is somewhat subjective. Recent work with the protein biomarker S100A7 has shown some predictive value for the transformation of dysplasia to cancer. The objective of this study is to determine a correlation between the expression of S100A7 and the histologic grade of oral epithelial dysplastic lesions using immunohistochemistry. Our hypothesis is that the expression of S100A7 will correlate with the histologic grade and can be used as a reliable marker for the progression of such lesions.

Methods: 90 formalin fixed paraffin embedded specimens, including several follow-up biopsies, from 27 subjects were obtained from the Western University Pathology Department tissue archives, from 2002-2015. Specimens were stained for S100A7 protein using a standard immunohistochemistry protocol. Expression of S100A7 was assessed semi-quantitatively, using an intensity and proportion scale, as well as by image analysis. An algorithm will be applied to determine the risk of transformation to malignancy.

Results: Preliminary results suggest that S100A7 has higher expression in high risk lesions (moderate and severe epithelial dysplasia) than low risk lesions (mild epithelial dysplasia).

Conclusions: 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: Oral epithelial dysplasia, oral cancer, S100A7.

Effect of Estrogen and Glucocorticoid Signaling on Th2 cells – Implications for Severe Asthma

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Introduction: The incidence and severity of asthma is greater in women than men and women are more susceptible to developing corticosteroid-resistant asthma. Despite this, the underlying mechanism(s) for the sex-specific prevalence of severe asthma is still poorly understood. Asthma is a T helper (Th) 2 cell-associated inflammatory disease where Th2 cytokines (IL-4, -5 and -13) influence asthma pathogenesis. Our laboratory has shown that Th2 cells are higher in severe asthmatic women than men. Furthermore, a positive correlation was seen between Th2 cells and the dose of inhaled glucorticosteroids (GC) in women but not in men. This suggests a potential interaction between sex hormones and GC, producing enhanced Th2 cell responses in women. The objective of this study is to examine the potential cross-talk between estrogen and glucocorticoid signaling.

Hypothesis: Cross-talk between estrogen and GC receptor signaling promotes CRTh2 expression and Th2 cell function.

Methods: The influence of estrogen and GC receptor signaling on CRTh2 expression was assessed by treating the Th2 cell line (CCRF CEM) with ER agonists in the presence of dexamethasone (Dex), a glucocorticosteroid analog (0.1μM). The ERα-selective agonist propylpyrazole-triol (PPT) and ERα-selective agonist diarylpropionitrile (DPN) were used to assess the role of each receptor alone and in combination with Dex. CRTh2 protein and mRNA expression were assessed using flow cytometry and qRT-PCR.

Results: We found an increase in surface expression of CRTh2 in Th2 cells treated with PPT alone (1.4 - fold), though there was no additive effect when Th2 cells were cultured with PPT + Dex (2 – fold). No increases were observed for CRTh2 when Th2 cells were cultured with DPN alone or in combination with Dex.

Discussion and conclusion: These data show that ERα signaling enhances CRTh2 expression and suggest that in vivo estrogen may have a similar influence on Th2 cells. Since CRTh2 activation inhibits apoptosis, an estrogen-mediated increase in CRTh2 may represent a mechanism by which Th2 cells are sustained in women, but not men, with severe asthma.

Keywords: Allergic disease, CRTh2, Estrogen, Glucocorticosteroid responsiveness, Inflammation, Severe Asthma, Sex difference, T cells, Th2 i

Characterization of Growth Hormone Secretagogue Receptor 1a for cardiac imaging

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Introduction: Developing cardiac-specific biomarkers are essential for detecting early molecular changes in heart failure (HF). There is current interest in the hormone ghrelin and its receptor, growth hormone secretagogue receptor 1a (GHSR1a). GHSR1a levels are increased in HF patients due to its cardioprotective roles. Metabolically, ghrelin is released during a fasted state and induces a substrate preference for glucose over lipids in cardiac tissue and modulates intracellular Ca2+ currents through GHSR1a. Receptor downregulation is expected during a fasting state. Our lab is characterizing a PET imaging probe, 18F-GHS, in fed vs. fasted mice and wild-type vs. GHSR1a-/- against GHSR1a as a potential cardiac-specific imaging agent. Histological verification is required to characterize probe binding. We hypothesize that GHSR1a levels will be decreased in fasted mice.

Methods: Histochemistry for GHSR1a and immunohistochemistry for ghrelin, CD36 (lipid transporter), GLUT4 (glucose transporter), and SERCA2a, will be performed on heart samples. SERCA2a (Ca2+-ATPase) is downregulated in cardiac tissue during a fasted state, and increased CD36 may be responsible to compensate for the loss of Ca2+ signaling. Fluorescence microscopy will be performed and protein levels will be quantified using a custom-made script in ImageJ.

Anticipated Results: We expect increased ghrelin, decreased GHSR1a, increased GLUT4, and decreased CD36 in fasted mice along with increased ghrelin, decreased GLUT4, and increased CD36 in GHSR1a-/- mice. We expect increased SERCA2a levels in fasted mice and decreased SERCA2a levels in GHSR1a-/- mice.

Discussion: Using GHSR1a as a biomarker will provide a cardiac-specific target that will enable clinicians to detect the early molecular perturbations in HF, and thus provide earlier intervention to prevent progression of the disease.

Keywords: Growth hormone secretagogue receptor 1a, ghrelin, heart failure, cardiac imaging, biomarker, metabolism, CD36, GLUT4, SERCA2a, fibrosis

HRAS G12V predicts for innate resistance to PI3Kα inhibition in head and neck squamous cell cancer

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer, with a 50% mortality rate for advanced disease. The mutational landscape of HNSCC has been recently elucidated, introducing the possibility for targeted therapeutics. PIK3CA—which encodes the α-catalytic subunit of PI3K—is the most frequently-altered actionable target in HNSCC, however it is not clear which patients benefit most from PI3Kα-inhibition. BYL719 is a leading PI3Kα inhibitor in clinical development for HNSCC. We previously examined the responses of a large panel of genetically-characterized HNSCC cell lines to BYL719 and identified activating HRAS G12V mutations as predictors for BYL719 resistance. Here we examine if this mutation is able to individually modulate BYL719 response and aim to elucidate the mechanistic underpinnings.

Methods: To determine if HRAS G12V was able to modulate BYL719 sensitivity, we knocked down HRAS in HRAS G12V and HRAS wild-type (WT) cell lines, and then treated cells over 10-point dose ranges with BYL719. Sensitivity was determined by calculating IC50 values at 72 hours using PrestoBlue®. Constructs expressing wildtype (WT) HRAS or HRAS G12V were transfected into BYL719-sensitive cells for overexpression studies. Proliferation and BYL719 sensitivity were measured. Immunoblotting and quantitative PCR was used to examine activity of the PI3K and MAPK pathways with/without BYL719 treatment.

Results: HRAS knockdown sensitized HRAS G12V lines to BYL719 (lower IC50), but did not affect the response of WT HRAS cells. WT HRAS and HRAS G12V overexpression significantly increased cellular proliferation and promoted BYL719 resistance. In HRAS G12V cell lines, although pAKT was be blocked by BYL719, proliferation was unaffected.

Discussion: These findings highlight a predictive role for HRAS G12V in mediating 'innate' BYL719 resistance; this may have important implications for patient candidacy for BYL719 therapy. Understanding the molecular mechanisms mediating this resistance will help identify more promising therapeutic targets for HRAS-mutant patients.

