

PATHOLOGY AND LABORATORY MEDICINE RESEARCH DAY 2019

PROGRAM GUIDE



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PATHOLOGY AND LABORATORY MEDICINE
RESEARCH DAY 2019



Our Pathology and Laboratory Medicine Research Day is a cornerstone calendar event of our department, and it emphasizes our commitment to knowledge creation, sharing and its application to real-world health issues. This day is dedicated to celebrating our research accomplishments and our people. I would like to congratulate our trainees in the undergraduate programs, thesis-based graduate studies, professional graduate program, dual Oral and Maxillofacial Surgery MSc program, dual MD/PhD program, and Pathology postgraduate programs for their fantastic work over the past year. Our multi-disciplinary approach to studying health and disease is highlighted by 79 presentations this year.

We welcome Dr. Anthony Magliocco to our Research Day as the Keynote speaker. Dr. Magliocco is a Senior Member and Chair, Department of Anatomic Pathology at Moffitt Cancer Center, Florida, as well as Executive Director of Esoteric Laboratory Services and the Morsani Molecular Diagnostic Laboratory, and Scientific Director of the Moffitt Tissue Core. In his research, Dr. Magliocco has developed new digital image analysis methods and has created numerous new diagnostic tests for use in better selecting treatment plans for patients with breast, ovary, lung, bladder and other cancers. It is a pleasure that Dr. Magliocco is visiting us as a Keynote speaker and sharing his clinical and research expertise.

Many members of our department have dedicated considerable time to ensure that this day is an exceptional experience for our members and the community. I would like to personally thank Nancy Chan, Martin Duennwald, Manal Gabril, Zia Khan, Chandan Chakraborty, Christopher Tran, Rachel Halaney, Tracey Koning, Cheryl Campbell, Susan Underhill, Jina Kum, Vy Ngo, Kathilyn Allewell, and Kayla Anderson. Lastly, I would like to thank the judges for interacting with the 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

9:00 – 9:15 am	Welcome and Opening Remarks University Hospital, Aud. A Dr. Subrata Chakrabarti Chair/Chief, Pathology and Laboratory Medicine, Schulich Medicine & Dentistry, Western University and London Health Science Centre
9:15 – 10:15 am	Keynote Address University Hospital, Aud. A Dr. Anthony Magliocco Senior Member and Chair, Department of Anatomic Pathology, Moffitt Cancer Center, Florida
10:15 – 10:30 am	Nutritional Break University Hospital, Aud. A
10:30 – 12:00 pm	Oral Presentations University Hospital, Aud. A
12:00 – 12:15 pm	Group Photo University Hospital, Aud. A
12:30 – 1:15 pm	Lunch The Great Hall, Somerville House
1:15 – 2:15 pm	Poster Presentations – Session A The Great Hall, Somerville House
2:30 – 3:30 pm	Poster Presentations – Session B The Great Hall, Somerville House
3:30 – 3:45 pm	CME Evaluation The Great Hall, Somerville House
3:45 – 4:00 pm	Travel to Windermere Manor
4:00 – 7:00 pm	Award Ceremony Windermere Manor

Learning Objectives

1. Discuss the role that pathology has in supporting precision medicine
2. Understand how new technologies such as image analysis, genomics, and liquid biopsy will impact cancer care
3. Understand how to convert a research test into a test that can be used for patient care

Time	First Name	Last Name	Title
10:30 am	Christopher	Tran	Evaluation of additional lymph node sampling in the identification of colorectal cancer metastases
10:45 am	Michael	Roes	Elucidating the Molecular Mechanisms of Resistance to Enzalutamide in Prostate Cancer
11:00 am	Thomas	Shi	Application of the p16-Ki67-HMB45 Scoring System to the Diagnosis of Conjunctival and Uveal Melanoma
11:15 am	Vy	Ngo	Oxidative stress-induced aggregation of Keap1 impairs Keap1-dependent Nrf2 regulation
11:30 am	Paul	Plantinga	Urine Cytology-Histology Correlation Before and After Implementation of The Paris System for Reporting Urinary Cytology
11:45 am	Saumik	Biswas	The Long Non-Coding RNA HOTAIR is an Important Angiogenic Mediator in Diabetic Retinopathy

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 (4.75 hours). Each participant should claim only those hours of credit that he/she actually spent participating in the education program. Twenty-five % of this program is dedicated to participant interaction. This program has no commercial support.

Evaluation of additional lymph node sampling in the identification of colorectal cancer metastases

Christopher Tran, Christopher Howlett, David Driman

Department of Pathology and Laboratory Medicine, London Health Sciences Centre

Introduction: Pathological staging in colorectal cancer informs prognosis and guides adjuvant treatment. One component of staging includes the evaluation of regional lymph nodes for metastasis. A standard minimum of 12 lymph nodes should be evaluated, and if not achieved, reexamination of the specimen for additional lymph nodes is typical. No previous studies have evaluated the effectiveness of additional lymph node sampling in cases with less than 12 lymph nodes, in identifying lymph node metastases.

Methods: All cases of primary unifocal colorectal adenocarcinoma cases requiring additional lymph node sampling, for insufficient lymph nodes, from July 2008 to July 2018 were included. For each case, variables relating to the patient (demographics), specimen (dimensions, tissue location), gross room personnel, malignancy (TMN staging, microscopic features), and lymph node dissection were collected. Histologic sections from lymph node positive cases were retrieved to determine whether metastatic nodes were retrieved in initial or subsequent lymph node sampling.

Results: A total of 400 cases had fewer than 12 nodes (mean: 4.4 range: 0-23). Of these, 100 cases already had lymph node metastases. Additional sampling yielded additional nodes (mean and range). In 23/400 cases (5.75%), nodal metastases were found only in the additionally sampled nodes, resulting in a change of TMN classification in 19 cases and stage group in 9 cases. There was no association between patient, specimen, personnel or malignancy features and the identification of positive lymph nodes in subsequent sampling.

Discussion: The 12 lymph node standard is a well-established quality measure at the population level. In cases with fewer than 12 lymph nodes, this study showed that additional sampling was effective in identifying metastases and upstaged a significant proportion of patients. Additional lymph node dissections should be performed on all colorectal specimens with fewer than 12 lymph nodes.

Keywords: colorectal cancer, surgical pathology, gross pathology

Elucidating the Molecular Mechanisms of Resistance to Enzalutamide in Prostate Cancer

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Introduction: Molecularly targeted cancer therapies (MTCT's) are effective in improving patient outcomes and act by inhibiting molecular dependencies of cancer cells. However, resistance to MTCT's is common. Prostate cancer cells can acquire resistance to the anti-androgen enzalutamide (EZ) by switching lineages from an adenocarcinoma to a neuroendocrine (NE) cell type that no longer depends on androgens for growth. Studies have identified the retinoblastoma (RB) protein to be important in this mechanism. RB mutations cause epigenetic and stem cell plasticity, suggesting that RB loss promotes transdifferentiation through gene misregulation. However, a molecular mechanism of how this occurs has yet to be defined. In this study, I hypothesize that mutations in RB and additional unidentified proteins cooperate to promote transdifferentiation and EZ resistance in prostate cancer.

Methods: A genome-wide CRISPR knockout (KO) screen was performed in the prostatic adenocarcinoma cell line, LNCaP. Pools of KO cells were treated with either EZ or DMSO over two months to identify loss of function mutations that cause increased survival under EZ treatment.

Results: Analysis of the screen results identified RB as one of the top hits. Other hits include members of the F-box, zinc-finger, and ring-finger proteins. Interestingly, EZ treated cells collected from the final time point were not more resistance to EZ compared with control cells at various concentrations of EZ. Furthermore, EZ treated cells had increased transcript levels of the stem cell reprogramming factor SOX2, but no difference in the neuroendocrine marker NSE compared with control cells.

Conclusions: These findings suggest that gene KO's of the top hits from the screen may promote a state of stem cell plasticity, but are not sufficient to cause NE transdifferentiation or EZ resistance. Future work will determine whether select hits from the screen have functional roles in regulating stem cell plasticity and neuroendocrine transdifferentiation.

Keywords: Molecular Targeted Therapies, Resistance, Retinoblastoma Protein, Prostate Cancer, CRISPR Screen, Enzalutamide

Application of the p16-Ki67-HMB45 Scoring System to the Diagnosis of Conjunctival and Uveal Melanoma

Thomas Shi, Subrata Chakrabarti, Manal Y Gabril

Department of Pathology and Laboratory Medicine, Schulich School of Medicine & Dentistry, Western University

Introduction: Distinguishing malignant melanoma from benign nevus can be challenging. Previous research has established a p16-Ki67-HMB45 scoring system that can distinguish skin melanoma from nevi with high sensitivity and specificity. We wish to apply this system to uveal and conjunctival cases

Methods: 14 cases of conjunctival nevus, 3 cases of conjunctival melanoma, and 13 cases of uveal melanoma were retrieved. 2 cases of uveal melanoma were discarded due to lack of viable tumor. Immunohistochemistry for MelanA, p16, Ki67 and HMB45 were performed. Ki67 was scored as: < 2% = 0, 2-5% = 1, 6-10% = 2, 11-20% = 3, > 20% = 4; p16 was scored as: > 50% of tumor cells = 0, 50-11% = 1, < 10% = 2, total absence = 0. The original HMB45 scoring called for 0 if positive gradient was present (junctional cells were positive but not deep cells), 1 for inclusive, and 2 for negative gradient. The modified HMB45 scoring is: absent = 0, focal positivity = 1, diffuse positivity = 2. In cases of focal positivity, we also noted if positivity was superficial, deep, or both. The total score, sensitivity and specificity were then calculated.

Results: Using the original scoring system, the specificity was 100% but the sensitivity was 28.5% due to difficulty applying the HMB45 gradient score to uveal melanoma, causing false negatives. With modified HMB45 scoring and using a cutoff of > 3, the sensitivity and specificity were both 100%. Increasing the cutoff from 3 to 4 had the same specificity (100%), but lower sensitivity (78.6%). A 2 parameter scoring system (Ki67 and p16 only) was inferior to the 3 parameter system (sensitivity = 71.4%, specificity = 85.7%).

Discussion: In our small sample, a modified scoring system reliably distinguished melanoma from nevus. In the future, a validation study with larger sample size could be considered.

Keywords: Melanoma, conjunctiva, p16, Ki67, HMB45

Oxidative stress-induced aggregation of Keap1 impairs Keap1-dependent Nrf2 regulation

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Introduction: Nrf2 is the master regulator of the oxidative stress response needed to eliminate reactive oxygen species (ROS) in cells. It is negatively regulated by Keap1, a substrate adaptor protein that allows for the polyubiquitination and ensuing degradation of Nrf2. Cancer cells exhibit persistently high levels of ROS because of genetic and metabolic instability that is compensated for by increased antioxidant abilities. This is Nrf2's dark side in cancer: hyperactive Nrf2 protects normal, but also malignant cells, from ROS and chemotherapeutic agents. This has been linked to poor prognosis in numerous cancers, therefore, elucidating the mechanisms of Nrf2 regulation in cancer is of utmost importance.

Methods: To mimic the environment of a cancer cell, we assess the Keap1-Nrf2 interaction under high ROS conditions. We postulate that Keap1's unusually high content of cysteine residues, which can easily be chemically modified by ROS, makes Keap1 susceptible to oxidative stress-induced protein misfolding by disulfide bond formation. To study Keap1-dependent Nrf2 regulation, we have established in vitro cell culture models and furthermore use purified proteins to biochemically assess protein misfolding.

Results: Our in vitro data show that Keap1 misfolds and forms dense protein aggregates upon exposure to oxidative stress by hydrogen peroxide treatment. Biochemical evidence also suggests that significant disulfide bond formation is present when purified Keap1 protein is treated with hydrogen peroxide.

Discussion: The oxidative stress-induced aggregation of Keap1 may render the protein inactive, preventing its interaction with Nrf2. This failure to mediate Nrf2 degradation could lead to Nrf2 hyperactivation and chemoresistance, as seen in many human cancers. Our research provides new insight into previously unexplored aspects of Nrf2 regulation by protein aggregation and introduces Keap1 aggregation as a novel therapeutic target in cancer therapy.

Keywords: oxidative stress, protein misfolding, chemoresistance, cancer

Urine Cytology-Histology Correlation Before and After Implementation of The Paris System for Reporting Urinary Cytology

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Objectives: Urinary tract cytology is a simple, minimally invasive, commonly used test for screening and monitoring urothelial carcinomas. Prior to the implementation of The Paris System for Reporting Urinary Cytology (TPS), there was a lack of standard diagnostic criteria, resulting in a widely variable “atypia” rate between pathologists, cytotechnologists, and different institutions, leading to challenges in clinical management. The aim of our study is two-fold. First, diagnostic rates over 6 month periods before and after implementation of TPS are examined. Second, cases with histological follow-up at LHSC were correlated with the cytology diagnoses in both time periods.

Methods: All urine cytology diagnoses at LHSC in the first 6 months of 2016 (pre-TPS) and 2017 (post-TPS) are reviewed. Cases with surgical follow-up diagnoses at LHSC were correlated with the cytology diagnoses in each period.

Results: Comparison between pre- (3215 cases) and post-TPS (3107 cases) time periods showed a decrease in “Atypical” (16.8% to 8.2%), with an increase in “Negative” diagnoses (79.4% to 88.3%). There were 234 follow-up histology cases in each period. With a focus on “Atypical” cytology diagnoses, a decrease was seen in the number of cases with high-grade urothelial carcinoma (HGUC) on follow-up (52.3% vs. 40.4%), with a decrease in “Atypical” cases with histological follow-up (65 vs. 42).

Conclusions: Implementation of TPS resulted in a decrease in “Atypical” diagnoses, and an increase in “Negative” diagnoses. The amount of surgical follow-up cases remained the same, with fewer “Atypical” cases followed up, and fewer “Atypical” cases showing HGUC on histology.

Keywords: urine, cytology, urologic pathology, high-grade urothelial carcinoma, atypia

The Long Non-Coding RNA HOTAIR is an Important Angiogenic Mediator in Diabetic Retinopathy

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Introduction: With increasing incidence of diabetic retinopathy (DR), the need for broadening the therapeutic scope for its management becomes fundamental. Recent advances in genomic technology have demonstrated several epigenetic alterations in DR. Long non-coding RNAs (>200 bps; lncRNAs), which are emerging as key epigenetic regulators in various diseases, possess no protein-coding potential and can alter chromatin configuration. In DR, numerous lncRNAs are aberrantly expressed in the retina; however, the majority of lncRNAs are not characterized. In this study, we have attempted to elucidate the epigenetic mechanism(s) of a particular lncRNA, HOTAIR, in DR. We hypothesize that HOTAIR regulates angiogenic processes through alternative epigenetic mechanisms.

Methods: We confirmed the upregulation of HOTAIR in human retinal endothelial cells (HRECs) following incubation in high glucose (25 mmol/L, HG) compared to normal glucose (5 mmol/L, NG). Total RNA was extracted using TRIzol and RT-qPCR was used to confirm the expressions of HOTAIR and angiogenic transcripts (VEGF-A and ET-1). HRECs were similarly examined following siRNA-mediated HOTAIR knockdown, or treatment with histone (DZNep) and DNA methylation (5'-aza-dC, zebularine, and siDNMT1) blockers. DNA methylation patterns and RNA-protein interactions were analyzed and we assessed HOTAIR in human diabetic and non-diabetic vitreous.