Keywords: resistance, head and neck cancer, BYL719, HRAS, AKT, targeted therapy

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

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Introduction: Heart Failure 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 characterizing the growth hormone secretagogue receptor (GHSR) and its ligand ghrelin. We have developed a fluorescent analog of ghrelin, Cy5ghrelin(1-18), which specifically binds to GHSR in cardiac tissue and can detect changes during cardiomyocyte differentiation. I am characterizing GHSR, ghrelin, fibrosis, and natriuretic peptide Type B (BNP) a known biomarker of HF, in human cardiac tissue and correlating corresponding levels to HF severity.

Methods: Samples of cardiac tissue from cardiac transplant patients (n=10) were obtained from the cardiology unit of London Health Sciences Center. GHSR levels were measured using Cy5-ghrelin(1-18) and ghrelin and BNP were measured using fluorescent antibodies where all levels were quantified using fluorescence microscopy. Masson's trichrome stain was used to test fibrotic tissue in all samples. Levels of all markers were compared between the explanted heart and the healthy biopsies using two-tailed t-test, two-way ANOVA and Tukey's test ($p<0.05$). We will then correlate these levels to the clinical data.

Results: GHSR and fibrosis levels increased in explanted hearts when compared to the healthy biopsies. Ghrelin and BNP levels were slightly elevated in end stage HF. Within individual patients there were significant differences in GHSR levels between the explanted heart and healthy heart biopsies.

Discussion: Elevated expression of GHSR in end stage HF indicate the potential of GHSR as a cardiac specific biomarker. While fibrosis and BNP, clinically established markers of HF, did show elevation, GHSR expression levels showed a more drastic change in expression. Ultimately the establishment of GHSR as a cardiac specific biomarker can greatly impact the diagnosis of HF and will help with personalized medicine.

Keywords: Heart failure, ghrelin, fluorescence microscopy, immunohistochemistry, GHSR, BNP, fibrosis

Salivary Gland Neoplasia – Culturing a Potential Model Cancer Cell Line Out of A-253 Cells

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Introduction: Salivary gland neoplasms (SGNs) are uncommon head and neck tumours that are histomorphologically complex and diverse, making proper classifications and diagnoses difficult. Pathologists currently struggle to clearly define the origin of each type of SGN (histogenesis), resorting to classification based on morphological patterns. This study aims to create a cell culture (from the A-253 cell line, obtained from a salivary gland epidermoid carcinoma) that mimics the phenotype of epithelial cells in SGNs, confirming the cell line's ability to act as an in vitro model for SGNs, specifically mucoepidermoid carcinomas (MECs).

Methods: A-253 will be cultured per protocol available on the ATCC's (American Type Culture Collection) website. Cells will be allowed to attach and spread on a plastic culture dish, after which they will be sub-cultured at a ratio of 1:4. Cell cultures will have their epithelial phenotype, growth kinetics, and protein-specific expression profiles (for osteopontin, kallikreins, cytokeratins, mucins, and p63) characterized, for future comparisons to various SGNs (specifically MECs).

Results: The cells are expected to have an epidermoid phenotype, similar growth kinetics to those of other epidermoid carcinomas, as well as a similar protein expression profile to that of MECs. Positive staining for pan-cadherin suggests an epidermoid phenotype, confirmed by positive staining for epithelial markers E-cadherin, β-catenin, and CK7 (found in MECs). Cells demonstrated strong expression of the proliferative marker Ki67, explaining the observed rapid population doubling time (28.5 hours).

Conclusions: These findings would support the use of A-253 as a pathophysiological model for MECs – this can lead to more accurate diagnostic and classification schemes, resulting in more specific prognoses and treatments. Modifying specific gene expression in this model system can provide insight into their respective effects on the cancer phenotype and pathogenesis of MECs. Furthermore, elucidating the histogenesis of MECs (and potentially other SGNs) will allow for future investigations on preventative therapies.

Keywords: salivary gland ; salivary gland neoplasia ; mucoepidermoid carcinoma ; epidermoid carcinoma ; A-253 ; osteopontin ; kallikreins ; mucins ; cytokeratins ; cell culture

Aberrant Polysialylation of Proteins Potentially leads to Prostate Cancer Metastases

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Glycosylation is post-transcriptional modification, the process which links glycans to terminal ends of proteins mainly through N-linkage and O-linkage. It provides functional diversity to the proteins for variety of biological functions. However, glycosylation pattern undergoes dramatic alterations in tumor microenvironment allowing neoplastic cells to invade and spread to distant sites in the body. Polysialylation is a specific type of glycosylation in which sialic acid chains are enzymatically introduced onto the cell molecules with help of polysialyltransferases (PTs). We hypothesize that aberrant polysialylation of proteins gives metastatic potential to primary tumor cells by enhancing cancer cells survival and migration. The expression of PTs was determined in classical prostate cancer cell lines PC3, LNCap, DU145 by using immunofluorescence, western blotting and RT-qPCR. We observed significant upregulation of PTs gene expression in different prostate cancer cell lines compared to the benign prostate cells (BPH). Likewise, there was upregulation in PTs protein expression in different prostate cancer cell lines. We speculate that overexpression of PTs potentially enhances cancer cell migration or extravasation. It would be interesting to perform cell extravasation assay by injecting cancer cells having intact and knocked down expression of genes encoding for PTs in chick embryos. It will show that polysialylation gives metastatic potential to the primary tumor cells by supporting cancer cell survival and migration. Henceforth, the PTs could be developed as a novel therapeutic target for halting prostate cancer metastases.

Keywords: prostate cancer, glycosylation, polysialyltransferases, extravasation, metastases

Transforming growth factor-β1 pathway regulates differentiation of bone marrow-derived progenitor cells

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Introduction: Diabetes is characterized by hyperglycemia that ultimately results in dysfunction of select organs due to blood vessel impairment. We have shown that vascular dysfunction and inadequate repair in diabetes entails vasculogenic impairments that may be due to depletion of regenerative stem cells in the bone marrow. Furthermore, we and others have shown enhanced marrow adiposity and reduced bone mass in both human and experimental diabetes. These findings indicate that changes to the marrow composition potentially depletes stem cells. To understand the mechanism of enhanced marrow adiposity, I screened various signaling pathways during mesenchymal cell differentiation and identified transforming growth factor-β1 (TGF-β1) pathway as a potential regulator. Therefore, I hypothesize that altered TGF-β1 signaling in diabetes causes mesenchymal cell differentiation in the bone marrow leading to enhanced adipogenesis and loss of regenerative stem cells.

Methods: To test my hypothesis, I cultured primary human bone marrow-derived cells in adipogenic differentiation media with/without TGF-β1 and its inhibitor, GW788388, and assessed for cellular and molecular alterations.

Results: My results to date show that TGF-β1 challenge of bone marrow-derived cells cultured in adipogenic differentiation media caused reduced differentiation of adipocytes and lipid accumulation. In contrast, inhibition of TGF-β signaling enhanced adipogenic differentiation. I then identified the induction of the master regulator of adipogenesis, peroxisome proliferator-activated receptor γ (PPARγ) in my model system. Bone marrow-derived cells exposed to TGF-β1 in adipogenic differentiation media showed significantly decreased PPARγ levels. Similar to the lipid accumulation studies, inhibition of TGF-β signalling significantly increased PPARγ induction.

Conclusions: TGF-β1 is known to play a role in cell differentiation. My results show that activation of TGF-β1 pathway inhibits differentiation in bone marrow-derived precursor cells into adipocytes, whereas inhibiting TGF-β1 pathway enhanced differentiation. I will build from these findings to determine whether TGF-β1 activation and dampened adipogenesis can rescue regenerative stem cells in diabetes.

Keywords: TGF-β1, adipogenesis, differentiation, bone marrow-derived cells, diabetes

The Effect of Glucocorticosteroid Treatment on Th2 cells

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Introduction: Th2 high asthma is referred to as “steroid sensitive” due to the ability of glucocorticosteroids (GCs) to reduce type 2 cytokines and induce death in eosinophils. Despite being labeled “steroid sensitive” Th2 high asthma is associated with the most severe form of asthma. Severe asthmatics have a higher percentage of Th2 cells in the circulation and airways, further highlighting the importance of these cells in mediating disease severity. Chemoattractant homologous receptor of Th2 cells (CRTh2) is a marker for Th2 cells and binding prostaglandin D2 (PGD2) inhibits apoptosis. Interestingly, CRTh2 signalling blocks interference of anti-apoptotic Bcl-2/Bcl-xL activity, both proteins are downregulated by GCs. I hypothesize that high dose GC is required to induce Th2 cell apoptosis.

Method: We treated an immortalized Th2 cell line (CCRF-CEM) with dexamethasone (0.1-1μM) for 24h and 48h. Annexin V and 7AAD flow cytometry assays were used to determine apoptosis and cell death, respectively. Expression of CRTh2 and GC responsive genes (i.e. GR, FKBP5) were determined using qRT-PCR.

Results: We showed that high dose (1μM) dexamethasone for 48 hours of treatment was required to reduce cell growth (28 %, $p<0.05$) and induce Th2 cell apoptosis (17%) compared to media alone (5%, $p <0.01$). Interestingly, both low and high dose GC increased CRTh2 mRNA expression (1.8 fold).

Discussion: Our results suggest that high dose GCs are needed to trigger cell death in Th2 cells. The fact that GC also induced CRTh2 expression suggests this treatment could increase Th2 cell responsiveness to PGD2. If so, this could represent a mechanism by which CRTh2 signaling sustains Th2 cells in patients taking GC treatment.

Keywords: CRTh2, Th2, glucocorticosteroids, asthma, allergy

Defining the cellular origin of infantile hemangioma

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Introduction: Infantile hemangioma (IH) is the most common vascular tumour of infancy, characterized by a unique life cycle consisting of robust proliferation followed by regression. We have shown that IH arises from multipotential stem cells termed hemangioma stem cells (HemSCs). However, the origin of HemSCs is not well understood. During embryogenesis, blood vessels and blood cells develop from a common precursor called the hemangioblast, which is believed to migrate from the yolk sac, the aorta-gonadmesonephros region, and the placenta to the fetal liver before colonizing the bone marrow at birth. We hypothesize that abnormal homing of hemangioblasts to an incorrect anatomical site results in IH.

Methods: To test this hypothesis, we compared the gene expression profile and differentiation potency between fetal liver stem cells (FLSCs), HemSCs and bone marrow-derived precursor cells (BM-MPCs).

Results: Our data shows that HemSCs show unique and robust expression of stem and pluripotency genes, and repressed expression of mesenchymal program genes compared to both FLSCs and BM-MPCs. Furthermore, FLSCs and BM-MPCs demonstrate upregulated endothelial and mesenchymal gene expression not found in HemSCs.

Conclusions: Preliminary data indicates that HemSCs are distinct from FLSCs and BM-MPCs in pluripotent, endothelial and hematopoietic gene expression, suggesting a deviation from normal hematopoietic development. Studies are currently underway to compare the differentiation ability of all three cell types into hematopoietic and mesenchymal cell lines.

Keywords: infantile hemangioma, stem cells, hemangioblast, fetal liver, bone marrow, hematopoiesis

Evolutionary arms race in cancer: Murine melanoma as a model for tumor heterogeneity

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Introduction: Core biopsies and aspiration cytology are current gold standard techniques for the diagnosis of primary tumors in cancer patients, but there is a recent push for non-invasive, "fluid biopsies". Currently, clinical tests for circulating tumor DNA (ctDNA) and tumor-derived microparticles (MPs) are in development for bladder and colorectal cancer. However, an emerging phenomenon that tumor cells evolve under selective pressures, or 'tumor heterogeneity', has yet to be considered. Our current study examines the applications of fluid biopsies by evaluating the strengths and limitations of enumerating tumor-derived MPs and ctDNA.

Methods: We engineered a murine melanoma cell line (B16-F10) expressing fluorescently tagged histone DNA and modeled the in vitro release of purified MPs by nanoscale flow cytometry (A20). Afterwards, we adopted a pre-clinical model by injecting fluorescent cells and drawing blood from the chorioallantoic membrane of Gallus gallus domesticus embryos. We detected tumor-derived ctDNA and MPs by A20 and confocal microscopy. Our next steps are to pair the fluorescent cells with a syngeneic model using C57BL/6 mice to visualize release from the primary tumor.

Results: Purified tumor-derived MPs showed a heterogeneous distribution after size-exclusion chromatography and were strongly positive for the tetraspanin family exosome marker CD9, when compared to the isotype control, with small subpopulation of oncosomes >800 nm. Similarly, in G. gallus embryos, we captured fluorescent MPs in plasma and found a significant release over time when compared to wildtype cells. We also found histone-tagged DNA fragments in MPs and positive histone-tagged events from imaging fluorescent DNA by confocal microscopy.

Discussion: Our results suggest that the time-dependent release of MPs from cancer cells may play a pivotal role in cancer prognosis and that a portion of this DNA may be compartmentalized in histone-containing MPs. This has potential implications for development of combined clinical tests for cancer-derived MPs and ctDNA.

The Relation of Injury Severity for Child Occupants under the Age of Six and the Time of Day of Motor Vehicle Collisions

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Introduction: Previously it was believed that putting children in the rear seats of a vehicle was considered the safest option, however, new research shows that this may no longer be the case. Children and youth are primarily seated in the rear rows and are at an elevated risk for injury than their front seat counterparts. Many researchers have found that if an infant/child is using a child restraint system (CRS) properly during a motor vehicle collision (MVC) they are less likely to sustain a severe injury. The present study involves investigating children under 18 years old as rear seat occupants and what influences their injury patterns. We hypothesize that youth under 6 years using a CRS will be more severely injured if the MVC happens between 1600 and 2000h.

Methods: To test this hypothesis a retrospective methodology was used to look at injuries sustained in MVCs. This data was recorded by Transport Canada crash investigations and hospital emergency and admissions records from Daily Level 1 Pediatric Trauma Centre (PTC) reports. The injuries were assessed using the Abbreviated Injury Scale (AIS) 2005. The population of interest was rear seated occupants under the age of 6 years involved in a MVC.

Results: Our results show that the majority of MVCs happen between 1300-2000h. About half of the young individual population were injured in a MVC had collisions occur between 1600 and 2000h. The mean severity of the injuries sustained by these individuals was an AIS of moderate severity. The mean severity for injuries sustained not during 1600-2000h was an AIS of severe severity. There was not a statistically significant difference in the severity of injury between 1600-2000h and any other time.

Conclusions: These findings show that the time of the MVC occurring does not have a statistically significant impact on the injury severity of the occupants.

Keywords: injury, MVC, collision, children, trauma, youth, time

The Validation of a 3D Bioartificial Tissue of the Tenon's Capsule

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Introduction: Excessive wound healing has been a persistent complication for glaucoma patients and surgeons alike with few novel preventative measures. The Tenon's capsule (TC) is a fibrous connective tissue that contains fibroblasts essential to the healing process after filtration surgeries. Previous studies investigating human Tenon's capsule fibroblast (HTCF) modulation during wound healing indicate that excessive proliferation is involved in glaucoma filtration surgery failure. Using a collagen lattice, conditioned media, and HTCFs to create a 3D bioartificial tissue of TC, we hypothesis that this model will demonstrate HTCF in vivo properties that cause excessive wound healing such as increasing collagen density when stressed.