Results: HG caused the upregulation of HOTAIR, VEGF-A, and ET-1 transcripts in HRECs and promoted a strong physical association between HOTAIR and two epigenetic RNA-binding proteins, p300 and EZH2. Our DNA methylation array demonstrated that HG can evoke hypomethylation in CpG regions across HOTAIR; while, DZNep, 5-aza-dC, zebularine, siDNMT1 and knockdown of HOTAIR altered mRNA expressions of VEGF-A and ET-1. Furthermore, HOTAIR levels were elevated in the diabetic vitreous.

Conclusions: Our findings suggest that HOTAIR may have a role in regulating these angiogenic mediators in DR through histone and DNA modifications. These results may uncover a novel mechanism for HOTAIR in DR progression and potential therapeutic targets.

Keywords: diabetic retinopathy, epigenetics, lncRNAs, histone methylation, endothelial cells, angiogenesis

Poster Presentations

Session A

1:15 - 2:15 pm

#	First Name	Last Name	Title
1	Maedeh	Naghibosadat	Ghrelin and des-acyl ghrelin binding in cardiac tissue are altered with cardiovascular inflammation in Duchenne muscular dystrophy
2	Adnan	Qamar	Mitochondrial Permeability Regulates Heart Graft Ischemia-Reperfusion Injury and Rejection
3	Lei	Yan	Pattern of Tau Burden in Progressive Supranuclear Palsy and Corticobasal Degeneration
4	Ahmed	Salem	Matrin3 and the functional domains mediating its neurotoxicity in sporadic & familial amyotrophic lateral sclerosis
5	Vitali	Veramkovich	The Role of GDF15 in T cell function
6	Meirui	Li	Laryngeal Surface Chondrosarcoma: A Case Report
7	Xitao	Chen	High Expression of Rsf-1 Desensitizes Non-small Cell Lung Cancer to Cisplatin
8	Sanghun J.	Kim	A scoping review: Identifying and comparing cardiac rehabilitation quality indicators
9	Tammy	Ng	Treeswithintrees: a new R phylogenetic tool for simulating HIV inter- and within-host evolutionary dynamics

#	First Name	Last Name	Title
10	Ali	Sherazi	Validating Cobas e 801 Tumour Marker Immunoassays for Use with Non-Blood Fluids
11	Hayley	Good	Role of Cyclooxygenase in the Initiation of Colitis-Associated Cancer
12	Jeff	Soparlo	Evaluating S100A7 in oral dysplasia and squamous cell carcinoma as a screening tool for predicting transformation in potentially malignant oral lesions
13	Christopher	Tran	Evaluation of two HER2 antibodies and a Concurrent Scoring Method in the evaluation of Gastroesophageal Adenocarcinoma
14	Jieting	Liu	The role of mitochondrial SIRT1 in glucose-induced endothelial aging: regulation by specific nucleus-derived miRNAs
15	Erica A.	Li	Effects of acetylsalicylic acid on myofibroblast differentiation in human Tenon's capsule fibroblasts
16	Christine	Caron	Characterizing T-cell phenotype in patients with hypersensitivity reactions to sulfonamides and beta-lactam antibiotics
17	Mohammad	Esmaili	Oxidized Parkin, its misfolding and aggregation via formation of aberrant intra- and intermolecular disulfide bonds produce cellular toxicity

#	First Name	Last Name	Title
18	Andres	Benavides	Quantitative Analysis of 6 months of Intraoperative Consultations at London Health Sciences Centre
19	Karissa	French	A Case of Fatal Peristomal Bleeding
20	Hind	Amzil	Determination of Rho Guanine Nucleotide Exchange Factor's (RGNEF) role in the regulation of ALS related protein TDP-43
21	Sara	Gitto	Shroom3 Deficiency and the Effect on Murine Heart, Kidney, and Gut Development
22	Jiaoyang	Wu	Creating a Human Choroid Plexus Cell Model of Metachromatic Leukodystrophy using CRISPR/Cas9 Genome Editing
23	Wan-yu	Chao	Diabetes induced liver fibrosis may potentially be mediated by of long non-coding RNA ANRIL
24	Rebecca	Dang	Increased acetylation of Vacuolar H ⁺ ATPase V0 D1 subunit during doxorubicin-induced cardiotoxicity
25	Jenna	Fortunato	The Influence of Estrogen Signalling on Th2 cell Sensitivity to Glucocorticoid
26	James	Armstrong	Acetylation of COX-2: an immunoresolving therapy
27	Jeffrey	Lovell	S100A7 as a biomarker for predicting transformation in a potentially malignant lesion: lichen planus

#	First Name	Last Name	Title
28	Nima	Nalin	Nicotinamide Riboside, a Vitamin B derivative, ameliorates doxorubicin toxicity in cardiomyocytes by restoring lysosomal pH
29	Dana	O'Brien	Impact of Circular RNA FSCN1 on Dendritic Cells
30	Lachlan	McLean	Evaluating Utility of Protein S100A7 in Predicting Progression of Oral Epithelial Dysplasia
31	Amanda	Oakie	c-Kit and IR co-stimulation does not lead to additive signalling or proliferation in INS-1 cells
32	Boris	Virine	Optimal Biopsy Site and Number for the Diagnosis of Microscopic Colitis
33	Bowen	Zhang	Validation of QuPath Based Machine Learning Cell Classifier for Neuronal Quantification
34	Madison	Wallace	The Role of Time-Dependent PaSC Activation on Islet Inflammation and Fibrosis in T2DM
35	Soumiya	Suresh	The Characterization of Brain Metastatic Tumours Arising from Primary Lung Adenocarcinomas
36	Wonjae	Lee	Sensitivity of Ovarian Clear Cell Carcinoma Spheroids to mTOR inhibition
37	Alex	Dionne	Cell-free DNA release during programmed cell death in ischemia reperfusion injury

Ghrelin and des-acyl ghrelin binding in cardiac tissue are altered with cardiovascular inflammation in Duchenne muscular dystrophy

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Introduction: Duchenne muscular dystrophy (DMD) is a severe neuromuscular disease caused by the absence of dystrophin, leading to skeletal and myocardial degeneration. Eventually, dilated cardiomyopathy develops from ischemia, inflammation and fibrosis. There is a need to characterize cardiovascular inflammation in order to develop therapies for the cardiomyopathy of DMD. Both cardiomyocytes and vascular endothelial cells produce the hormone ghrelin and its receptor, the growth hormone secretagogue receptor (GHSR) which may be indicators of cardiovascular inflammation in DMD.

Methods: We used a mouse model of DMD in which both dystrophin and utrophin have been deleted (mdx:utrn^{-/-}). We have previously detected cardiomyopathy at 15-17 weeks of age in these mice. In both littermate control and mdx:utrn^{-/-} mice (n=3), myocardial tissues were assessed for GHSR using the fluorescent peptide analog Cy5-ghrelin (1-19). Des-acyl ghrelin binding was detected with Cy5-des-acyl ghrelin (1-19). To determine whether GHSR levels correlate with an inflammatory phenotype, levels of interleukin-6 (IL-6), F4-80 (Macrophage marker), and the fatty acid transporter CD36 were quantified by immunofluorescence. Isolectin was used to detect vasculature, and fibrosis was detected using Masson's trichrome staining. Fluorescence intensities were determined using NIS Elements AIR 5.02.00 software. Two-way ANOVA was used to compare the levels of GHSR between the DMD and aged-matched control groups. To correlate the level of inflammation with GHSR, linear regression analysis was run on the F4-80 vs. GHSR data and on IL-6 vs. GHSR data.

Results: There were significant increases in levels of GHSR (p<0.0001), IL-6 (p<0.0001) and CD36 (p<0.005) in the myocardium of mdx:utrn^{-/-} mice. There was strong positive correlation between GHSR and F4-80 (p<0.0004, r=0.966) and GHSR and IL-6 (p<0.002, r=0.926). Large amounts of collagen deposition as well as regions of necrosis were evident throughout the cardiac tissue, indicating severe fibrosis. CD36 correlated positively with fibrosis and necrosis (p<0.02, r=0.925). Interestingly, large cardiac vessels, but not microvessels, were strongly positive for Cy5-des-acyl ghrelin (1-19) in DMD mice (p<0.02).

Conclusion & Significance: We report that GHSR associates with cardiac inflammation and that both CD36 and des-acyl ghrelin binding associate with vascular inflammation in mdx:utrn^{-/-} mice. Des-acyl-ghrelin could be a marker of inflammation in large blood vessels in the heart. We propose that GHSR and des-acyl ghrelin are markers of cardiovascular inflammation in DMD.

Keywords: GHSR, inflammation, cardiac, vasculature

Mitochondrial Permeability Regulates Heart Graft Ischemia-Reperfusion Injury and Rejection

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Introduction: A growing body of evidence indicates that mitochondrial permeability transition pore (mPTP) formation plays a crucial role in necroptosis – a programmed form of necrosis. We have recently shown that inhibiting cyclophilin D (CypD), a critical mediator of mPTP, decreases necroptosis and that CypD-deficient heart grafts exhibit prolonged survival. In this study, we extended our findings to a clinically relevant scenario of prolonged cold ischemic organ storage followed by transplantation.

Methods: To model ischemic insult in vitro, endothelial cells (ECs) were exposed to hypoxia in oxygen-depleted glucose-free medium. To model the reperfusion event, ECs were then transferred to a normoxic incubator in oxygenated glucose-rich medium. Necroptosis was induced using TNF α and a pan-caspase inhibitor and monitored by live cell imaging system and flow cytometry. For in vivo studies, C57BL/6 heart grafts were treated with cold ischemia for 4 hours before transplantation into BALB/c mice. Histopathological grading of ischemia-reperfusion injury (IRI) was done by a pathologist.

Results: Our data indicate that necroptosis plays a significant role in hypoxia induced EC death and that inhibition of CypD decreased hypoxia induced necroptosis. Interestingly, apoptosis inducing factor (AIF) silencing also decreased hypoxia induced necroptosis. Following IRI, AIF, a mitochondrial oxidoreductase, translocates to the nucleus and induces DNA fragmentation. We found that pre-transplant ischemia aggravates heart transplant rejection. Ischemia-treated heart grafts had shorter survival times compared to non-ischemic grafts (n=4, p=0.03). Interestingly, CypD deficiency in donor heart grafts attenuates such graft rejection (n=8, p=0.008).

Discussion: Our studies indicate that CypD and AIF play significant roles in EC necroptosis following IRI and that AIF may be the downstream effector of necroptosis. Targeting mitochondrial permeability and the downstream pathways involved in necroptosis is critical in formulating clinically applicable intervention strategies aimed at reducing IRI induced PCD associated with organ transplantation and promoting donor organ survival.

Keywords: Endothelial Cells, Necroptosis, Heart Transplantation, Mitochondria, mPTP, CypD, AIF, IRI

Pattern of Tau Burden in Progressive Supranuclear Palsy and Corticobasal Degeneration

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Introduction: Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) overlap in both clinical symptoms and pathological changes. One of the most common clinical features in both disorders is parkinsonism. It is generally accepted that parkinsonism is caused by neuronal loss in substantia nigra. However, there is growing evidence showing that parkinsonism is associated with brain network involving multiple regions. In the present study, we are exploring the extent of Tau and β -amyloid burden in different brain regions and to determine the correlation of regional difference and clinical symptoms in PSP and CBD.

Methods: In this pilot study, 19 neuropathology confirmed Tauopathy autopsy cases were examined, including 4 PSP, 4 CBD and 11 Alzheimer disease (AD) cases. All PSP and CBD cases presented with parkinsonism. There are no movement symptoms reported in the 11 AD cases. The digital image analysis software QuPath was used to quantify Tau- and amyloid burden on scanned whole slide images. We used the DAB staining intensity as a surrogate marker to measure expression of abnormal phosphorylated tau protein and beta-amyloid accumulation. Six different brain regions were examined, including putamen, globus pallidus (GP), claustrum, subthalamic nucleus (STN), substantia nigra (SN), and anterior cingulate (AC).

Results: Both PSP and CBD have high Tau burden in SN, STN, and GP, which is significantly higher than AD-high level cases. In contrast to CBD, there is a much lower Tau expression in the claustrum, putamen and anterior cingulate of the PSP cases. AD-high level cases show similar amount of Tau expression as CBD in these 3 regions. Compared with AD, β -amyloid is either absent or very low in both CBD and PSP groups.

Conclusions: PSP and CBD share an overlapping but distinct Tau-pathology expression pattern.

Keywords: Parkinsonism, PSP, CBD, AD, LBD, tau, amyloid

Matrin3 and the functional domains mediating its neurotoxicity in sporadic & familial amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is an uncommon neurodegenerative disease caused by degeneration of upper and lower motor neurons. Mutations in the gene encoding Matrin3 protein have been identified as a cause of familial amyotrophic lateral sclerosis (FALS). Post mortem analysis of central nervous system (CNS) tissue of ALS patients with Matrin3 mutations revealed the formation of neuronal cytoplasmic inclusions (NCIs) in motor neurons. However, the involvement of Matrin3 in both sporadic and familial ALS cases caused by mutations in other proteins and the role that matrin3 domains play in its neurotoxic effect have not been fully assessed.

To determine the role of Matrin3 in ALS cases that do not carry mutations in the Matrin3 encoding gene, we performed immunohistochemistry (IHC) analyses using Matrin3 antibody to determine Matrin3 pathology and inclusion formation in the both spinal cord and brain tissue of these ALS patients.

To investigate the role of Matrin3 functional domains in mediating its neurotoxic effect. Here, we established a yeast model of matrin3-mediated cellular toxicity and aggregation to determine which domains of Matrin3 are required for toxicity by systemically ablating each of the following Matrin3 domains: the two RNA recognition motifs and the Zinc finger domains.

Our results show Matrin3 positive NCIs in the spinal cord & brain tissue of patients with SALS & FALS which indicates a general role of Matrin3 aggregation in ALS. They also provide an evidence that deleting the specific Matrin3 domains specifically the RNA recognition motifs will result in decreased Matrin3 toxicity in the yeast model in accordance with previous studies showing that RNA recognition motifs are required for the toxicity caused by two other ALS proteins, TDP-43 and FUS.

Keywords: ALS, Matrin3, Inclusions, Toxicity, Immunohistochemistry, Yeast Model

The Role of GDF15 in T cell function

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Introduction: Elevated plasma levels of growth differentiation factor 15 (GDF15) have been observed in patients suffering from a wide variety of ailments. While GDF15 is implicated in immunomodulation, there is limited research on the effects of GDF15 on lymphocytes. In the present study, we attempt to characterize the effects and potential mechanisms involved in the activity of GDF15 on T cells. We hypothesize that GDF15 suppresses CD4 and CD8 T cell proliferation, increases T cell viability and increases CD4 T cell differentiation into regulatory T cells. In melanoma, GDF15 promotes tumor growth through immunosuppression resulting in reduced immune response.

Methods: In order to evaluate the effects of GDF15 on T cell proliferation and differentiation we are using knockout and transgenic mouse models. A combination of flow cytometry, PCR and colorimetric assays is used to evaluate the cell cycle progression, differentiation and cytokine secretion profiles of CD4 and CD8 positive T cells.