Methods: Primary HTCFs were cultured using Dulbecco modified Eagle's minimal essential medium with 10% Fetal Bovine Serum and 1% penicillin/streptomycin. HTCFs were seeded on an untreated discoid flexcell plate pre-augmented with rat-tail collagen, waymouth medium, and NaOH. Cells were given 3 days to acclimate before being subjected to biomechanical stress that mimicked circadian variation of intraocular pressure. Each bioartificial tissue was surgically resected from the discoid flexcell plates between days 3 to 14, stained with Masson's Trichrome or Picosirius Red, and imaged with a 20X objective lens of a light microscope.

Results: The bioartificial tissues stained with Masson's Trichrome from day 3 to 14 displayed HTCF viability and altered collagen organization in the later replicates. Picosirius Red staining indicated that stain intensity was proportional to duration of biomechanical stress. Picosirius Red stains collagen and thus demonstrated that collagen density increased in proportion to the duration of stress.

Conclusion: Masson's Trichrome and Picosirius Red staining determined that the HTCFs in bioartificial tissues produced collagen when stressed. In vivo HTCFs excessively produce collagen after filtration surgeries, which reverse therapeutic feats. As such, our novel model is suitable to study methods to improve therapeutic outcomes for HTCFs post-surgical wound healing.

Keywords: glaucoma, Tenon's capsule, human Tenon's capsule fibroblast, filtration surgery, bioartificial tissue, Masson's trichrome, Picosirius red, collagen

Histological Elucidation of Tumour Deposits in Colorectal Adenocarcinoma

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Introduction: Pericolonic tumour deposits (TDs) in colorectal carcinomas are discrete nodules of tumour within the pericolic or perirectal fat. They are within the lymphatic drainage of the primary carcinoma, away from the leading edge of the tumour, and lack residual lymph node tissue. Currently, colorectal adenocarcinomas with TDs are classified – similary to positive lymph nodes – as pN1c (stage III), according to the 7th edition of the TMN Classification of Malignant Tumours, and as such patients would typically receive adjuvant therapy. However, in the updated 8th edition, the definition has been clarified, such that, if a vessel wall or neural structure is identified, these nodules will no longer be classified as TDs, and patients would be stage II, for which adjuvant therapy would likely not be administered.

Methods: To examine the origin of TDs, 50 historical cases classified as pN1c are being identified and retrieved from the LHSC Pathology database. The presence of TDs will be confirmed and cases with no identifiable TD will be excluded. Blocks containing TDs will undergo deeper sectioning to exclude cases that represent direct extension from the primary tumour. In addition, elastin stains and S100 protein immunohistochemistry will be performed to identify vein walls and neural tissue, to aid in classification as venous or perineural invasion.

Results: Preliminary results of 11 cases containing 28 TDs reveal 5 with perineural and 2 with venous invasion, with 1 additional TD showing both. The remaining 20 TDs remain classified as TDs after further examination.

Discussion: These preliminary results show that several TDs, as currently classified, in fact would be regarded as venous and perineural invasion, thereby down-grading patient stage from stage III to stage II. If there is a similar trend for the remaining cases, this will aid pathologists by encouraging the further examination of so-called TDs in colorectal adenocarcinoma, and may reduce unnecessary treatment.

Keywords: colon cancer, rectal cancer, tumour deposits, colorectal adenocarcinoma

Deciphering the Role of the UBL Domain in Parkin Degradation

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Introduction: Parkinson's is a neurodegenerative disorder involving the loss of dopaminergic neurons found in the substantia nigra pars compacta. This disorder often involves mutations in the gene PARK2. The gene PARK2 codes for the protein parkin, which is a ubiquitin-protein ligase. This ligase is part of a larger construct know as the ubiquitin proteasome system. Without the proper functioning of this pathway protein aggregates begin to accumulate and cause oxidative damage. This is believed to be a possible precursor to Parkinson's disease. We are proposing a new study to see exactly how the ubiquitin binding ligase domain plays a role in the degradation of parkin as this domain is believed to be its regulatory mechanism.

Hypotheses: We Hypothesize that the ubiquitin like domain regulates parkin proteolysis and cleavage and that the ubiquitin like domain is a degron in parkin.

Methods: Parkin fragments where created where the ubiquitin binding ligase domain was both truncated and completely cleaved off. These genes where attached to GFP and transformed into yeast cells (BY4742, W303MATA). Once these proteins where expressed in yeast, with spot assays and western blots the regulating domain was reviewed by observing the fragments toxicity. The proteins where aged systematically and reviewed under a microscope.

Results: We expect to see the proteins without the ubiquitin binding like domain to have toxicity and therefore reduced yeast growth. With the yeast cells not containing the domain we expect aggregates to build up and be observed under a microscope. The truncated fragments may have different regulation and upon their review further studies will be conducted.

Conclusion: With the findings from the other fragments the regulation sites of the gene can be better understood. These findings may help to demonstrate how the regulation of parkin plays a role in Parkinson's disease.

Keywords: Parkin, ubiquitin, ubiquitin binding like domain, proteasome, Lewy bodies

ANRIL : a regulator of VEGF in Diabetic Retinopathy

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Introduction: Long noncoding RNAs (lncRNAs) previously thought to be "dark matter" of the genome, play key roles in various biological processes. LncRNA ANRIL is located at a genetic susceptibility locus for coronary artery diseases and Type 2 Diabetes. We examined the role of ANRIL in diabetic retinopathy, through study of its regulation of vascular endothelial growth factor (VEGF) both in vitro and in vivo.

Methods: Human retinal endothelial cells (HRECs) were subjected to incubation in high glucose for various durations to mimic diabetes. ANRIL expression was measured following knockdown with siRNA in HRECs. ANRIL knockout mice, with or without streptozotocin (STZ)-induced diabetes were also investigated. Cell and tissues were measured for VEGF mRNA and protein expression. Functional alterations in VEGF were determined through tube formation, cell proliferation and retinal vascular permeability assays. VEGF regulation through ANRIL's interactions with polycomb repressive complex 2 (PRC2) components and p300 were studied thorough PRC2 blocker, siRNA, and RNA-IP assays.

Results: High glucose and diabetes caused ANRIL upregulation in HRECs and in the retina. Glucose-mediated elevation of ANRIL, on silencing, prevented VEGF expression. Such regulation involved ANRIL-mediated control of PRC2 components, p300 and miR200b. Direct binding of ANRIL to p300 and enhancer of zeste homolog 2 (EZH2; PRC2 component) were elevated following exposure to high glucose levels.

Conclusions: Our results demonstrate for the first time that ANRIL regulates VEGF expression and function in diabetic retinopathy. This regulation is mediated by p300, miR200b and EZH2.

Keywords: ANRIL, VEGF, angiogenesis, diabetic retinopathy, p300, EZH2

The Impact of miR-128 in Cold Ischemia-Reperfusion Injury in Cultured Rat Cardiomyoblasts

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Introduction: In transplantation, ischemia-reperfusion (I/R) injury shortens the transplanted organ's lifespan and is a major cause of its dysfunction or non-function. To date, no effective treatments for I/R injury exist. Our previous study showed decreased microRNA-128 (miR-128) expression in I/R injured heart tissues. Here, we aim to investigate miR-128's role in cold I/R injury and understand its underlying mechanisms through a series of in vitro studies. We hypothesize miR-128 plays a protective role in cold I/R injury and that over-expression of miR-128 will prevent heart cell death in this injury via downregulation of polo-like kinase 2 (PLK2).

Methods: To test our hypothesis, H9c2 cells (Rattus norvegicus heart myoblast cell line) were cultured and transfected with miR-128 inhibitors and mimics before being subjected to cold I/R injury treatment in vitro. Live-cell imaging using SYTOX green staining and an IncuCyte system analyzed and compared the amounts of cell death in the various treatment groups. Cells were stained with both annexin-V and propidium iodide (PI) and analyzed by flow cytometry to further assess cell apoptosis and necrosis.

Results: Our flow cytometry and live-cell imaging results showed that 18 hours of cold ischemia followed by 24 hours of reperfusion induced cell death. Live-cell imaging showed an increase in cell death in the miR-128 treated cells and a decrease in cell death in miR-128 inhibitor treated cells.
Discussion: We demonstrate an in-vitro model of cold I/R injury in H9c2 cells validated by both flow cytometry and live-cell imaging. In addition, we show that miR-128 plays a detrimental effect in cultured H9c2 cells following cold I/R injury. Future directions: We hope to repeat our experiments in order to perform statistical analysis, determine miRNA and protein expressions by RT-qPCR and western blot, respectively, and show that miR-128 targets PLK2 using a luciferase assay.

Keywords: miR-128, PLK2, ischemia-reperfusion injury, H9c2, cardiomyoblast

Role of Doublecortin-like kinase 1 (Dclk1) positive tuft cells in colitis-associated colorectal cancer

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Introduction: The cell-of-origin and the cellular processes regulating the initiation of CRC remains largely unexplored. Our previous work demonstrated that mature intestinal tuft cells, demarked by doublecortin-like kinase 1 (Dclk1), can serve as a cellular origin of colonic tumors upon dextran sodium sulfate (DSS)-induced inflammation. This study attempts to characterize Dclk1+ tuft cells as colon cancer-initiating cells with various types of tissue injury. We hypothesize that colonic inflammatory insults lead to reprogramming of Dclk1+ tuft cells to a stem cell state, making the tuft cells susceptible to tumor initiation.

Methods: To determine which forms of colonic injury transforms quiescent tuft cells to form tumors, we employed Dclk1-CreERT2; ROSA26-mTmG; APCflox/flox (Dclk1-CreERT2/APCflox/flox) mice to examine the effects of inflammatory (DSS, trinitrobenzenesulfonic acid (TNBS), oxazolone) and infectious (Citrobacter rodentium) insults on colonic tumor formation.

Results: Myeloperoxidase (MPO) activity assay and histology confirmed the presence of colonic inflammation with DSS, TNBS, oxazolone, and C. rodentium treatment. Our preliminary data show a decrease in the frequency of Dclk1+ cells with DSS and oxazolone-induced colitis and an increase in the frequency in TNBS and C. rodentium-induced colitis. We have also confirmed that DSS treatment in Dclk1-CreERT2/APCflox/flox mice leads to Dclk1+ cell-derived colonic tumors with 100% efficiency. C. rodentium inoculation in Dclk1-CreERT2/APCflox/flox mice does not lead to colonic tumorigenesis 30 weeks post first inoculation; however, it gives rise to Dclk1+ cell-derived oral tumor. TNBS and oxazolone-colitis experiments are currently under way.

Discussion: Our data suggest that C. rodentium and DSS may be acting through different mechanisms to induce colitis, and this may affect their ability to activate Dclk1+ tuft cells. Overall, this study will identify the type of inflammatory injuries required for the initiation of colitis-associated CRC and ultimately highlight the cellular mechanisms that regulate the activation of the tuft cells.

Keywords: Colitis-associated colorectal cancer, Tuft cells, Dclk1, Inflammatory bowel disease, Colitis, Adenomatous polyposis coli

Carboplatin Paradoxically Increases Angiogenic Factors in Ovarian Cancer Cells

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Introduction: Ovarian cancer is the leading cause of death among gynecological malignancies. Current treatment of ovarian cancer involves carboplatin-based therapy. Despite an initial response to treatment, a large proportion of patients undergo relapse. The mechanisms underlying carboplatin treatment failure are not fully understood. One mechanism that is instrumental to the growth and viability of an ovarian tumour is angiogenesis (the expansion of blood vessels). In exciting preliminary studies, we have observed significant vascular proliferation after carboplatin treatment in ovarian serous adenocarcinoma patient tumour specimens compared to patient-matched, pre-treatment biopsies. We hypothesize that platinum-based chemotherapy alters ovarian cancer cells leading to neovascularization.

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

Results: Our results to date show that carboplatin reduces the viability of both ovarian cancer cells and endothelial cells. We have also found several angiogenesis-associated genes to be altered in ovarian cancer and endothelial cells following carboplatin exposure, including placental growth factor (PGF).

Discussion: Induction of PGF by carboplatin in both ovarian cancer and vascular endothelial cells is remarkable. PGF is variably upregulated in tumours and is known to bind to vascular endothelial growth factor receptor 1, which is directly involved in angiogenesis. We plan to modulate PGF in ovarian cancer cells and/or endothelial cells in an effort to investigate the functional significance after carboplatin exposure through endothelial proliferation and tubule formation assays. Ultimately, our findings may lead to the identification of a novel therapeutic target to improve chemotherapy treatments for patients with ovarian cancer.

Keywords: ovarian cancer, chemotherapy, angiogenesis, PGF

Immunological Impact of CLI095 on Dendritic Cell Maturation and Hypoxia-re-oxygenation induced inflammation injury

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Introduction: Ischemia reperfusion injury (IRI) activates innate immunity through the engagement of Toll-Like Receptors (TLRs) by endogenous ligands. TLR4 expressed within the kidney is a potential mediator of innate activation and inflammation. Stimulation of TLR4 induces distinct patterns of gene expression, which not only leads to the activation of innate immunity but also to the development of acquired immunity. CLI095, a novel synthetic small-molecule, suppresses production of multiple cytokines by inhibiting TLR4 signaling. In this study, we have determined the role of TLR4 in hypoxia and re-oxygenation injury model, which mimics IRI invitro, and investigated the effect of CLI095 (a specific TLR4 inhibitor) on TLR4 mediated inflammation and maturation of dendritic cells (DCs) and macrophages like cell line (RAW 267.4).

Hypothesis: TLR4 signaling plays an important role in activation of innate immunity, and that targeting its pathway will prevent innate immunity activation.

Method: Bone marrow derived dendritic cells were stimulated by lipopolysaccharide (LPS) or hypoxia re-oxygenation with or without CLI095. Expression of TLR4, proinflammatory cytokines, and dendritic cells maturation markers were then tested by Flow Cytometry, qRT-PCR, and ELISA.

Results: We have shown that CLI095 is able to reduce the expression of pro inflammatory cytokines (IL6 and TNFα) in response to LPS activation and hypoxia re-oxygenation. In addition, DCs that were pretreated with CII095 showed low expression of maturation markers in comparison to cells that were only subjected to LPS or hypoxia re-oxygenation. Furthermore, our results showed that CLI095 blocks the TLR4 signaling pathway.

Conclusion: TLR4 is involved in innate immunity activation in response to IRI or hypoxia re-oxygenation and CLI095 able to block TLR4 signaling pathway and suppress the activation of the inflammatory response. Therefore, targeting TLR4 by new therapies such as CLI095, which specifically targets TLR4, may have potential implication in reducing IRI in clinical transplantation.