Results: Using GDF15 knockout (KO), Wild Type (WT) and GDF15 Transgenic (TR) mice we determined that lack of GDF15 promotes T cell proliferation in vitro. Furthermore, GDF15 enhances T cell viability and promotes the expression of Treg associated cytokines. In melanoma, GDF15 reduces tumor antigen response of T cells and GDF15 expression in mice promotes tumor growth. The expression of GDF15 in tumor cells results in reduced tumor growth.

Conclusion: Preliminary data suggests that GDF15 is involved in T cell viability, proliferation and immunomodulation. More specifically, GDF15 increases T cell viability and decreases T cell proliferation in vitro. Furthermore, GDF15 affects Treg cells both in vitro and in vivo. In the context of a tumor, GDF15 functions as an inhibitor of tumor growth when overexpressed in the tumor, and promotes tumor growth when overexpressed in the host.

Keywords: GDF15, TGF- β , Melanoma, T cell, Treg, Immunity, Lymphocytes

Laryngeal Surface Chondrosarcoma: A Case Report

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Introduction: Primary chondrosarcoma of the larynx is a rare tumour of the laryngeal cartilages that most commonly involves the cricoid cartilage and arises from the central/intramedullary region. Patients typically present with hoarseness, dyspnea or a neck mass, with a mean time of 28.1 months between symptom on-set to presentation. Initial biopsy cannot always distinguish between benign enchondroma and low-grade chondrosarcoma. Definitive management is complete surgical resection.

Case: A 67-year-old man presenting with hoarseness had an initial CT scan that revealed no mass in the neck. Over the next 2 months, he developed increasing dysphagia as well as respiratory distress which required an open tracheotomy. A subsequent MRI identified a mass involving the left arytenoid cartilage, aryepiglottic fold, and superior cricoid cartilage. A well-differentiated hyaline cartilage neoplasm was diagnosed from biopsies taken from the aryepiglottic fold. The patient then underwent a total laryngectomy. Histopathological examination of the laryngectomy specimen identified a surface chondrosarcoma.

Discussion: Primary laryngeal chondrosarcoma is usually intramedullary and low-grade. Surface chondrosarcoma of the larynx, or other bones, is distinctly uncommon. Distinguishing between low-grade chondrosarcoma and enchondroma is challenging, particularly on biopsy sampling, owing to histological similarities. A definitive diagnosis may only be confirmed with total resection of the lesion. Although patients with laryngeal chondrosarcoma typically follow a slow course of progression from symptom on-set, our case illustrates that even a low-grade laryngeal chondrosarcoma can progress quickly and lead to significant pre-operative complications and risks.

Keywords: laryngeal chondrosarcoma, surface chondrosarcoma, enchondroma

High Expression of Rsf-1 Desensitizes Non-small Cell Lung Cancer to Cisplatin

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Introduction: Cisplatin based chemotherapy occupies a very important position in treating advanced lung cancer. However, its therapeutic efficacy is restricted by the increasing frequency of chemotherapeutic resistance. High expression of Rsf-1 has been reported to enhance drug resistance in ovarian cancer. However, the role of Rsf-1 in cisplatin resistance to NSCLC has not been demonstrated.

Methods: H1299, H460, H1299 Rsf-1 KO and H460 Rsf-1 KO cells were incubated with cisplatin (4 nmol/ml) for 24 hours. Cell viability and activated caspase-3 proteins were determined using CCK8 assay kit and western blot analysis, respectively. NF-κB P65 and BCL2 protein were determined by western blot analysis. Female nude mice (6 weeks of age) were subcutaneously injected with H460 and H460 Rsf-1 KO cells in the back. The length (L) and width (d) of tumors were measured every day. The volumes of the tumors were calculated using the formula $V = 0.5 \times L \times d^2$, and growth curves of the tumors were plotted. Cisplatin was injected through the tail vein (10 mg/kg in every other day) after the tumors formed. Thirty days after tumor formation, the tumor size was determined.

Results: The protein levels of Rsf-1 were much higher in H460 and H1299 compared with HBE, A549 and SPC cells. Rsf-1 knockout increased the cytotoxic effect of cisplatin in H1299 and H460 cells, while augmenting the protein levels of activated caspase3. Rsf-1 knockout also decreased the protein levels of NF-κB P65 and Bcl2. In vivo study showed that the implanted tumor size was much smaller in nude mice receiving Rsf-1 knockout H460 cells than wild-type H460 cells after cisplatin treatment.

Conclusion: These results demonstrate that high expression of Rsf-1 desensitizes the sensitivity of non-small cell lung cancer to cisplatin. Rsf-1 knockout increases cell apoptosis. Thus, Rsf-1 may be a potential target for the treatment of NSCLC.

Keywords: Rsf-1, non-small cell lung cancer, NSCLC, cisplatin, resistance

A scoping review: Identifying and comparing cardiac rehabilitation quality indicators

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Introduction: Following an event such as an acute myocardial infarction, standard-of-care requires that a physician refer the patient to cardiac rehabilitation (CR), a comprehensive disease management program designed to improve cardiovascular health through medical assessments, structured exercise training, patient education, and risk factor management. The current literature supports vast benefits of CR programs, such as improving the quality of life and reducing the recurrence of heart disease. Equitable access to high quality CR programs is essential to the publicly funded Canadian health care system and quality and performance indicators are often used to measure the quality of care being delivered and how optimally the care systems are delivering. The purpose of this study is to conduct a comprehensive review to assemble and compare quality and performance indicator for CR programs that have been established by national and professional associations or organizations.

Methods: We conducted a scoping review of the peer-reviewed medical literature with a gray literature search of government and professional reports. PubMed, CINAHL, Scopus and Web of Science were queried for studies in English published between January 1st, 1995 and February 8th, 2019 which investigated the quality and performance indicators of CR programs. This search yielded 542 studies, which were screened independently by 2 reviewers using Abstrackr and interrater reliability determined through Cohen's Kappa.

Results: Approximately 50 studies and reports will be included with the majority arising from North American and European countries. Only a very few studies report the actual implementation of these indicators and quality of the CR programs using the developed quality and performance indicators.

Discussions: This study will help to identify efforts underway across countries to assure that their populations can access these cost-effective services and that the services are of high quality.

Keywords: cardiac rehabilitation, cardiovascular disease prevention, quality indicators, quality assurance, quality performance, quality control

Treeswithintrees: a new R phylogenetic tool for simulating HIV inter- and within-host evolutionary dynamics

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The rapid evolution and diversity of human immunodeficiency virus (HIV) on the level of infected individuals (hosts) is a major contributor to its viral pathogenicity. Investigating the largely unexplored dynamics involved in HIV diversity is important to developing strategies and therapies to reduce and prevent viral adaptation within hosts. Treeswithintrees (twi) is an R package for the coalescent (reverse-time) simulation of host and pathogen phylogenetic trees according to a set of user-given parameters. This tool allows for a deeper exploration of the parameters involved in compartmentalized within-host virus evolution like the effect of migration rates between the blood and genital tract. Trees are simulated in the presence of a population of unsampled infected compartments with unique branching and migration rates as well as multiple lineages per compartment. These additional features are normally ignored or simplified with unrealistic assumptions in other coalescent simulation frameworks, but are incorporated in twi to increase the variability in reconstructing HIV evolutionary behaviour. Twi has also been designed to be used to answer population-level questions using epidemiological models and other evolutionary relationship questions regarding cospeciation using cophylogeny models. Accurately modeling viral evolutionary events through a simulation tool like twi is the current approach to better understand the processes behind HIV diversity.

Keywords: HIV dynamics, evolution, phylogenetics, R software package

Validating Cobas e 801 Tumour Marker Immunoassays for Use with Non-Blood Fluids

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Introduction: The Roche Cobas e 801 immunoassay analyzer can measure tumour marker concentrations in blood, which could be useful in helping make a diagnosis, monitoring tumour remission after treatment, or observing tumour recurrence. However, tumours may metastasize to or originate in cavities without entering blood. There are currently no published studies which have validated Cobas e 801 tumour marker immunoassays on non-blood fluids. This study is aimed at validating Cobas e 801 carbohydrate antigen 19-9, cancer antigen 125, carcinoembryonic antigen, alpha-fetoprotein, and human chorionic gonadotropin tumour marker immunoassays for use in cerebrospinal, peritoneal, and pleural non-blood fluids.

Methods: Plasma samples containing high concentrations of tumour markers and non-blood fluids will be collected from core lab at Victoria Hospital (London, Ontario). Basal concentrations of each tumour marker will be measured by Cobas e 801 for each individual non-blood fluid sample to determine reference intervals. Fluids will then be pooled and aliquoted. Each aliquot will be spiked with a small volume of plasma samples with high tumour marker concentrations to obtain desired concentrations for precision, linearity, and recovery testing. Aliquots of varying concentration will then be analyzed by Cobas e 801, and precision and accuracy parameters will be calculated. Results: We expect that a) we will determine appropriate reference intervals for tumour markers in non-blood fluids, and b) Precision, linearity, and recovery will be in line with the manufacturer's claims for measuring these tumour markers in blood, thus validating the use of tumour marker immunoassays in non-blood fluids.

Conclusions: Our reference intervals could provide guidance to health care staff at London Health Sciences Centre by defining a range of normal tumour marker concentrations in non-blood fluids. By validating the tumour marker immunoassays, labs worldwide will potentially be more reassured and confident in the measurement of tumour markers in these non-blood fluids.

Keywords: Cobas e 801, tumour markers, non-blood fluids, validation, reference intervals, precision, linearity, recovery.

Role of Cyclooxygenase in the Initiation of Colitis-Associated Cancer

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Introduction: Inflammatory bowel disease (IBD) is a major risk factor for colorectal cancer (CRC). Despite the clear link between inflammation and cancer, the mechanism by which this occurs is unknown. We previously showed that Dclk1+ tuft cells are long-lived and resistant to proliferation even upon mutation of the tumor suppressor APC. Following colitis, however, APC-mutated tuft cells initiate tumorigenesis. Interestingly, Dclk1+ cells express high levels of cyclooxygenase (COX)-1 and -2, enzymes responsible for prostaglandin synthesis. COX is the direct target of non-steroidal anti-inflammatory drugs (NSAIDs) which are known chemopreventative drugs in sporadic CRC. Thus, we aimed to determine the effect of NSAIDs in colitis-associated cancer.

Methods: Dclk1CreERT2/APCfl/fl mice were administered tamoxifen and the colitis-inducing agent DSS, followed by daily treatment with Aspirin (non-selective COX inhibitor), celecoxib, rofecoxib (COX-2 inhibitors), SC-560 (COX-1 inhibitor), or vehicle. Additionally, the effect of these NSAIDs were tested in the AOM/DSS model of CAC, where azoxymethane (AOM) and DSS were administered to induce tumorigenesis. 16 weeks post-tamoxifen/AOM, colonic tumor number and size were analyzed. Acutely, prostaglandin levels were measured by LC-MS, and extent of colitis was assessed by myeloperoxidase activity, histology, and qRT-PCR.

Results: Treatment with Aspirin, but not the COX-2 inhibitors, significantly reduced tumor number in both Dclk1/APC and AOM/DSS models. SC-560 also reduced the number of tumors. We detected no difference in tumor size or colitis severity. LC-MS revealed that Aspirin and SC-560, but not celecoxib, significantly reduced prostaglandin levels in colitis. Interestingly, Aspirin was associated with a reduction in Dclk1+ cells, suggesting an important role for prostaglandins in tuft cell viability.

Conclusions: These findings suggest an important role for COX and downstream prostaglandins in initiation of CAC. Our results suggest that Aspirin is effective in chemoprevention of CAC, potentially through the reduction of COX-1-derived prostaglandins that may be critical for Dclk1+ cell survival.

Keywords: colorectal cancer, colitis, inflammation, Dclk1, cyclooxygenase

Evaluating S100A7 in oral dysplasia and squamous cell carcinoma as a screening tool for predicting transformation in potentially malignant oral lesions

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Introduction: Recently, S100A7 has been shown to be a potential useful marker for identifying oral lesions at risk of transformation from dysplasia to squamous cell carcinoma. Our hypothesis is that potentially malignant oral epithelial lesions have significant expression of S100A7. The objective of our study is to evaluate the level of S100A7 expression in dysplastic lesions which have transformed into oral squamous cell carcinoma using immunohistochemistry. In addition, we will attempt to demonstrate a correlation with dysregulation of phosphorylated MAPK pathway proteins ERK1/2, p38, and JNK, and upregulation of S100A7.

Methods: Formalin fixed paraffin embedded specimens from 55 patients with oral squamous cell carcinoma, where from the same site, a previous non-cancerous biopsy had been previously obtained, were included in the study. For comparison, 44 patients with multiple biopsies identifying dysplasia which had not advanced to squamous cell carcinoma, and 25 patients with a diagnosis of hyperkeratosis were included as control groups. Specimens were stained for S100A7 protein using a standard immunohistochemistry protocol. Expression of S100A7 will be assessed semi-quantitatively, using an intensity and proportion scale, as well as by image analysis by Stratocyte. As S100A7 is active correlated with the MAPK signaling pathway activity, phosphorylated proteins from the ERK1/2, p38, and JNK pathways will also be evaluated via immunohistochemistry.

Results: Preliminary staining of squamous cell carcinoma and previously non-malignant, dysplastic biopsies from those same patients suggests that S100A7 is upregulated in lesions with high likelihood of malignant transformation.

Discussion: S100A7 protein staining may be a reliable marker to determine the risk of malignant transformation in potentially malignant oral lesions and may aid in improved patient outcomes.

Keywords: S100A7, Oral Squamous Cell Carcinoma, Oral Dysplasia, MAPK, Pathology, immunohistochemistry

Evaluation of two HER2 antibodies and a Concurrent Scoring Method in the evaluation of Gastroesophageal Adenocarcinoma

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Introduction: Patients with HER2 overexpressed gastroesophageal adenocarcinoma show improved survival with trastuzumab and as a result, HER2 has become an important predictive biomarker. Immunohistochemistry (IHC) is used as the first line test, with in situ hybridization methods used to reconcile equivocal cases. Multiple HER2 IHC antibodies are available for use, but previous studies have shown variable performance. In this study, we evaluated the performance of two HER2 IHC antibodies, SP3 and A0485. We also tested the use of a concurrent score in evaluating HER2 expression, determined by the greater score of the two antibodies.

Methods: All cases of gastroesophageal adenocarcinoma diagnosed in 2015 were included. External consultation cases requesting HER2 testing in the same time period were also included. IHC and fluorescent in situ hybridization (FISH) were performed on all case. HER2 positivity was considered in cases with an IHC score of 3+, or scores of 2+ with FISH amplification.

Results: The study population included 173 cases, and the number of HER2 overexpressed cases was 75 (43.4%) for SP3, 67 (38.7%) for A0485, and 80 (46.2%) for the concurrent score. There was a good concordance between SP3 and A0485 (K = 0.69, 95% CI 0.59, 0.81). SP3 showed the greatest specificity, while the concurrent score was the most sensitive test. There were 52 cases with discordant A0485 and SP3 scores, with 21 leading to differences in HER2 status. Of these 14 of the positive cases were from SP3 (13 FISH positive) and 7 were from A0485 (5 FISH positive).

Discussion: The use of two IHC antibodies in a concurrent score improved the sensitivity for HER2 amplification. A sizeable proportion of HER2 overexpressed patients would be potentially missed if one of the two antibodies were used. Careful selection and testing of HER2 IHC antibodies is required to improve accuracy and improve patient outcomes.