Keywords: Kidney, Ischemia Reperfusion Injury, hypoxia re-oxygenation, Toll like receptors, Innate immunity, CLI095

The examination of prolonged high-fat diet on islet function in adult MIP-βIRKO mice

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Introduction: The presence of insulin receptor (IR) on insulin-secreting β-cells suggests an autocrine role for insulin signaling. Mice with β-cell-specific IR knockout (βIRKO) from conception show glucose intolerance and reduced islet function, yet our lab has found that mice with a postnatal loss of IR do not develop hyperglycemia. In this study, we examine mice with postnatally-induced IR knockdown on long-term high-fat diet (HFD) to investigate the role of IR signaling in a high glucose environment.

Methods: Male mice were administered tamoxifen (4mg/20g BW, intraperitoneal) at 4 weeks of age to induce β-cell-specific, mouse insulin promoter-controlled Cre recombinase excision of IR (MIP-βIRKO). Wild-type (WT) and MIP-βIRKO mice were placed on normal chow or 60% HFD at 2 weeks post-tamoxifen for an 18-week duration. Glucose tolerance and insulin release were measured through the use of IPGTT and GSIS. Histological analyses of HFD WT and MIP-βIRKO mice focused on islet morphology, β-cell-specific transcription factors, and SNARE proteins.

Results: MIP-βIRKO mice under HFD treatment showed significantly increased glucose intolerance compared to HFD-fed WT mice. In vivo GSIS in HFD mice found that MIP-βIRKO mice had reduced insulin secretion. Islet morphology studies revealed slight, but insignificant, increases in β-cell mass and proliferation in HFD MIP-βIRKO, and increased staining of Igf-1R in HFD MIP-βIRKO was also noted. Immunofluorescence staining in HFD MIP-βIRKO mice found no changes in the transcription factor Pdx-1 and Nkx6.1. However, decreased levels of SNARE proteins VAMP2, SNAP25, and Munc18-a were found, suggesting defects in the machinery that regulates insulin release from islets.

Discussion: IR signaling in β-cells under HFD is required to maintain normal insulin secretion and exocytotic molecules that control glucose homeostasis. Future studies will focus on in vitro cell line experiments and MIP-βIRKO isolated islets to determine the mechanisms that link IR stimulation with SNARE proteins.

Keywords: Diabetes mellitus, β-cell function, Insulin Receptor, tamoxifen-inducible Cre recombinase, high-fat diet, SNARE complex

Clinical Validation of a NGS Pipeline that Outperforms Sanger Sequencing and MLPA Analysis

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Introduction: Conventional analysis for the molecular diagnosis of genetic conditions involves a tiered approach for the assessment of sequence and copy number variants by a number of technologies including Sanger sequencing, MLPA or microarray. However, the recent advances in next generation sequencing (NGS) allows for the parallel analysis of multiple genes with a more cost-effective and rapid methodology. Objective of this study was to develop and validate a clinical NGS pipeline that outperforms the combined approach of Sanger sequencing and MLPA.

Methods: Our approach utilizes genomic fragmentation and a custom targeted probe design for capture-based DNA library enrichment, resulting in high-depth sequencing with inter-sample sequence coverage uniformity, allowing for highly sensitive sequence and copy number variant detection. Each gene panel probe design includes all coding nucleotides as well as 20 nucleotides of both 5' and 3' flanking intronic sequence.

Results: Validation of the custom NGS BRCA1 and BRCA2 gene panel resulted in 183 unique sequence and copy number variants in the 402 patient retrospective (n=95) and 240 patient prospective (n=88) cohort, demonstrating 100% sensitivity and a very high degree of specificity. We applied the same approach for an expanded hereditary cancer panel of 25 genes as well as eight other larger gene panels (up to 69 genes tested in parallel), with similar sample to sample reproducibility in coverage uniformity, and sensitivity and specificity for detection of sequence and copy number variants.

Conclusion: We validated a clinical-grade, single-tiered NGS pipeline which outperforms the combined approach of Sanger sequencing and MLPA for sensitive and specific detection of sequence and copy number alterations.

Keywords: NGS, molecular diagnosis, genetics, cancer, BRCA1/BRCA2, gene panels

Investigating antigen presenting cell-phenotype of granular cell tumors

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Introduction: Granular cell tumours (GCTs) are relatively uncommon solitary benign subepithelial lesions with a high incidence 65-85% in the dorsolateral tongue. They present as raised yellowish lesions 1-3 cm in diameter. GCTs consist of sheets or ribbons of large polygonal cells, dense hyperchromatic nuclei and cytoplasmic eosinophilic granules thought to be lysosomes. First described in 1926 as Abrikosoff's tumor, they were thought to be of myogenic origin; but are currently thought to be of neural origin due to expression of S100 protein and other neural markers. Recently, our laboratory has shown that GCTs express HLA-DR suggesting an antigen presenting cell (APC)-phenotype. The aim of this study is to further characterize the phagocytic phenotype of GCTs using immunohistochemistry and reverse transcriptase polymerase chain reaction (RT-PCR) in support of a possible antigen presenting cell origin.

Methods: Twenty-two cases of GCTs and 10 cases of schwannomas (controls) from the oral cavity were assessed immunohistochemically for protein expression of APC-phenotype associated genes CD68, HLA-DR, CD163, CD40, and CD11c. Tumours were scored for intensity and number of reactive cells. Paraffin embedded formalin fixed tumour tissue punches were obtained from 10 GCTs for RT-PCR. RNA was successfully extracted in sufficient quantity in 7/10 tumour blocks for RT-PCR analysis.

Results: Our results show that 22/22 granular cell tumours stained densely positive for HLA-DR, and CD68 while CD163, CD40 and CD11c were negative. All schwannoma cases stained positive for HLA-DR and CD163. CD40 and CD11c immunoreactivity was not detected in GCTs or schwannomas. RT-PCR results are pending.

C**onclusions:** Our results show evidence suggesting a phagocytic phenotype for the cells in GCTs, which has not been previously explored. Further work involving additional antibodies and assessing the RNA profile using RT-PCR will give further insight into the differentiation phenotype of GCTs.

Keywords: Granular Cell Tumour, Schwannoma, Immunohistochemistry, CD68, HLA-DR, CD163

Cardiac-specific over-expression of Tcap reduces doxorubicin-induced cardiotoxicity in mice

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Introduction: Doxorubicin is widely used as a first-line chemotherapeutic drug for various malignancies. However, the clinical use of doxorubicin is limited because of its severe cardiotoxicity. Tcap is mainly located at Z-discs in cardiac and skeletal muscles, and plays an important role in sarcomere assembly and t-tubule function. A recent study reported that Tcap facilitated p53 degradation and inhibited apoptosis in a mouse model of heart failure. We showed that doxorubicin decreased histone deacetylase (HDAC) activity, and inhibition of HDAC enhanced p53 acetylation and apoptosis induced by doxorubicin. Thus, it is possible that Tcap may protect HDAC activity, leading to p53 de-acetylation.

Methods: A novel line of transgenic mice with inducible cardiac-specific over-expression of human Tcap (hTcap) was generated (Tg-Tcap/tTA). Cardiotoxicity was induced in Tg-Tcap/tTA mice and their wild-type littermates by doxorubicin. Myocardial function was assessed by echocardiography. Primary neonatal and adult cardiomyocytes were stimulated with doxorubicin. An adenoviral vector containing Tcap was used to over-express Tcap in cardiomyocytes. Apoptosis was determined by caspase-3 activity, DNA fragmentation and annexin-V staining. The protein levels of Tcap, p53 and acetylated-p53 were analyzed by western blot. HDAC activity was measured in cardiomyocytes.