Keywords: breast cancer, immunohistochemistry, HER2, FISH

The role of mitochondrial SIRT6 in glucose-induced endothelial aging: regulation by specific nucleus-derived miRNAs

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Introduction: Cellular senescence may be modulated by various stressful stimuli, such as DNA damage and the formation of reactive oxygen species (ROS). Hyperglycemia generates oxidative stress that causes premature aging of endothelial cells, which ultimately contributes to tissue damage. Since the mitochondria play a pivotal role in endothelial damage during diabetes, the epigenetic mechanisms involved in this pathogenesis are not entirely understood. Therefore, this study aimed to investigate the regulatory mechanisms of mitochondrial SIRT6 and their targeting miRNAs in high glucose-induced endothelial cell senescence.

Methods: Expressions of SIRT3, SIRT4 and SIRT5 and their targeting miRNAs were examined using real time qPCR in human cardiac microvascular endothelial cells (HCMECs). The cells were exposed to 25 mM glucose (high glucose; HG) with or without transfection of specific SIRT-targeting miRNAs and were then compared to 5 mM glucose (normal glucose; NG). Aging in HCMECs was investigated using the senescence-associated β -gal (SA β -gal) stain. 8-OHdG was also used as an oxidative stress marker.

Results: qPCR analyses showed glucose-induced downregulation of SIRT 3 and SIRT5 mRNA and upregulation of miRNA-1 (targets SIRT3) and miRNA-19b (targets SIRT5) in HCMECs. No changes were seen in SIRT4 expression in HG. Transfection with miRNA antagomirs prevented the downregulation of their respective SIRTs. SA β -gal positive staining showed early senescence in HCMECs treated with HG and such positivity was prevented by transfection of specific miRNA antagomirs. In parallel, 8-OHdG positivity in HG was also prevented following the administration of miRNA antagomirs.

Conclusions: These experiments demonstrate a novel mechanism by which specific mitochondrial SIRT6 regulate glucose-induced cellular aging. Furthermore, we showed that specific nuclear miRNAs regulate these mitochondrial SIRT6. Identifying such mechanisms may lead to potential RNA-based treatment for diabetic complications.

Keywords: SIRT6, miRNAs, diabetic complications, epigenetics, endothelial cells, aging

Effects of acetylsalicylic acid on myofibroblast differentiation in human Tenon's capsule fibroblasts

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Introduction: Scarring in the eye after glaucoma filtration surgery is associated with excess inflammation and high rates of surgery failure. Differentiation of fibroblasts into myofibroblasts in the highly inflammatory micro-environment of the human Tenon's capsule can result in scarring after filtration surgery. Acetylsalicylic acid (ASA) is a promising anti-scarring agent, however its downstream effects on transforming growth factor beta (TGF β)-induced myofibroblastic changes in human Tenon's capsule fibroblasts (HTCFs) are unknown. We hypothesized that ASA will decrease TGF β -induced myofibroblast proliferation and protein expression in human Tenon's capsule fibroblasts by modifying COX-2 function and by inducing anti-inflammatory lipid mediators.

Methods: HTCFs were co-treated with 2 ng/mL of TGF β and ASA ranging from 50 μ g/mL to 3200 μ g/mL. MTT/LDH and LIVE/DEAD assays were conducted to assess the ability of ASA in reducing HTCF viability. HTCFs were also co-treated with 2 ng/mL of TGF β and lipid mediators 5/11/15-hydroxyeicosatetraenoic acid (5/11/15-HETE) and 17-hydroxydocosahexaenoic acid (17-HDHA) at 10 nM, 100 nM, and 1000 nM. Western blot and immunohistochemistry were used to examine the effects of ASA and the lipid mediators on expression of pro-fibrotic proteins such as alpha-smooth muscle actin (α -SMA), matrix metalloproteinase-9 (MMP-9), and collagen 1.

Results: ASA decreased TGF β -induced myofibroblast growth, while ASA in a non-inflammatory micro-environment was non-toxic to the cells. TGF β -induced myofibroblasts displayed a dose-dependent decrease in α -SMA and MMP-9 protein levels after exposure to ASA. These TGF β -induced myofibroblasts also displayed a decrease in α -SMA, MMP-9, and collagen 1 protein levels after treatment with lipid mediators.

Discussion and Conclusion: ASA represses TGF β -induced myofibroblastic changes in human Tenon's capsule fibroblasts. Thus, ASA may mitigate the cellular events responsible for many ocular pathologies. The well-established safety profile of ASA in humans will allow for rapid translation of these findings into human patients.

Keywords: Human Tenon's capsule fibroblasts, myofibroblasts, glaucoma, aspirin, acetylsalicylic acid, lipid mediator

Characterizing T-cell phenotype in patients with hypersensitivity reactions to sulfonamides and beta-lactam antibiotics

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Introduction: Delayed drug hypersensitivity reactions (DHRs) are idiosyncratic, mediated by T-cells, and resulting skin rashes can occur days after initial exposure to the culprit drug. Many hypotheses attempt to explain how T-cell activation and the pathomechanisms occur in vivo and in vitro, but there is no consensus. Previous research groups different skin reactions, often without accounting for the type or severity of the clinical presentation, although some clinical presentations have different cytokine profiles and T-cell subset involvement. This project will address this issue and determine DHR mechanisms in context of both the drug and resulting clinical presentation. We hypothesize that differences in activated peripheral T cell subsets lead to the different clinical presentations observed in drug hypersensitivity reactions to sulfamethoxazole and beta lactam antibiotics.

Materials and Methods: Peripheral blood mononuclear cells (PBMCs) are isolated from the blood of participants with diagnosed DHRs to either sulfamethoxazole or beta-lactam antibiotics. PBMCs are stimulated with the culprit drug or anti-CD3. T-cell subset proliferation is assessed by T-cell specific surface markers, 3H-thymidine incorporation, and secreted effector cytokines.

Results: Proliferation and surface staining of isolated lymphocytes have been optimized. 3H-thymidine for measuring T-cell proliferation and flow cytometry for T-cell CD69 expression/activation using control blood samples have provided satisfactory results. A freezing method has been confirmed to yield live PBMCs compatible with optimized methods. Participants with drug hypersensitivities are being recruited to participate in this project. It is anticipated that different clinical presentations occur due to response of different T-cell subsets and effector cytokines.

Discussion: This study will provide insight into the underlying pathophysiology of DHRs. Establishing differences in cytokine profiles between skin rashes in DHRs to sulfamethoxazole and beta-lactam antibiotics can help with developing reliable tests for prediction and diagnosis that are less invasive than current gold standard tests.

Keywords: Drug hypersensitivity, beta-lactam antibiotics, sulfamethoxazole, pathomechanism, T-cell, cytokines

Oxidized Parkin, its misfolding and aggregation via formation of aberrant intra- and intermolecular disulfide bonds produce cellular toxicity

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Introduction: Parkinson's disease (PD) is a neurodegenerative disorder characterized by selective neuronal loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) and decreased DA levels in the nigrostriatal DA pathway in the brain. In this study, we hypothesized that oxidative stress damages parkin resulting in its misfolding and altered cellular localization, which contributed to UPS and autophagy dysfunction and thus to PD pathogenesis.

Methods: We obtained key results from studies in HEK 293 cells stably expressing parkin and SH-SY5Y cells expressing endogenous parkin, by co-transfecting the most interesting parkin truncation with PINK1 and monitor the co-localization using fluorescent protein tagged proteins. We performed western blot analyses with protein lysates from cells to evaluate that parkin is modified by oxidative damage under normal growth conditions and under oxidative stress.

Results: Untreated SH-SY5Y cells expressing endogenous parkin and N2a cells transiently transfected with parkin-YFP have several small dots and crescent-shaped of parkin observing throughout of cytoplasm. However, upon stress treatment, endogenous parkin and parkin-YFP accumulates excessively in foci, which often appears interconnected with one another, localized throughout of cytoplasm and nucleus not necessarily on or near mitochondria. More than 80% of our analyzed cells, rather than showing normal diffuse parkin fluorescence, exhibited a large or several smaller discrete parkin spots in untreated or exposure to stress treatment. Under these experimental conditions, analysis performed with the software image J showed that roughly 60 % of these spots colocalized with the mitochondrial protein TOM20, and the rest appeared throughout of the cells.

Conclusions: We found that alteration of parkin solubility induced by various extrinsic and intrinsic stressors provides a mechanism for parkin misfolding and dysfunction may be relevant to the pathogenesis of sporadic PD.

Keywords: Dopaminergic neurons, Gain of function, Loss of function, Misfolding, Oxidative stress, Parkinson's disease

Quantitative Analysis of 6 months of Intraoperative Consultations at London Health Sciences Centre

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Introduction: Intraoperative consultations (IOCs) are an essential point of contact between the Operating Room and the Pathology Department. The purpose of these consultations is to provide the surgeon with prompt information regarding a surgical specimen with immediate impact on the rest of the operation. The information requested may include: diagnosis of a lesion, assessment of completeness of excision, or a confirmation of tissue type.

Methods: The LHSC Pathology Database was searched to identify IOCs performed from January 1st to June 30th 2016. These were classified by type (gross examination only, microscopic examination ("frozen section"), lymphoma protocol, or neuropathology), and assessed for subspecialty, subspecialty of reporting pathologist, requirement for internal pathology consultation, and rates of deferred diagnosis and error.

Results: Twenty-four LHSC pathologists performed 1234 IOCs, excluding neuropathology cases. Primary subspecialties of these Pathologists included: Head & Neck, Skin, Gynecology, Liver/Pancreaticobiliary, Lung, Bone/Soft Tissue, and GI, among others. Seventy-nine percent of IOCs involved frozen section, 8% were lymphoma protocols, and 1% were gross assessments. The most common referring specialty was Head & Neck (41%) followed by Skin (14%). Seventy-six percent of IOCs were performed by a Pathologist from a different subspecialty; 92% did not include an internal consultation with another pathologist. The deferral rate, excluding lymphoma protocols, was 2% and the error rate when compared with permanent sections was 2.5% (total) and 1.1% (major).

Conclusion: Head & Neck specimens accounted for most IOCs, although only 4/24 pathologists sign out these specimens regularly. Despite this, the major IOC error rate was low at 1.1%; this is lower than the published acceptable error rate of 3%. The rate of IOCs performed by pathologists of a different subspecialty is high, supporting a need for potential methods of inter-pathologist consultation when pathologists are working at distant sites.

Keywords: Intraoperative consultation, frozen section, quality assurance, error rate, deferred diagnosis, internal consultation

A Case of Fatal Peristomal Bleeding

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Introduction: Varices are a known complication of advanced liver disease, usually occurring at the esophagus and rectum. They have also been rarely reported adjacent to stoma sites in the setting of previous ileostomy, and may be characterized by bleeding from the edge of the stoma site.

Case: A 71 year old man with a long-standing history of ulcerative colitis, with ileostomy formation more than 40 years ago, had recently been diagnosed with primary sclerosing cholangitis. He was unexpectedly found dead in his home with a large amount of blood at the scene and no obvious traumatic injuries. At post mortem examination there was fresh blood present in the stoma bag, without evidence of hemorrhage in the more proximal gastrointestinal tract. The peristomal skin showed signs of irritation. Microscopic examination of the stoma showed prominent dilatation and congestion of mucosal and submucosal vessels with scattered microthrombi, suggestive of peristomal varices.

Discussion: Peristomal variceal bleeding is a rare complication of advanced liver disease which can require multiple blood transfusions, and which may be fatal if not treated. The macroscopic findings at post mortem examination can be subtle but this rare cause of death must be kept in mind when dealing with bleeding in a deceased person with a stoma.

Keywords: forensics, sudden death, peristomal varices, bleeding

Determination of Rho Guanine Nucleotide Exchange Factor's (RGNEF) role in the regulation of ALS related protein TDP-43.

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Introduction: Amyotrophic Lateral Sclerosis (ALS) is a progressive, adult-onset disease characterized by the degeneration of motor neurons. The presence of neuronal cytoplasmic inclusions (NCIs) composed of RNA-binding proteins, neurofilaments and other proteins, is considered to be the disease's neuropathological hallmark. Amongst ALS-related proteins, Rho guanine nucleotide exchange factor (RGNEF) has been identified as a key element in the pathogenesis of ALS as it has been observed to form pathological NCIs in motor neurons. Additional evidence supporting this pathogenic role is the co-aggregation of RGNEF with the RNA-binding proteins TAR DNA binding protein of 43kDa (TDP-43) and fused in sarcoma/translocated in liposarcoma (FUS/ TLS) within motor neuron NCIs in ALS. RGNEF is a 190 kDa RNA-binding protein that acts as a pro-survival factor under stress conditions. It has five important domains: a leucine-rich domain (L-rich) and a cysteine-rich Zn-binding domain in the amino terminal half of the protein; a Dbl homology domain (DH), a Pleckstrin homology domain (PH) and an RNA-binding domain in the carboxy-half of the protein. Considering that RGNEF has been observed to regulate the levels of NEFL mRNA, we studied the regulatory effect of this protein over the mRNA stability and protein levels of TDP-43.

Material and Methods: To accomplish this, we first analyzed the regulation of the Firefly luciferase linked to the 3'UTRs of TDP-43 and FUS/ TLS by RGNEF using a luciferase reporter assay. Then, in order to study the expression levels of these proteins in the presence of RGNEF, we measured the amount of TDP-43 and FUS/TLS in a stable cell line of HEK293T expressing a full length RGNEF-myc.

Results: First, we observed a down-regulation in luciferase activities linked to the 3'UTR of TDP-43 and FUS/TLS, suggesting that RGNEF regulates the mRNA stability of both proteins. However, in the western blots, we observed a down-regulation in TDP-43 protein only.

Discussion and Summary: These results suggest that RGNEF regulates the stability of other RNA-binding proteins involved in ALS and that at least with TDP-43 this effect implies a down-regulation at the mRNA and protein levels. Additionally, the results suggest that the inter-regulation between ALS related proteins could be something common which led us to investigate further mechanisms by which RGNEF regulates the activity of TDP-43 such as protein-protein interactions.

Keywords: RGNEF, TDP-43 regulation, protein-protein interaction, ALS.

Shroom3 Deficiency and the Effect on Murine Heart, Kidney, and Gut Development

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Introduction: Shroom3 is a protein responsible for the regulation of apical constriction and apicobasal elongation during embryogenesis. Previous studies have shown that Shroom3 contributes to neural tube closure, kidney glomerulus and podocyte development, heart ventricle formation, and gut rotation and pseudostratification. As such, the protein has been investigated in the context of many diseases including spina bifida, chronic kidney disease, heterotaxy syndrome, and congenital heart defects. The aims of the present study are to investigate the kidneys, heart, intestines and stomach of a transgenic mouse population, and examine the developmental abnormalities on a more comprehensive scale. This approach will provide a unifying interpretation of the abnormalities and could shed additional light on systematic pathogenesis.

Methods: A population of ten Shroom3+/Gt, Shroom3Gt/Gt, and wild-type E18.0 mice were embedded, stained and sectioned. The sections were then histologically examined using light microscopy, and multiple measurements of the heart, kidneys and gut were taken using Lumenera Infinity Analyze software. Qualitative observations were also made to assess cellular shape and arrangement.

Results: Initial results indicate that Shroom3 did not have a significant effect on renal corpuscle size or heart ventricular thickness at E18.0. However, the gut epithelium and muscularis differed significantly between Shroom3Gt/Gt and wild-type mice. The gut of Shroom3Gt/Gt mice also appeared to have a more disorganized cellular arrangement.

Discussion: These findings indicate that in the gut, Shroom3 may play a significant role in apicobasal elongation and maintenance of cellular organization. Additional research is needed to investigate the contributions of Shroom3 to heart and kidney development.

Keywords: Shroom3, murine embryogenesis, apicobasal elongation, apical constriction, developmental abnormalities

Creating a Human Choroid Plexus Cell Model of Metachromatic Leukodystrophy using CRISPR/Cas9 Genome Editing

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Introduction: Metachromatic leukodystrophy (MLD) is an autosomal recessive lipidosis caused by deficiency of lysosomal enzyme arylsulfatase A (ARSA). ARSA deficiency leads to accumulation of substrate galactosylceramide 13-sulfate (sulfatide), a major sphingolipid of myelin. MLD is characterized by myelin degeneration in both central and peripheral nervous systems. Currently, no treatment exists for MLD, and research is limited. Our laboratory has previously shown that lentiviral vectors overexpressing ARSA can effectively transduce mouse choroid plexus cells in vivo. The aim of this project is to establish a human choroid plexus (hCP) cell line representative of MLD by performing an ARSA knockout using CRISPR/Cas9 techniques. The knockout will lead to a deficiency of ARSA activity that mimics MLD in cells.

Methods: A CRISPR/Cas-9 knockout kit was designed to target four ARSA gene sequences by single guide RNAs (sgRNAs). hCP cell cultures were grown using supplemented Epi-Cell media. CRISPR/Cas9 transfection via lipofectamine was performed on hCPs for a mutation to truncate ARSA. DNA was extracted and analyzed using Sanger sequencing for mutation locations and relevance. Finally, ARSA activity was measured to determine impact of knockout.

Results: hCP cell population experienced considerable cell loss after transfection, which provided low numbers for analyses. Three of four sgRNA sequences yielded results in the mutation analysis, which was inconclusive: frameshift mutations of unknown significance in two samples, and a deletion mutation in one. A secondary transfection was performed, which had increased cell survival. ARSA enzyme assay showed low enzyme activity in all samples indicating successful knockout. Further analyses will be performed on cells from the secondary transfection.

Conclusion: CRISPR/Cas9 does introduce mutations in the targeted ARSA gene in hCPs that diminishes ARSA activity, mimicking what is in MLD. However, the transfection protocol needs to be further optimized for hCPs, to allow for more analyses on its effects and efficacy.

Keywords: Metachromatic leukodystrophy, arylsulfatase A, choroid plexus, CRISPR/Cas9, gene knockout

Diabetes induced liver fibrosis may potentially be mediated by of long non-coding RNA ANRIL

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Introduction: The lncRNA ANRIL is encoded on a locus that has been identified as one of genetic susceptibility for many different cancers, cardiovascular disease, and type 2 diabetes. A previous study has shown increased expression of ANRIL in human retinal endothelial cells (HRECs) due to high glucose conditions and diabetes regulates extracellular matrix protein and vascular endothelial growth factor (VEGF) expression in diabetic retinopathy and cardiomyopathy. The present study aims to explore the role of ANRIL in non-alcoholic fatty liver disease (NAFLD) and its impact on fibrosis. We hypothesize that ANRIL expression has a role in promoting liver fibrosis and the upregulation of extracellular matrix proteins such fibronectin (FN) and collagen1a4 (Colla4).

Methods: To test this hypothesis, RNA will be isolated from liver tissue samples harvested from ANRIL knockout male mice (ANRILKO) and littermate controls with or without streptozotocin (STZ)-induced diabetes after 2 months post onset of diabetes. RT-PCR will be used to determine the expressions of ANRIL, FN, and Colla4, with p15 used as a surrogate marker for ANRIL. Finally, an ELISA will be performed to measure the expression of FN and Coll1a4 protein.

Keywords: Liver, lncRNA, ANRIL, NAFLD, fibronectin, collagen1a4, fibrosis, mice, diabetes mellitus

Increased acetylation of Vacuolar H⁺ ATPase V0 D1 subunit during doxorubicin-induced cardiotoxicity

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Introduction: Doxorubicin (DOX) is an established anti-cancer treatment prescribed to a wide spectrum of cancers in adults and children. However, DOX usage is limited because it induces severe damage to the heart. Emerging studies support that imbalance between histone acetylation and deacetylation interferes with autophagy leading to doxorubicin-induced cardiotoxicity (DIC). Autophagy is a protective mechanism functioning to degrade damage protein and organelles through engulfment. Lysosomal transmembrane proteins called Vacuolar H⁺ ATPase (V-ATPase) create an acidic environment for proteases to degrade cargo in late autophagy. The V-ATPase V0 D1 subunit is important for allowing cytosolic H⁺ entrance into the lysosome. It has been reported that DOX disrupts the lysosomal acidic condition, thereby blocking autophagic flux and leading to cell injury. The precise mechanism involved in DIC and whether acetylation of V-ATPase V0 D1 plays a role in the progression to DIC remains unknown.

Methods: Adult mice were injected with DOX, then 5 days later, mouse hearts were sacrificed and collected. Co-immunoprecipitation and western blots were completed to assess the level of V-ATPase V0 D1 acetylation. In an in vitro study, immortalized rats' cardiomyocytes were treated various concentrations of pharmacological inhibitors and DOX for 24 hours. Assays including caspase-3 and lactate dehydrogenase were performed to assess cell death.

Results: DOX treatment group had increased the levels of acetylated V-ATPase V0 D1 in mice hearts. Ongoing studies will (1) reveal whether V-ATPase V0 D1 acetylation compromises V-ATPase function during autophagy in cardiomyocytes and (2) confirm that DOX induces acetylation of V-ATPase V0 D1 in cultured cardiomyocytes.

Discussion and Future Directions: The preliminary results suggest that DOX cardiotoxicity may be associated with increased acetylation of the V-ATPase V0 D1 subunit. A future study to determine which acetylase is involved in V-ATPase V0 D1 acetylation will be conducted by utilizing selective histone acetyltransferase inhibitors.

Keywords: Cardiotoxicity, Doxorubicin, Vacuolar ATPase, Autophagy, Lysosome, Acetylation

The Influence of Estrogen Signalling on Th2 cell Sensitivity to Glucocorticoid

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Introduction: Allergic asthma is mediated by allergen activation of Th2 cells and their respective mediators (cytokines IL4, IL5, and IL13). Glucocorticosteroids (GC) are the main treatment for asthma, improving symptoms by suppressing Th2 cytokine production and inducing apoptosis, however, severe asthmatics do not experience significant improvements. Women are more likely to be diagnosed with severe asthma, and we found these women had elevated levels of Th2 cells. Estrogen signalling drives Th2 cytokine production, and may interfere with the ability of GCs to reduce inflammation, suggesting that women may be less steroid responsive. I hypothesized that Th2 cell sensitivity to GC-induced apoptosis is reduced by estrogen signalling.

Methods: CD4⁺ T cells were isolated from donors and differentiated to Th2 cells. Th2 cells were treated with the GC dexamethasone (DEX) with/without an ER α selective agonist (propylpyrazole triol, PPT) for 48 hours. Apoptosis was assessed by flow cytometry staining for Annexin-V, 7-Aminoactinomycin D (7-AAD), Fluorochrome Inhibitor of Caspases (Flica) and Propidium Iodide (PI). The anti-apoptosis gene B-cell lymphoma 2 (BCL-2) was also measured using qRT-PCR.

Results: Th2 cells treated with DEX underwent apoptosis (Annexin V+/7-AAD-; 20-30%) and when co-treated with DEX (0.1-1 μ M) and PPT (1 μ M) showed significant reductions (13.0%, 9.3%, and 7.8%, respectively), compared to DEX alone. Staining for Caspase activity indicated a similar trend with Th2 cells co-treated with DEX (0.1-0.5 μ M) and PPT (1.0 μ M) showing significant reductions (48.3%, 22.7%). BCL-2 gene expression was significantly increased with the addition of PPT to DEX (0.1 - 0.5 μ M) compared to Th2 cells treated with DEX alone.

Discussion: Our findings show that activating ER α inhibits GC-induced apoptosis of Th2 cells and increases the level of mRNA for the anti-apoptotic factor BCL2. In conclusion, these data suggest a potential mechanism that women with severe asthma may have more Th2 cells due to estrogen promoting their survival.

Keywords: Asthma, allergic inflammation, glucocorticoid, estrogen-signalling, Th2 cell response, apoptosis, sex differences

Acetylation of COX-2: an immunoresolving therapy

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Introduction: Phospholipase A2 (PLA2) generates substrates for inflammation/resolution mediator biosynthesis, and is upstream of the cyclooxygenase (COX) and lipoxygenase (LOX) pathways. Broadly, COX products trigger the cardinal signs of inflammation and LOX products are essential endogenous signals mediating the resolution of inflammation. Acetylsalicylic acid (ASA) acetylates Ser530 on COX2, such that its activity becomes lipoxygenase-like. However, the LOX-derived mediators produced by acetylated-COX2 in vivo is limited, likely by the salicylate ion of ASA remaining to competitively inhibit the acetylated enzyme. We hypothesize that ASA co-treated with a PLA2 agonist will increase upstream COX2 metabolites which will out compete salicylate for acetyl-COX2's active site and ultimately increase the amount of acetyl-COX2 products produced.

Methods: Human ocular fibroblasts were induced with 1ug/ml each of INF γ , TNF α , IL-1B and TGF β 1 for 24 hours before being treated with: 1) vehicle, 2) ASA 200ug/ml or 3) ASA and Melittin 5ug/ml. Next, supernatant was collected (6, 12, 24h) and analyzed for PLA2, COX and LOX products using LC-MS/MS. Total protein was used for normalization of lipid mediators and for western blot analysis of myofibroblast associated proteins.

Results: ASA did not cause a significant increase in acetylated-COX2 products compared to control, however it did significantly inhibit prostaglandin production. The PLA2 agonist significantly increased arachadonic acid, eicosapentaenoic acid and docosahexaenoic acid relative to control. The combined ASA and PLA2 agonist resulted in a 200 to 400 times increase in acetylated-COX2 derived products compared to ASA only treated replicates, further there was only a 20 times increase versus control in the production of prostaglandins. Western blot revealed a significant reversal of INF γ /TNF α /IL-1B/TGF β 1 induced α SMA, collagen 1 and MMP9 expression. These data were subsequently supported by SMAD and PPAR γ activation patterns.

Conclusions: To our knowledge, this is the first treatment strategy that modulates autacoid signalling to promote early engagement of the resolution axis of inflammation and the abolition of certain myofibroblast specific transcription programs.

Keywords: inflammation, eicosanoids, resolvins, scarring, myofibroblasts

S100A7 as a biomarker for predicting transformation in a potentially malignant lesion: lichen planus

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Introduction: Oral potentially malignant disorders (PMD) are changes in the oral mucosa that are clinically recognizable. One PMD of specific interest is oral lichen planus (OLP). OLP has a malignant transformation rate of approximately 1.09%. Current diagnosis and management of lichen planus and other PMD's includes incisional biopsy, grading of dysplasia, and clinical follow up or lesion excision depending on the presence and severity of dysplasia. Current literature reports that incisional biopsy and grading of dysplasia are not reliable diagnostic or predictive tools for malignant transformation (ML Goodson 2015). As a result, novel and more accurate methods for predictive risk of malignant transformation in these lesions should be examined. Tissue biomarkers, such as S100A7, may provide a more accurate method of risk determination.

Hypothesis: We hypothesize that S100A7 is increased in the epithelium of Lichen Planus lesions which transform into squamous cell carcinoma. The proposed mechanism is through phosphorylation of proteins EGFR and ERK1/2 of the MAPK signalling pathway.

Methods: Tissue samples of cases of OLP, oral epithelial dysplasia's and normal epithelial controls will be obtained from the department of Pathology and Laboratory Medicine at Western University and University Hospital. These will be stained via immunohistochemical methods. The staining will be quantified by semi-quantitative means using an immunoreactivity score based on the proportion of epithelial cells staining, and the intensity of staining. The area of staining will also be measured using image analysis, and an algorithm applied to determine low and high risk levels in the non-cancerous tissues. Tissue samples will also be analyzed by RT-PCR to determine the presence of S100A7 mRNA. Patient samples will be anonymized and the demographic information such as gender, age, smoking status, alcohol consumption, site of lesion, and histopathological diagnosis will be acquired. Follow up data with regard to prognosis and cases that have transformed into malignancy will be obtained; and will be correlated with S100A7 and ERK1/2 expression.

Results: Undetermined

Significance: Demonstrating that Oral Lichen Planus is a PMD may alter clinical management of this lesion including risk factor modification and clinical management/ follow-up. Risk stratification may also be accomplished through quantification of biomarker S100A7 in tissue samples.

Keywords: Potentially malignant disorder, Lichen planus, Dysplasia,

Nicotinamide Riboside, a Vitamin B derivative, ameliorates doxorubicin toxicity in cardiomyocytes by restoring lysosomal pH

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Introduction: The cardiotoxicity associated with the anthracycline, Doxorubicin (DOX), has eclipsed the therapeutic efficacy of the drug in the chemotherapeutic regimen. DOX has been reported to block the autophagic flux in cardiomyocytes by inhibiting lysosome acidification. Our recent study showed that Nicotinamide Riboside (NR) improved autophagic flux thereby preventing DOX cardiotoxicity. NR is a vitamin B derivative and a nicotinamide adenine dinucleotide (NAD⁺) precursor found in human diets. NAD precursors have been shown to trigger autophagy in cells by activating sirtuins (protein deacetylases). We therefore hypothesized that NR could be beneficial in preventing DOX toxicity by restoring the acidic lysosomal pH.

Materials and Methods: H9c2 cells were treated with DOX (1 µM) with or without NR (500 µM) for 24 hours and control cells were left untreated. As a positive control, Bafilomycin A (100 nM), an inhibitor of the lysosomal proton pump, vacuolar H⁺-ATPase (V-ATPase) was used. Lysosomal pH was quantified using LysoSensor™ Yellow/Blue DND-160. The lysosomal pH was expressed as the ratio of intensity of emission at 460 nm and 535 nm.

Results: DOX and Bafilomycin A increased lysosomal pH in H9c2 cells compared to the control group. Treatment with NR restored lysosomal pH in DOX-incubated cells. The protective effects of NR could be via the sirtuin mediated deacetylation of proteins targeted by DOX and studies related to the identification of these targets are currently in progress.

Discussion: We have demonstrated that the NAD⁺ precursor, NR preserves the pH of cells during DOX treatment and this could potentially restore the autophagic flux in cells. NR is commercially available as an over-the-counter oral formulation under the trade name Niagen™ (ChromaDex Inc.). Owing to its safe toxicity profile and wide therapeutic efficacy reported, NR deserves to be further explored and exploited in the clinical settings as well.