Results: Transgenic over-expression of hTcap was verified in Tg-Tcap/tTA mouse hearts. Administration of doxorubicin decreased myocardial function in wild-type mice, which was restored in Tg-Tcap/tTA mice. In cultured cardiomyocytes, doxorubicin reduced the protein levels of Tcap and induced apoptosis. These effects of doxorubicin were prevented by over-expression of Tcap. Mechanistically, up-regulation of Tcap restored the HDAC activity and prevented p53 acetylation in doxorubicin-stimulated cardiomyocytes. Moreover, inhibition of HDAC activity enhanced doxorubicin-induced p53 acetylation and apoptosis.

Conclusions: Up-regulation of Tcap reduces doxorubicin-induced cardiotoxicity by preserving HDAC activity and de-acetylating p53. Thus, Tcap may be a novel therapeutic target for doxorubicin-induced cardiotoxicity.

Keywords: Doxorubicin, cardiotoxicity, Tcap, HDAC, p53

Histogenesis and Immunohistochemical Profiles of Granular Cell Tumours

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Introduction: Granular cell tumours (GCTs) are very rare, slow growing, benign lesions. GCTs have a wide anatomical distribution, but about 50% of cases are found in the head and neck region, with the tongue being the most common site. Following a benign GCT diagnosis, a complete surgical excision of the lesion is considered curative. The most distinct feature of GCT cells is the presence of eosinophilic coarse granules in the cytoplasm, which are believed to be the accumulation of secondary lysosomes.

These lesions were first characterized by Dr. Abrikossoff in 1926, who thought they were of striated muscle origin. Over the years, this theory of origin has been debunked and in place, many different alternatives have been proposed. Scientists have explored possible neural, endomesenchymal, metabolic and antigen-presenting cell origins for GCTs. However, the currently accepted view suggests GCTs express a neuroectoderm phenotype. The neural origin hypothesis of GCTs requires further evidence.

Methods/Results: Our study aims to immunohistochemically profile a range of oral GCTs (n=22) in order to gain a better understanding of this lesion's origin and lineage. A series of neural origin markers: S-100, neuron specific enolase (NSE), and SOX10 were examined using monoclonal/polyclonal antibodies on tissue sections. A series of oral Schwannomas (n=10) were used as our neural tumour control. In all available cases, GCTs and Schwannomas showcased immunopositivity for S-100 and SOX10, two schwannian differentiation markers, and for NSE, a neuronal lineage marker.

Conclusions: The positive expression of neural markers in the GCTs, especially for SOX10 which is considered a highly specific marker of neural cell origin, supports a neural phenotype. We hope the findings of our study will contribute to updating the known immunohistochemical profile of GCTs, which can impart important diagnostic value.

Keywords: Granular cell tumours; immunoprofile; origin; Schwannomas; neuroectodermal

Endotheliocyte-specific deletion of capns1 reduces diabetic cardiomyopathy in mice by improving angiogenesis

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Introduction: Diabetic cardiomyopathy is a serious clinical condition. Calpain-1/2 were implied in diabetic hearts. They required capns1 for assembly and activity. We recently reported that deletion of cardiomyocyte capns1 reduces adverse cardiac changes in mouse models of diabetes, suggesting a critical role of cardiomyocyte calpain in diabetic cardiomyopathy. However, it remains unknown whether endothelial calpain plays a role in diabetic cardiomyopathy. We hypothesize that endotheliocyte-specific deletion of capns1 disrupts calpain-1 and calpain-2, protects endothelial cells and attenuate adverse cardiac changes in diabetes.

Methods: A novel line of endotheliocyte-specific capns1 knockout mice were generated. Pre-diabetes, type-1diabetes and type-2 diabetes were induced in capns1 knockout mice and their wide-type littermates. Myocardial function was assessed. The capillary density and aortic ring sprouts were analyzed. Cultured mouse cardiac microvascular endothelial cells (MCECs) were stimulated with palmitate. Apoptosis and angiogenesis were analyzed.

Results: Deletion of capns1 disrupted calpain-1 and calpain-2 in endotheliocyte capns1 knockout mice. Cardiac collagen deposition and cardiomyocyte size were significantly increased in pre-diabetic and diabetic mouse hearts, accompanied by myocardial dysfunction. These cardiac changes were attenuated by disruption of calpain in capns1 knockout mice. Deletion of endotheliocyte capns1 prevented a reduction in capillary density and increased coronary blood flow in pre-diabetic and diabetic hearts. Ex vivo analysis revealed more microvessel sprouts in aortic rings from diabetic capns1 knockout mice compared with their wild-type littermates. In cultured MCECs, inhibition of calpain restored tube formation and wound healing in response to diabetic conditions.

Conclusions: Disruption of endothelial cells calpain improved angiogenesis in diabetic hearts and reduce diabetic cardiomyopathy. Thus, calpain may be an important therapeutic target for diabetic cardio complications.

Keywords: capns1, endothelial cells, angiogenesis, diabetic cardiomyopathy

Characterization of 5-Lipoxygenase expressing Epithelial Cells in Colitis-associated Colorectal Cancer

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Introduction: Doublecortin-like kinase 1 (DCLK1) positive intestinal epithelial tuft cells are long lived quiescent cells that may act as the origin of some colitis-induced colorectal cancers. We have recently observed increased expression of the pro-inflammatory enzyme 5-lipoxygenase (5-LO) in these cells. The present study is aimed at assessing the viability of using 5-LO as a superior marker of these cells. We hypothesize that 5-LO is a viable marker of intestinal epithelial tuft cells.

Methods: To assess 5-LO expression, we used a novel transgenic 5-LO-GFP-Diphtheria Toxin Receptor-Cre ERT2-TdTomato (5-LO-GDC) murine model and visualized cells in the epithelium of stomach, small intestine, and colon at four endpoints. Tamoxifen inducible Cre-Lox activation of TdTomato fluorescent protein, as well as anti 5-LO and anti-DCLK1 antibodies, were used to locate and characterize 5-LO expressing cells. Diphtheria was used for cell knockout to further confirm 5-LO expression.

Results: Our results show that GFP was observed in epithelial cells in the crypts and villi along the gastrointestinal tract. TdTomato was not observed to overlap with GFP-positive cells. However, overlap was observed within the stromal tissue, indicating functioning Cre in other 5-LO expressing cells. Diphtheria treatment did not show a significant decrease in the number of 5-LO expressing epithelial cells. Anti-DCLK1 and 5-LO antibodies did not stain these cells.

Conclusions: Our findings show that there is a population of cells within the intestinal epithelium that are 5-LO expressing due to GFP expression. However, these cells do not demonstrate the other effects downstream of the promoter, therefore limiting our results.

Keywords: Tuft cells, 5-lipoxygenase, inflammation, colorectal cancer, cell markers

Analysis of CD123 and Retinoblastoma (Rb) protein immunohistochemistry in Blastic Plasmacytoid Dendritic Cell Neoplasm

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Blastic plasmacytoid dendritic cell neoplasm (BPDCN), formerly known as CD4+CD56+ haematodermic neoplasm, is a recent entity identified in the 2008 World Health Organization classification and is a very aggressive and rare subtype of acute myeloid leukemia. BPDCN is thought to originate from clonal proliferation of precursor plasmacytoid dendritic cells (PDC) and is believed to share a phenotypic commonality with PDC by having a strong expression to CD123 (IL-3 receptor α chain). Karyotypic analyses of these tumour usually reveals a complex karyotype, however a few recurrent chromosomal abnormalities have been identified, including loss of 13q in over half of cases. It has been postulated that the deregulation of the G1-S cell cycle checkpoint is a common abnormality in these tumours, and the loss of the retinoblastoma (RB1) gene (located at 13q) possibly playing a key role in this process. This quality assurance study will be used to analyze CD123 expression in multiple cases of bone marrow and skin BPDCN, including calculating its sensitivity and specificity, as well as validating the use of CD123 immunohistochemistry in the clinical laboratory for future diagnosis of BPDCN. In addition, immunochemistry for the RB protein (pRB) will be attempted, and correlated with karyotype results.

Keywords: Blastic plasmacytoid dendritic cell neoplasm, CD123, RB protein

Alteration of Mitochondrial Sirtuin Expression in Endothelial Cells Under High Glucose Conditions

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Introduction: One of the most severe complications of Diabetes Mellitus is the development of vascular pathology mediated by endothelial cell (EC) dysfunction. Production of reactive oxygen species (ROS) in the mitochondria is a major outcome of high glucose (HG) utilization by ECs. Upregulated ROS production can cause damage of various macromolecules, and accelerate cell aging. Sirtuins are NAD-dependent deacetylases, or ADP-ribosyl transferases of target proteins. SIRT1 downregulation has previously been shown in EC cell culture grown under HG conditions. The effects on mitochondrial sirtuins are not as well established. We hypothesize that HG conditions downregulate the expression of the mitochondrial sirtuins, namely SIRT3 and SIRT5, in ECs, and as a result accelerate cell aging patterns.

Methods: Human cardiac microvascular ECs (HCMECs) and human retinal microvascular ECs (HRECs) have been cultured in a 25mmol/L D-glucose solution to model the effect of HG levels. ECs will also be cultured at HG conditions along with Resveratrol, a known Sirtuin activator. SIRT3 and SIRT5 expression was measured using RT-qPCR, and cell aging changes will be determined by staining for a senescence marker Senescence Activated-β-galactosidase (SA-β-gal).

Results: Our results showed that exposure of ECs to HG levels decrease SIRT3 and SIRT5 expression in HRECs. Our expected findings are that the decreased expression of mitochondrial sirtuins will be associated with accelerated EC aging. Also we expect that treatment with Resveratrol will restore SIRT3 and SIRT5 expression, and these cells will show less aging than the HG group.

Discussion: HG levels lead to decreased mitochondrial sirtuin expression in ECs. Our remaining results will show whether restoration of SIRT3 and SIRT5 levels reduces cell aging in ECs. These findings may elucidate a novel mechanism of EC aging and dysfunction in diabetic patients, which can lead to a potential future target for therapeutic modalities in vasculogenic diabetic complications.

Keywords: Diabetes mellitus, endothelial cells, mitochondria, reactive oxygen species, sirtuins

A Case Report of Sudden Death from Intracardiac Leiomyomatosis

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Introduction: Intracardiac leiomyomatosis (ICLM) is the extension of an intravenous leiomyoma into the heart. ICLM causing sudden death is extremely rare. The mechanism of death is thought to be right heart failure.

Case: A 50-year-old female was found deceased in her bathroom. Her only medical history was an episode of transient right hemiparesis 17 years earlier from either a transient ischemic attack (TIA) or a migraine episode. Investigations found no cause.

At autopsy, there was a yellow, cylindrical intravascular mass attached to the distal inferior vena cava that extended into the right heart. There were also 3 yellow, interconnected nodules under the tricuspid valve leaflet. The distal right main pulmonary artery was occluded. There were 3 masses in the right ovarian broad ligament. Microscopy of all these lesions showed they were spindle cell tumors with a myxoid and hyalinized fibrotic stroma containing blood vessels. The tumor was positive for smooth muscle actin, muscle specific actin, desmin, and vimentin. Based on the distribution of the tumor, and its histological and immunohistochemical features, the diagnosis was intravenous leiomyomatosis.

Discussion: ICLM was first described in 1907. Reports of sudden death are rare. ICLM is exclusively seen in middle-aged females.
There are some similarity in expression profiles between ICLM and leiomyosarcoma, which may explain the quasi-malignant behavior of ICLM compared to uterine leiomyoma. There are two theories regarding the origin of intravenous leiomyomatosis. It originates either from an uterine leiomyoma that extends into veins or within a vein de novo. Microscopic demonstration of continuity with the vessel wall is consistent with the latter. In the present case, the uterus was grossly normal. Site of attachment were seen by microscopy in the lower inferior vena cava and paratubal veins, consistent with intravenous origin possibly in one or more of these sites. Separate foci of tumor under the tricuspid valve and in the pulmonary artery can be interpreted as tumor emboli.

Keywords: intracardiac leiomyomatosis, sudden death

Detecting virus compartmentalization within hosts from genetic sequence variation

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Introduction: In the study of virus genetic diversity, a compartment is a genetically distinct subpopulation of virus caused by cell or tissue-specific selection and/or restricted viral migration. Quantifying viral compartmentalization is important for understanding the pathogenesis of viruses such as HIV, which utilizes a variety of tissues and cell types in the human host. However, different methods for measuring compartmentalization from genetic sequences can give conflicting results on the same data.

Methods: We performed a simulation study of existing methods for detecting compartmentalization, and used these simulations to validate a new method. By varying the migration rate of cell-free virus between compartments, we can model random mixing or strong compartmentalization. Virus phylogenetic trees were simulated from the model where the tips of the tree were labeled with the compartment from which the respective virus was sampled. We evaluated the ability of various methods to detect compartmentalization from these simulated trees, including a kernel method being developed in our lab.

Results: We confirmed that the distribution of labels on tips in simulated trees varied with migration rate parameters. The Slatkin-Maddison test was effective for detecting migration rates at 0.01 and lower against the null hypothesis of random mixing, but provided no means of estimating rates. A PCA plot of the kernel score matrix generated from the same data illustrates separation of trees with respect to migration rates that is consistent with the S-M test.

Conclusions: This result implies that the kernel method is able to capture the same information as current test statistics. Our next objective is to evaluate a potential key strength of the kernel method to estimate compartment-specific dynamic rate parameters. Such a method would play an important role in understanding the roles of different compartments as HIV latent reservoirs, with implications for HIV cure/eradication strategies.

Keywords: virus evolution, compartmentalization, phylogenetics, HIV, kernel methods

The Effects of Repeated Exposure to Whole Body Vibration on Murine Intervertebral Disc and Knee Joint Health

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Introduction: High-frequency, low-amplitude whole body vibration (WBV) is being used clinically for the treatment of musculoskeletal disorders and in the fitness industry to promote weight loss and increased muscle strength. Despite the increasing popularity of WBV platforms, there is an alarming lack of research that directly assesses the effect of WBV on load-bearing joint tissues. Previous research by our lab has shown that exposing ten-week old male CD1 mice to repeated WBV leads to osteoarthritic-like changes in the knee and degeneration of the intervertebral disc. The current study aims to determine whether the deleterious response of skeletal tissues to WBV is consistent between mice of different ages and genders. We hypothesize that six-month old mice and male mice will have increased susceptibility to vibration-induced damage in cartilaginous joint tissues.

Methods: To test this hypothesis, ten-week old and six-month old male and female mice were subjected to WBV for 30 min/day, 5 days/week for 8 weeks using protocols that emulate those used clinically (45 Hz, peak-to-peak amplitude 74 μm, peak acceleration 0.3 g). After exposure to WBV, intervertebral disc and knee tissues were examined using histological analysis and gene expression quantified using real-time PCR.

Results: Our results show that articular cartilage damage was similar in mice at 10 weeks and 6 months of age. In addition, less articular cartilage damage was seen in female than male mice at 10 weeks and 6 months of age.

Conclusions: These findings suggest that age does not alter WBV-induced damage in the knee joint. In contrast, gender may alter the effects of WBV.

Keywords: whole body vibration, knee joint, intervertebral disc, cartilage degeneration, disc degeneration

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