Keywords: Doxorubicin, cardiotoxicity, autophagy, lysosomes, Nicotinamide Riboside, sirtuins

Impact of Circular RNA FSCN1 on Dendritic Cells

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Introduction: Dendritic cells (DCs) stimulate T cell activation and proliferation and are therefore a critical to initiating the adaptive immune response. Circular RNA (circRNA) has roles in gene regulation and is expressed differentially by in DCs at different stages of maturation. This study investigates circRNA from the FSCN1 gene, which is involved in migration and motility of mature DCs, to determine its impact on DC development and function. We expect a difference in the expression of circFSCN1 expression in mature and immature DCs, and to observe more immature-like functions in DCs on knockdown of this circRNA.

Methods: Bone marrow cells from a C57BL/6 mouse were cultured in GM-CSF and IL4 to induce production of DCs, and total RNA from mature and immature DCs was used for cDNA synthesis and RT-qPCR for an expression profile of circular RNA FSCN1 (circFSCN1). siRNA was used to alter circFSCN1 expression, and antibody staining was used to determine the extent of DC maturation. DCs were co-cultured with Balb/c mouse T cells to determine their capacity to activate T cells using a CFSE assay and their ability to induce regulatory T cell (Treg) production using flow cytometry and using qPCR.

Results: Both circFSCN1 and its parent gene were found through qPCR to be increased in mature DCs compared to immature DCs. qPCR following coculture of mature DC coculture with allogenic T cells showed decreased levels of T regulatory cell-associated genes including FoxP3, T-bet, and TGF beta, alongside increased levels of pro-inflammatory cytokines including IFN gamma. Data for experiments involving circFSCN1 knockdown is still being collected.

Discussion: This study suggests a role for circFSCN1 in the maturation and development of DCs, and therefore in the proper functioning of the immune system as a whole.

Keywords: Dendritic Cells, circular RNA, FSCN1, Fascin1, adaptive immunity

Evaluating Utility of Protein S100A7 in Predicting Progression of Oral Epithelial Dysplasia

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Introduction: Five-year survival of oral cancer has been relatively unchanged despite advancements in treatment, mostly because diagnosis is often made at an advanced stage of disease. The progression of 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 lends itself to some subjectivity. Recent work with the protein biomarker, S100A7, in oral dysplasia and squamous cell carcinoma has shown some predictive value for the transformation of dysplasia to cancer. The objective of this study is to determine if there is a correlation between the expression of S100A7 and the histologic grade of oral dysplastic lesions using immunohistochemistry. Our hypothesis is that the expression of S100A7 can be used a reliable marker for the progression of dysplastic lesions.

Methods: Formalin fixed paraffin embedded specimens, including several follow-up biopsies, were obtained from the Western University Pathology Department tissue archives. Included in this study is 28 cases of dysplastic progression and 45 non-progressing cases. Specimens were stained for S100A7, cyclin D1 and Beta Catenin proteins using a standard immunohistochemistry protocol. Expression of S100A7 was assessed semi-quantitatively, using an intensity and proportion scale, as well as by image analysis with an algorithm applied to determine the risk of transformation to malignancy. The data was analyzed to compare the manual semi-quantitative and the computer algorithm methods and to look for a correlation of S100A7 staining and progression of disease. Cyclin D1 and Beta Catenin were analyzed semi-quantitatively to assess expression in various grades of dysplasia.

Results: Preliminary analysis suggests S100A7 has increased expression in higher risk lesions, however, final statistical analysis still needs to be completed before conclusions can be drawn on the utility of S100A7 as a protein biomarker for predicting malignant transformation.

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

Keywords: S100A7, oral dysplasia, oral cancer

c-Kit and IR co-stimulation does not lead to additive signalling or proliferation in INS-1 cells

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Introduction: c-Kit and insulin receptor (IR) are individually able to maintain beta cell function through the PI3K/Akt pathway. Our previous study using a beta cell -specific c-Kit overexpression mouse model demonstrated increased IR/IRS and Akt signalling, which may indicate additive signalling. However, chronic stimulation through the Akt/mTOR axis can induce serine phosphorylation of insulin receptor substrate-1 (pIRS-1S), which produces negative feedback on Akt signalling. This study examines the in vitro interplay between c-Kit and IR co-stimulation in enhancing beta cell intracellular signalling and proliferation.

Methods: INS-1 832/13 cells were treated with c-Kit ligand stem cell factor (SCF), IR ligand insulin (Ins), or a co-treatment (SCF+Ins) in a time-dependent manner (15 minutes, 1 and 24 hours), with non-treated cells as controls. Cells were harvested and analyzed for receptor activation, intracellular signalling, and proliferation. Rapamycin treatment (2 hours) was used to examine inhibition of the Akt/mTOR axis.

Results: pIRS-1S612 levels were unchanged in all treatments at 15 minutes. SCF+Ins cells showed increased pIRS-1S612 at 1 hour when compared to controls. SCF+Ins cells that were pre-treated with rapamycin displayed reduced pIRS-1S612 levels at 1 hour. Both SCF only and Ins only treatment showed increased c-Kit and IR phosphorylation at 24 hours, but this was not observed in the SCF+Ins treatment. Intracellular signalling of pAktS473 was increased in all treated groups, but the SCF+Ins group treatment was not additive. Cyclin D1, which was used to examine cell proliferation, was not altered in any treated groups.

Discussion: SCF+Ins displayed similar levels of Akt phosphorylation at 24 hours compared to single ligand treatments, which may indicate inhibited PI3K/Akt signalling through IRS-1S. Rapamycin experiments demonstrate that this negative feedback loop can be inhibited with short-term treatment. Further experiments will focus on how c-Kit and IR co-stimulation and inhibition of negative regulatory sites may affect Akt signalling.

Keywords: Beta cell, INS-1 cell line, c-Kit, insulin receptor, intracellular signalling, rapamycin inhibition

Optimal Biopsy Site and Number for the Diagnosis of Microscopic Colitis

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Introduction: Lymphocytic and collagenous colitis are types of microscopic colitis (MC) and are a common cause of chronic watery diarrhea, often misdiagnosed as IBS in patients without macroscopic findings on colonoscopy. In the absence of endoscopic features to direct high yield biopsy sites, endoscopists often take multiple random biopsies from all sections of the colon, potentially over-sampling the mucosa with attendant increased colonoscopy time for the patient and slide review time for the pathologist.

Aims: To identify colonic regions that could offer highly sensitive biopsy sites for MC diagnosis and to determine the appropriate number of biopsies to take at these sites.

Methods: Consecutive cases of MC, negative for co-morbid colonic pathologies, were selected from the Department of Pathology's computerized database. All H&E stained slides were reviewed for the presence of lymphocytic or collagenous colitis, and the proportion of biopsies that were diagnostic, calculated at each biopsy site.

Results: Biopsies from 40 patients were assessed. There were 20 cases of collagenous colitis, 17 cases of lymphocytic colitis and 3 cases with a combination of the two. 100% of biopsy fragments from the cecum through to the splenic flexure were positive for MC. 93% of fragments from the left colon had MC, while 81% of biopsies from the sigmoid and 67% of biopsies from the rectum were diagnostic of MC. For biopsies submitted as "random", 91% were positive for MC.

Conclusion: These results suggest that the right and transverse colon are the most reliable diagnostic sites for MC. Additionally, at all sites, MC could be diagnosed using fewer biopsies than were collected.

Keywords: Microscopic colitis, lymphocytic colitis, collagenous colitis, biopsy site, endoscopy

Validation of QuPath Based Machine Learning Cell Classifier for Neuronal Quantification

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Introduction: Many neurological diseases are characterized by neuronal loss. Neuronal quantification is often a crucial diagnostic criterion. For surgically resected brain tissue, neuron quantification can be performed based on immunohistochemical (IHC) staining with neuron-specific biomarkers (NeuN, MAP2, etc). For post-mortem cases, IHC techniques cannot be reliably applied due to tissue autolysis. However, neuronal histology remains largely unaffected under mild to moderate autolysis. This allows pathologists to quantify neurons based on morphological features either by manual cell counting or eyeballing. These processes are often time consuming and have poor interobserver agreement. A more efficient, objective and reproducible analysis method is needed for clinical neuropathological practice and research.

Methods: We employed the digital image analysis software QuPath to quantify neurons in scanned whole slide images stained with hematoxylin and eosin (H&E) and cresyl violet (CV). The cell detection and classification functions of QuPath were used to identify neurons in neocortical regions and hippocampal subfields. Cell morphological parameters (cell size, nuclear shape, chromatin pattern, nucleolus) measured through the cell detection function were used to train a machine learning based cell classifier. The numbers of neurons per unit area (mm²) determined using the classifier in H&E, CV stained samples were contrasted with values obtained from positive cell detection analysis of NeuN immunohistochemistry.

Results: Neuronal cell counts determined using the machine learning based cell classifier were comparable to numbers obtained from positive cell detection of NeuN immunostained samples in both temporal neocortex and hippocampal CA4 sector. Furthermore, the classifier was able to distinguish between hippocampal sclerosis and normal controls based on neuronal quantification.

Discussion: Built-in QuPath functions can be used to construct a machine learning based cell classifier for fast and reproducible quantification of neurons in conventionally stained samples. With fine tuning, neuron quantification using QuPath software has applications in diagnosis and research of neurological disorders involving post-mortem brain tissue.

Keywords: QuPath, neuron quantification, cell detection, machine learning, epilepsy, hippocampus

The Role of Time-Dependent PaSC Activation on Islet Inflammation and Fibrosis in T2DM

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Introduction: Type 2 diabetes mellitus (T2DM) progression increases levels of oxidative stress, a factor that activates intra-islet pancreatic stellate cells (PaSCs). Activated PaSCs (α SMA+) produce extracellular matrices (ECM), growth factors, and cytokines that are potentially beneficial during β -cell compensation, but if overproduced can increase fibrotic and inflammatory responses within pancreatic islets.

Hypothesis: (1) initial activation of PaSCs increase β -cell proliferation, however, (2) chronic activation of PaSCs will lead to β -cell dysfunction via increased immune and fibrotic changes.

Methods: Six-week-old male C57BL/6J mice fed a 60% kcal high fat diet (HFD) to stimulate progressive T2DM were examined at 8 and 22-weeks for PaSC activation and subsequent effects on β -cell proliferation and function. Antioxidant N-acetylcysteine (NAC) was used to assess the effects of inhibiting PaSC activation in a prevention study starting 1-week before an 8-week HFD (pNAC-HFD), and an intervention study beginning week 12 of a 22-week HFD (iNAC-HFD). Age-matched controls were maintained on normal diet (ND) with parallel NAC treatment.

Results: Preliminary results demonstrated 8-week HFD mice had significantly increased glucose intolerance compared to ND. Glucose and insulin intolerance were significantly increased in 22-week HFD versus ND. Enlarged β -cell mass, increased islet size and increased intra-islet α SMA were seen in 22-week HFD group compared to 8-week HFD and 22-week ND groups, along with increased intra- and peri-islet ECM deposition. NAC intervention improved glucose tolerance in iNAC-HFD compared to 22-week HFD. Intervention also reduced iNAC-HFD intra-islet α SMA staining, β -cell mass and large islet numbers to the levels of 22-week ND. NAC prevention had no effect on glucose or insulin tolerance.

Discussion/Summary: Increased disease severity seen in 22-week HFD mice compared to 8-week HFD may be reduced by NAC intervention. Prevention with NAC has not shown alterations to metabolic outcome. Further analysis into effects of PaSC activation on β -cell function is currently underway.

Keywords: Pancreatic stellate cell, β -cell, HFD, T2DM, Fibrosis, Inflammation

The Characterization of Brain Metastatic Tumours Arising from Primary Lung Adenocarcinomas

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Introduction: Brain metastatic tumours are associated with poor prognoses and significant mortality. Recent studies determined the involvement of gap junction-forming proteins Connexin 43 and Pannexin 1 in developing a brain metastatic tumour-promoting environment in mouse models, human cancer cell lines, and brain metastatic tissue arising from primary breast cancer. This study aims to characterize the expression of Connexin 43 and Pannexin 1 in brain metastatic tumours derived from primary lung adenocarcinomas and correlate these proteins' expression patterns to patient clinical outcomes.

Methods: Tissue microarrays will be performed on brain metastatic tumour tissue and the respective tumour microenvironment, primary lung adenocarcinoma, and lymph node metastatic tumour tissues. Immunohistochemistry (IHC) staining will visualize the expression of Connexin 43 and Pannexin 1 in the various tissue samples. QuPath will be utilized to analyze and compare the staining intensities among the various tissue samples and correlate the staining patterns with patient outcomes.

Results: It is expected that the brain metastatic tumour and respective microenvironment will have increased expression of Connexin 43 and Pannexin 1 in comparison to the primary lung adenocarcinoma and lymph node metastatic tissue. Additionally, we expect to find a positive correlation between the expression of Connexin 43 and/or Pannexin 1 and poor patient clinical outcomes.

Discussion: These findings may demonstrate and confirm the importance of Connexin 43 and Pannexin 1 in the development of a tumour-promoting microenvironment for brain metastatic tumours derived from primary lung adenocarcinomas. This may lead to future research into developing therapeutics against these proteins to reduce the morbidity and mortality associated with brain metastatic tumours.

Keywords: Brain metastatic tumour, lung adenocarcinoma, Connexin 43, Pannexin 1, metastasis, central nervous system

Sensitivity of Ovarian Clear Cell Carcinoma Spheroids to mTOR inhibition

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Introduction: Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy. Clear cell carcinoma (OCCC) is a chemoresistant subtype of EOC that is understudied due to its rarity. A portion of OCCC patients possess mutations in PIK3CA and PTEN, which result in hyperactive AKT signaling and subsequent cellular growth and proliferation. EOC metastasizes by forming dormant cancer spheroids in the abdominal cavity, simultaneously resisting chemotherapeutic toxicity. Our lab has demonstrated that AKT is downregulated upon spheroid formation in high-grade serous ovarian cancer (HGSC), another type of EOC. This study aims to investigate the regulation of AKT in OCCC spheroids as well as sensitivity of these spheroids to inhibition of AKT signaling.

Methods: OCCC cell lines will be used to form in vitro spheroids using the ultra-low attachment (ULA) culture system. Activity of the PI3K/AKT/mTOR signaling axis will be analyzed using immunoblot before treating OCCC spheroids with the dual mTORC1/2 inhibitor, AZD8055, at concentrations of 10 – 1000 nM to assess whether sensitivity to these drugs correlates with AKT activity.

Results: The ability of OCCC cell lines to downregulate AKT signaling upon spheroid formation varies and does not necessarily correlate with PIK3CA and PTEN mutational status. Studies to assess sensitivity to AZD8055 in monolayer and spheroid cultures are underway and preliminary data suggests that OCCC cell lines with hyperactive AKT signaling are more sensitive to blockade of this pathway.

Conclusions: These findings may demonstrate that OCCC spheroids downregulate AKT signaling in a similar manner to HGSC spheroids, even though they carry mutations which activate AKT signaling. This study may reveal novel mechanisms of OCCC pathogenesis as well as make way for possible therapeutic improvements.

Keywords: Ovarian clear cell carcinoma, cancer spheroids, PI3K/AKT/mTOR pathway, tumour dormancy, mTORC1/2 inhibitor

Cell-free DNA release during programmed cell death in ischemia reperfusion injury

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Introduction: Transplantation is invariably associated with acute allograft injury caused by ischemia reperfusion injury (IRI). This injury causes cells of the allograft to undergo various forms of programmed cell death including apoptosis and necroptosis. During programmed cell death, immunogenic molecules are released from cells, one of which is cell-free DNA (cfDNA). We hypothesize that cfDNA is released by microvascular endothelial cells (MVECs) during programmed cell death of IRI and that cfDNA acts as both a biomarker for cellular injury as well as a biologically active molecule capable of amplifying inflammation and organ injury.

Methods: MVECs were subjected to various reagents to induce necroptosis and apoptosis in vitro followed by cfDNA isolation and quantification. A mouse model was used to characterize cfDNA release during IRI. NK cells were subjected to cfDNA in order to determine its biological activity.

Results: Our data show that cfDNA is released by MVECs under both apoptotic and necroptotic conditions in vitro, as well as during IRI in an in vivo mouse model. We have also shown that cfDNA release is ameliorated by blocking necroptosis in vivo with the use of RIPK3^{-/-} mice that are incapable of undergoing necroptosis. Lastly, we have shown that cfDNA is capable of activating immune cells, showing that NK cell activation markers are upregulated when purified NK cells are subjected to cfDNA in vitro.

Conclusions: Our results indicate that cfDNA is a potential biomarker of allograft injury in a renal transplant setting. Donor-derived cfDNA from blood or urine may give rise to novel non-invasive tests to diagnose graft damage. cfDNA also appears to exacerbate inflammation by activating immune cells to produce pro-inflammatory cytokines which further escalates inflammation. It may be prudent to inhibit the release of cfDNA in a transplant scenario, a goal our lab is currently working towards.

Keywords: cfDNA, Necroptosis, Apoptosis, Renal Transplantation, Endothelial Cells, NK Cells

Poster Presentations

Session B

2:30 - 3:30 pm

#	First Name	Last Name	Title
1	Moheem	Halari	A Postmortem Study of Injury Patterns in Pedestrian and Cyclist Fatalities as a Predictor of Motor Vehicle Collision Dynamics and Pedestrian Kinematics
2	Jeffrey	Liang	Predicting Recovery Neutrophil Count in Patients with Hematologic Malignancies after Chemotherapy and Stem Cell Transplant
3	Seana	Hill	Defining domains of differential chromatin compaction between human metaphase homologues: A higher-order chromosome structure link?
4	Rebecca	Sullivan	PET Imaging of the Cardiac Growth Hormone Secretagogue Receptor in a Large Animal Model of Heart Failure
5	Xiaoyun	Ji	Junctophilin-2 Protects Cardiomyocytes against Palmitate-induced Injury
6	Janice	Gomes	Development of a microparticle-based bio-marker of hemodialysis induced vascular injury
7	Mihai	Dumbrava	Oxidation, Aggregation, and Function of PARK7/DJ-1 in Parkinson's Disease

#	First Name	Last Name	Title
8	Lauren	Solomon	Estrogen Effects on Th2 cell phenotype: Key to Severe Asthma in Women?
9	Ron	Ng	Adapting the MiCall pipeline for influenza A virus
10	Srinitya	Gannavarapu	Hypomyelinating leukodystrophy: do heterozygous variants in HSPD1 deserve a closer look?
11	Alice	Shin	Role of Lgr5 in Dclk1+ cell-derived colitis-associated colon cancer
12	Ryan	Gillies	In-utero Δ 9-tetrahydrocannabinol Exposure Alters Pancreatic Development in Female Offspring and Impairs Glucose Tolerance in Adulthood
13	Sonja	DiGregorio	RGNEF protein misfolding and toxicity in ALS
14	Andrea	Wang	Identifying Wrong Blood in Tube in Hematology Samples Using Delta HMR on Sysmex XN Analyzer
15	Casey	Dion	Comparison of Surgical Pathology Quality Assurance Measures at Five Ontario Hospitals

#	First Name	Last Name	Title
16	Kevin	Zhou	Discovery of Novel and Recurrent Structural Genomic Rearrangements Detected by Microarray Analysis in Patient Population Tested at London Health Sciences Centre
17	Benjamin	Rogala	Evidence of a phagocytic phenotype in granular cell tumors
18	Sundip	Shah	Review of departmental usage of immunohistochemistry and special stains over a 3 year period
19	Stephanie	Newman	Viral Vector Therapy as a Therapeutic Option for Peripheral Nerve Disease Associated with Metachromatic Leukodystrophy
20	Yiming	Lin	Characterization of microvasculature in Duchenne Muscular Dystrophy
21	Jina	Kum	Glucose Modulates Transforming Growth Factor- β Signalling in Bone Marrow-derived Progenitor Cells to Enhance Adipogenic Differentiation

#	First Name	Last Name	Title
22	Haley	Jamison	Reconstitution of Human Mitochondrial Calcium Uniporter (MCU) Activity in Bacteria to Evaluate Cancer-Linked Mutations
23	Ruipeng	Lu	Meeting cytogenetic biodosimetry capacity requirements of population-scale radiation exposures with geostatistical sampling
24	Alaa	Badawi	Regulation of T helper 2 cell Function by Glucocorticoid and Estrogen Receptor Signaling
25	Basma	AlYamany	A Case of Sudden Unexpected Death in Epilepsy with Diffuse Leptomeningeal Glioneuronal Tumour
26	Megan	Noel	Development and Implementation of a Mobile Assessment Tool for Pathologists' Assistant Grossing Competency
27	Ahmed	Hassan	Effects of Impaired Fetal Development on Sepsis: Sex-Differences in Kidneys
28	Farzad	Asadi	The Neuronal Protein Stathmin-2 Regulates Glucagon Secretion from α -Cells

#	First Name	Last Name	Title
29	Matthew	Hintermayer	The role of pThr175tau and the N-terminal phosphatase activating domain in tau cytoplasmic inclusion formation
30	Asieh	Alikhah	Dysregulation of circRNAs in Amyotrophic Lateral Sclerosis
31	David	Piccoli	Temperature-Specific Surfactin Production and Biocontrol Potential of <i>Bacillus methylotrophicus</i> 1B-23
32	Sam	Taheri	Safety and effectiveness of cardiac rehabilitation delivered in non-hospital settings by non-physician healthcare professionals
33	Mohammad	Khazaneh	Post-Mortem Microbiological Analysis of Sudden Infant Death Syndrome
34	Sophie	Heinlein	The Impact of Intraoperative Consultation on Patient Management at London Health Sciences Centre
35	Komila	Zakirova	CRISPR-based Approach for Identification of Novel Genes Essential for Spheroid Dormancy in Epithelial Ovarian Cancer
36	Erin	McGrath	Retrospective review of CSF White Blood Cell Count and Viral PCR Results. A Laboratory Utilization Initiative

A Postmortem Study of Injury Patterns in Pedestrian and Cyclist Fatalities as a Predictor of Motor Vehicle Collision Dynamics and Pedestrian Kinematics

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Introduction: In 2018, the International Road Traffic Data and Analysis Group reported an increase of 21.1% and 7.3% in pedestrian and cyclist fatalities, respectively in 2016 compared to 2015. The purpose of this study is to understand the mechanism of injury by correlation of motor vehicle collision findings with trauma patterns sustained by fatally injured pedestrians and cyclists. A predictive model using patterns of injury can be used by pathologists to assist coroners and police investigators. We hypothesize that patterns of injury will assist in predicting motor vehicle collision dynamics and pedestrian/cyclist kinematics and distinguish hit upright vs run over pedestrian cases.

Methods: To identify the injury patterns sustained by pedestrians and cyclists involved in a motor vehicle collision, we commenced a medical literature review. An online systematic review application (Rayyan QCRI) was used. Records were screened by two reviewers and mapped for eligibility and inclusion using the PRISMA flow diagram. A data collection form was created and will be used to extract post-mortem trauma data from the Provincial Forensic Pathology Unit in the Forensic Services and Coroners Complex in Toronto from 2013 to 2018 to identify patterns of injury predictive of vehicle and collision types.

The secondary objective of the study is to analyze post-mortem computerized tomography (PMCT) scan data and compare pedestrians to distinguish hit upright vs run over cases.

Results: Initial screening and secondary screening for the literature review has been completed. Research Ethics Board application has been submitted for obtaining post-mortem trauma data from the Provincial Forensic Pathology Unit in the Forensic Services and Coroners Complex in Toronto and is currently awaiting approval.

Discussion: The post-mortem trauma data from 300 cases of pedestrian and cyclists will be used to identify patterns of injury by analyzing pedestrian kinematics and collision dynamics and correlating the injuries sustained.

Keywords: Motor Vehicle Collision, Pedestrian, Cyclist, Injury Pattern, Collision Dynamics, Pedestrian Kinematics, Vehicular Design.

Predicting Recovery Neutrophil Count in Patients with Hematologic Malignancies after Chemotherapy and Stem Cell Transplant

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Introduction: Chemotherapy induced neutropenia is a common side effect while treating patients with Bone Marrow Transplant (BMT) or Hematopoietic Stem Cell Transplantation (HSCT). Patients that have Absolute Neutrophil Counts (ANC) below 0.5×10^9 per L but otherwise clinically stable are required to stay in the hospital for extended periods of time. This increased duration in the hospital is accompanied with increase antimicrobial use which can lead to adverse drug reactions, and antimicrobial resistance. In the present study, we will attempt to create a multivariable model with Immature Granulocytes (IG), and Monocytes to predict neutrophil recovery. We hypothesize that this multivariable model will be able to predict neutrophil recovery and could be used with different chemotherapy induced neutropenia

Methods: To test this hypothesis, we will be identifying BMT patients through patient chart review. We will use the coding language Python to extract and sort the appropriate parameter data. We will then split our model into 3 distinct phases. During Phase 1, we will create a multivariable prognostic model with IBM Statistical Package for the Social Sciences (IBM SPSS) using IG and Monocyte with 25 of our 50 patients. Phase 2 will be testing for internal validity with the rest of our cohort – 25 patients and Phase 3 will be a prospective test in the clinic.

Results: Our current results predict that Monocytes or a combination of total Phagocytes (Monocyte + Neutrophil) have a relationship with Neutrophil Recovery. We expect to see Immature Granulocytes to have a positive correlation with Neutrophil Recovery as well.

Conclusion: Our results will provide physicians the confidence to discharge patients earlier and to stop antimicrobial use. Patients who are expected to recover based on the model will reduce hospital costs and reduce the chances of nosocomial diseases and antimicrobial resistance. These findings may improve the lives of both the patients and physicians.

Keywords: Neutrophil Recovery, Neutropenia, Bone Marrow Transplant, Stem Cell Transplant, Predictor, Multivariable Model, Immature Granulocyte, Monocyte, Immature Platelet Fraction, Reticulocyte

Defining domains of differential chromatin compaction between human metaphase homologues: A higher-order chromosome structure link?

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Introduction: During mitosis, higher-order chromatin structures are lost including topologically associated domains (TADs) necessary for correct gene regulation. How these structures are re-established after division is not well understood. We have proposed that unique, non-random localized condensation differences between metaphase homologues (termed differential accessibility or DA) represent a structural memory of the parental configuration. Detected by fluorescence in situ hybridization (FISH) using short, single-copy (sc) DNA probes, DA is observed in ~10% of scDNA. DA has been reported in short sc regions (1.5-4 kb), both within and between genes, widely dispersed throughout the genome. To better understand genomic representation of DA, this study expands confirmed DA regions to investigate the size and frequency of DA domains and their relationship to TADs.

Methods: To define genomic DA domains, scFISH probes (1.4-4.0kb) designed for multiple sc intervals adjacent to confirmed DA regions were hybridized to metaphase chromosomes prepared from lymphocytes. Differences in fluorescent intensity between homologues were analyzed. Correspondences between DA and equivalently accessible regions with interphase epigenetic features were identified with the ENCODE and 3D Genome browsers.

Results: Six DA domains (16 - 130kb) were identified using 2-4 different sc probes per region [gene anchor (chromosome band): XDH(2p23), HMGB1P5(3p24), FGF6(12p13), TPM1(15q22), COX5A(15q24), HMGB1P1(20q13.3)]. All individual probes (1459-3553bp) investigated show DA. Five domains (XDH, FGF6, TPM1, COX5A, HMGB1P1) defined in metaphase chromosomes were each within a separate TAD and show a high frequency of intra-TAD interactions between the chromatin within the domain and larger TAD area.

Discussion: DA spans genomic domains that extend beyond defined boundaries of individual scFISH probes. DA appears to be dispersed and common in the human genome. The presence of DA domains, each within a single TAD, is consistent with a possible role in the maintenance of structural chromosome memory through multiple cell generations.

Keywords: Metaphase homologous chromosomes, topologically associated domains, chromosome decondensation, epigenetics

PET Imaging of the Cardiac Growth Hormone Secretagogue Receptor in a Large Animal Model of Heart Failure

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Introduction: Cardiac imaging technologies including echocardiography and magnetic resonance imaging (MRI) enable non-invasive detection of anatomical changes in the heart that are seen after molecular changes. Developing targeted molecular imaging agents will address this need. The cardiac growth hormone secretagogue receptor (GHSR) could be a potential target. Our lab is characterizing imaging agents by positron emission tomography (PET). We hypothesize a novel imaging agent (18F-LCE00470) can detect changes in cardiac GHSR using hybrid PET/MRI in a canine model of heart failure.

Methods: A myocardial infarct (MI) was generated in female hounds by occlusion of the left descending coronary artery, followed by reperfusion. Dogs (n=4) were imaged with a combination of PET tracers at specific timepoints: 18F-LCE00470 for GHSR; 18F-FDG for inflammation; 13N-ammonia for perfusion. Dogs were injected with 100-150 MBq of tracer, followed by a 1-hour (18F-) or 30 min (13N-) dynamic PET scan using simultaneous PET/MRI.

Results: At baseline, distribution of 18F-LCE00470 was uniform throughout the LV, but at all other timepoints, tracer distribution excluded middle of the infarct (microvascular obstruction or MO) and infarct areas with increases in non-infarcted tissue. There was a significant decrease in binding in the infarct at days 3 (p<0.05) and 112 (p<0.01). This distribution is different from the binding patterns of both 18F-FDG and 13N-ammonia indicating a unique binding pattern of 18F-LCE00470 post-MI. 18F-FDG was significantly lower in the MO at days 1 and 7 (p<0.05).

Conclusions: In a canine model of MI, 18F-LCE00470 detected changes in the regional distribution of GHSR with different binding patterns than known tracers of perfusion and inflammation. Next steps are to analyze this data using compartmental modeling to better understand the function of this tracer. This work will help improve the knowledge of molecular changes in the heart prior to conventional imaging methods.

Keywords: GHSR, inflammation, heart failure, positron emission tomography, magnetic resonance imaging, 18F-FDG

Junctophilin-2 Protects Cardiomyocytes against Palmitate-induced Injury

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Introduction: Lipotoxicity is an important mechanism contributing to cardiac injury under metabolic stress. Excessive fatty acid uptake induces cardiac lipotoxicity, leading to cardiomyocyte death and myocardial dysfunction. However, the underlying mechanism remains not fully understood. Junctophilin-2 (JPH2), a membrane binding protein, bridges the physical gap between the plasma membrane and the sarcoplasmic reticulum in cardiomyocytes. JPH2 is down-regulated in diseased hearts and over-expression of JPH2 attenuates the progression of heart failure. In this study, we aimed to investigate whether and how JPH2 protects cardiomyocytes against lipotoxic injury.

Methods: Lipotoxic injury was induced in cultured neonatal mouse cardiomyocytes by palmitate. Adenoviral vectors were used to knock down or over-express JPH2 protein in cardiomyocytes. Caspase-3 activity, lactate dehydrogenase (LDH) and mitochondrial reactive oxygen species (ROS) were determined.

Results: Incubation with palmitate reduced the protein levels of JPH2 whereas oleate, as a control, did not change JPH2 protein levels in cardiomyocytes. The reduction of JPH2 was associated with increases in caspase-3 activity, LDH release and mitochondrial ROS generation in palmitate-stimulated cardiomyocytes. Over-expression of JPH2 attenuated palmitate-induced caspase-3 activity, LDH release and mitochondrial ROS generation in cardiomyocytes. Knockdown of JPH2 sufficiently induced caspase-3 activation, LDH release and mitochondrial ROS generation, and enhanced palmitate-induced caspase-3 activity, LDH release and mitochondrial ROS generation in cardiomyocytes. Furthermore, selective inhibition of mitochondrial ROS with mito-TEMPO reduced caspase-3 activity and LDH release in cardiomyocytes with palmitate incubation or JPH2 knockdown.

Discussion: Palmitate reduced JPH2 expression, increased mitochondrial ROS generation and induced injury in cardiomyocytes. Up-regulation of JPH2 protects cardiomyocytes against palmitate-induced injury by inhibiting mitochondrial ROS generation.

Keywords: Cardiomyocytes, cell injury, junctophilin-2, lipotoxicity, reactive oxygen species

Development of a microparticle-based bio-marker of hemodialysis induced vascular injury

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Background: For those who have Kidney Failure, hemodialysis is usually the only treatment option. Hemodialysis is a repetitive and ongoing treatment that creates complications such as hemodynamic instability, microvascular dysfunction, and damage to vulnerable vascular beds which can lead to irreversible multi-organ injury. This damage has been observed through gold standard imaging techniques such as Echo (Echocardiography), CT (Computed Tomography), MRI (Magnetic Resonance Imaging), and PET (Positron Emission Tomography), however there are no reliable blood based bio-markers available to identify this injury that might be suitable to use in general clinical practice. We therefore investigated the use of blood vessel and circulating blood cell derived microparticles as an indicator of vascular damage in a cohort of Hemodialysis patients under standard treatment condition and a state of reduced circulatory stress (using dialysate cooling).

Methods: An assay was created to assess the level of endothelial, platelet, erythrocyte, and leukocyte derived microparticles. By utilizing Nanoscale Flow Cytometry (Apogee A50), we measured microparticle levels within plasma samples obtained from patients receiving standard hemodialysis treatment (36.5 ° C, n=31) and 16 of the same patients receiving cooled dialysate treatment (35 ° C). Microparticles were enumerated at pre, during, and post treatment.

Results: CD31+/CD62E+ microparticles (derived from activated endothelium) correlated with ultrafiltration rate (as the primary driver of HD-based circulatory stress) (R²=0.1720, p<0.05). There was no relationship between erythrocyte, leukocyte, and platelet derived microparticles and ultrafiltration rate. Furthermore, we observed that 86% of patients experienced a reduction in CD62E+ (e-selectin) microparticle levels when administered cooled dialysate treatment in comparison to standard treatment (**p<0.01).

Conclusions: The use of microparticles as a bio-marker of HD induced ischemic injury shows promise and warrants further refinement and investigation to identify and risk assess patients receiving HD, as well as monitoring response to efforts to individualize and optimize HD treatment.

Keywords: Hemodialysis, Vascular Damage, Extracellular Vesicles, Microparticles, Biomarker, Cooled Dialysate

Oxidation, Aggregation, and Function of PARK7/DJ-1 in Parkinson's Disease

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Introduction: Parkinson's Disease (PD) is a neurodegenerative disorder that primarily affects the motor system and is characterized by progressive degeneration of dopaminergic neurons. While both genetic and environmental factors are suspected to contribute to sporadic forms of the disease, mutations in a number of genes of the PARK family result in familial early onset forms of PD. In particular, multiple mutations in the PARK7 gene encoding DJ-1 which disrupt DJ-1 dimerization or reduce DJ-1 stability have been linked to PD. We hypothesize that PD-associated point mutations cause DJ-1 misfolding and aggregation, resulting in a toxic gain of function that accelerates neurodegeneration.

Methods: To characterize the function of DJ-1 and elucidate which interactions are impaired by the PD-associated mutations, a yeast model for DJ-1 was established and genetic interactions were identified using a yeast deletion library screen. To validate the results from yeast, a neuronal model of DJ-1 and the PD-associated DJ-1 mutants was established using neuroblastoma-derived SH-SY5Y cells. In these mammalian cells, siRNA or CRISPR/Cas9 was used to inhibit PARK7 gene expression and transient transfection allowed for expression of DJ-1 mutant proteins. The cellular localization of DJ-1 was investigated using immunofluorescence microscopy.

Results: We identified several genes, including SOD1, SOD2, CTA1, and YAP1, which interact with DJ-1 and are involved in pathways that aim to reduce the levels of oxidative stress in the cell. Notably, we demonstrated that the point mutation L166P alters the localization of DJ-1 in the cell and prevents these interactions, leading to the pathogenesis of PD.

Conclusions: Our results compare, for the first time, the aggregation propensity, sensitivity to oxidative stress, localization, and function of DJ-1 WT versus PD-associated DJ-1 mutants. Notably, these findings provide a novel mechanistic understanding of the role of DJ-1 in both familial and sporadic PD and contribute to future therapeutic development.

Key Words: PARK7/DJ-1, Parkinson's Disease, Oxidative stress, Neurodegeneration, Protein misfolding, SH-SY5Y, *Saccharomyces cerevisiae*

Estrogen Effects on Th2 cell phenotype: Key to Severe Asthma in Women?

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Introduction: Allergic asthma is a T helper 2 (Th2) cell-associated inflammatory disease, driven by cytokines such as IL-4, IL-5, and IL-13. Th2 cells express the G-protein-coupled receptor CRTh2, a receptor for prostaglandin D2 (PGD2) that influences Th2 function and survival. Inhaled glucocorticosteroids (GC) are the primary treatment of allergic asthma and improve symptoms by inhibiting Th2 cytokine production and killing Th2 cells. Women are more likely than men to have severe asthma and have symptoms requiring a hospital visit. We observed that severe asthmatic women have more circulating Th2 cells than men with severe asthma, despite taking similar doses of glucocorticosteroid. We hypothesized female sex hormones could influence Th2 cell response to GC.

Methods: Using RNA-seq, gene expression in primary Th2 cells was quantified following exposure to glucocorticosteroids (0.1µM) in the presence or absence of an estrogen mimic, PPT (10µM). Gene expression was validated by qPCR. Prostaglandin D2 and IL-5 levels in culture supernatant were determined by ELISA.

Results: Gene expression in Th2 cells was examined following exposure to glucocorticosteroids in the presence or absence of an agonist for estrogen receptor alpha, PPT. While glucocorticosteroids repressed Th2 cytokines, regardless of addition of PPT, many glucocorticosteroid-mediated effects were suppressed or counter-acted by PPT. These included increased expression of genes identifying a "pathogenic" Th2 subset characterized by increased CRTh2, hPGDS and CD161 expression. Prostaglandin D2 in the media following treatment with a combination of glucocorticosteroid and PPT was significantly increased over glucocorticosteroid alone. Anti-apoptotic genes including BCL2 were also increased by co-treatment.

Discussion: Functional studies are planned to examine the combination of glucocorticosteroid and estrogen treatment in a feed-forward, pro-survival loop involving PGD2-CRTh2 signaling. These findings suggest glucocorticosteroid effects on Th2 cells are influenced by estrogen signaling which, in women, could represent a mechanism driving steroid insensitivity and development of severe asthma.

Keywords: Asthma, Th2 cells, steroid, sex hormones, prostaglandin

Adapting the MiCall pipeline for influenza A virus

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The MiCall pipeline is a next-generation sequencing tool designed for processing sequence data of samples based on human immunodeficiency virus (HIV-1), hepatitis C virus (HCV), and human leukocyte antigen (HLA). MiCall maps sequence data to a set of reference sequences to generate consensus sequences, variant calls, and coverage maps. Currently, the pipeline does not accommodate sequence data from other sample types. Reference data used by MiCall is generated from the Centre for Excellence in HIV/AIDS (CFE) database in British Columbia, Canada, making the reference data difficult to edit. The project aims to develop a user-interface to allow for a more accessible method of viewing and editing reference data, with influenza A virus as the subject of the test case.

Using JavaScript and HTML, a browser-based user interface will be created. It will allow for manipulation of the reference data. To test the ability to accommodate sequences from different samples, sequence data of influenza A virus from the Short Read Archive (SRA) of the National Centre for Biotechnology Information (NCBI) will be used. The MiCall pipeline will be run using that sequence data as the reference to determine if it will function.

Keywords: Next-generation sequencing, bioinformatics, virus evolution, user interface, influenza A virus

Hypomyelinating leukodystrophy: do heterozygous variants in HSPD1 deserve a closer look?

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Introduction: Heterozygous variants in HSPD1 (heat-shock 60kDa protein [HSP60]) are associated with adult-onset spastic paraplegia-13. While biallelic variants cause pediatric-onset hypomyelinating leukodystrophy, also known as mitochondrial HSP60 chaperonopathy (MitCHAP-60 disease). HSP60 participates in the folding and trafficking of proteins into mitochondria and is expressed abundantly in myelin-forming glial cells.

Case and Methods: Exome sequencing identified a de novo heterozygous HSPD1 variant (p.Leu47Val) in a 23 year old male with consanguineous parents. At 2 years, he presented with progressive ataxia, developmental delay and dysmyelination showing a symmetric tigroid pattern in the posterior fossa on his MRI. He also had ethylmalonic aciduria, with the presence of homozygous variants in ACADS, considered to be a benign trait. In silico analyses, immunoblotting and immunofluorescence (IF) were used to determine the predicted impact of the variant, HSP60 protein expression and subcellular localization. MitoTracker Red CMXRos was used to study mitochondrial morphology and membrane potential. All initial functional tests are being performed in proband-derived and control fibroblast cell lines.

Results: Prediction scores in CADD and WANNVAR all categorized the variant as pathogenic. IF images demonstrated co-localization of HSP60 and mitochondrion as expected. Preliminary findings suggest decreased HSP60 expression and mitochondrial membrane potential in the proband fibroblasts compared to unaffected, age-matched control cell lines.

Conclusions: Mitochondrial dysfunction resulting from impaired expression of HSP60 can critically impact myelin-forming neural cells, triggering apoptotic factors. We propose that certain heterozygous variants in HSPD1 may lead to more severe features resembling the biallelic phenotype, due to a dominant-negative effect or multigenic interactions. At present, analyses are underway to better characterize effects of this variant. We continue to explore additional contributors or interactors and seek more disease-relevant in vitro models, such as patient-derived induced neural progenitor cells.

Keywords: heat shock 60-kDa protein 1, hypomyelinating leukodystrophy, exome sequencing, ataxia, spastic paraplegia

Role of Lgr5 in Dclk1+ cell-derived colitis-associated colon cancer

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Introduction: Colorectal cancer (CRC) is the second leading cause of cancer death, with a major risk factor being chronic inflammation. However, the mechanism by which colitis leads to CRC is still not well understood. We previously showed that Dclk1-expressing tuft cells are long-lived quiescent cells in the colon that serve as a cellular origin of CRC upon colonic inflammation. In this study, we aim to explore the mechanism by which inflammation contributes to tuft cell-derived cancer initiation. We hypothesized that colonic inflammation leads to dedifferentiation of Dclk1+ tuft cells to an Lgr5-expressing stem cell state susceptible to tumor initiation.

Methods: To conduct Dclk1+ cell lineage tracing and cell-specific knock-out of the tumor suppressor adenomatous polyposis coli (APC), we crossed our Dclk1-CreERT2 mice to both ROSA26-tdTomato and APC^{fl/fl} mice (Dclk1/APC^{fl/fl}). To examine the role of dedifferentiation in colonic tumor initiation, the mice were further crossed to Lgr5-DTR-eGFP mice (Lgr5DTR;Dclk1/APC^{fl/fl}). These mice were given tamoxifen and dextran sodium sulfate (DSS) to induce colitis and subsequent tumorigenesis. The mice were additionally administered diphtheria toxin (DT) for six weeks post DSS injury to ablate Lgr5+ cells.

Results: DT ablation of Lgr5+ cells significantly reduced the number of colonic tumors but did not affect tumor size. Lgr5-expressing cells were readily observed within Dclk1+ cell-derived colonic tumors. Interestingly, two weeks post DSS-colitis, we could detect rare Dclk1+ cells that co-expressed Lgr5, and qRT-PCR analysis of colonic mRNA levels revealed significantly reduced Lgr5 and increased RSPO1 and RSPO3 levels in DSS-treated mice.

Conclusions: Upon DSS-induced colonic injury, Dclk1+ tuft cells express the stem cell marker Lgr5 prior to initiation of colonic tumorigenesis. These data suggest that dedifferentiation of Dclk1+ cells to a stem cell state may play an important role in the development of colitis-associated CRC and provides insight into the mechanism by which Dclk1+ cell derived colonic tumors arise.

Keywords: Dclk1, Lgr5, Tuft cell, Colitis, Colon cancer, Dedifferentiation, R-spondin

In-utero Δ 9-tetrahydrocannabinol Exposure Alters Pancreatic Development in Female Offspring and Impairs Glucose Tolerance in Adulthood

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Introduction: Epidemiological studies show that 7.0% of pregnant women in the United States use cannabis, with this number expected to increase with legalization. The main psychoactive component in cannabis, Δ 9-tetrahydrocannabinol (THC), has increased in concentration drastically in recent decades, and freely traverses the placental barrier to access fetal circulation. This compound causes placental insufficiency and symmetrical intrauterine growth restriction (IUGR), altering organ development and predisposing the offspring to a number of deleterious outcomes. THC may also aberrantly activate the endocannabinoid system in developing pancreatic β -cells, impairing the growth of these cells. We hypothesize that these effects of THC in utero will result in altered pancreas development leading to glucose intolerance in adulthood.

Methods: We treated pregnant Wistar rats with either 3.0 mg/kg/day THC extract or vehicle, by intraperitoneal (i.p.) injection, from gestational day 5.5 (GD5.5) through GD21.5. Pancreata were harvested from male and female offspring aged 21 days and 6 months, then embedded in paraffin and sectioned. We assessed pancreas morphology by immunohistochemistry. Glucose tolerance was assessed in 5-month-old offspring by i.p. glucose challenge.

Results: β -cell mass is reduced in THC-exposed female offspring at weaning, while THC-exposed male offspring show no change. This reduction stems from decreased islet number, and occurs without alteration in α -cell mass. THC-exposed female offspring also show impaired glucose tolerance in adulthood. No change in glucose tolerance was observed in THC-exposed male offspring.

Discussion: There is striking sexual dimorphism in the effects of in-utero THC exposure on pancreas morphology at weaning and glucose tolerance in adulthood. While the mechanism remains to be elucidated, these results raise concern over the long-term metabolic health of offspring, particularly female offspring, exposed to THC in-utero.

Keywords: Cannabis sativa, Δ 9-tetrahydrocannabinol, symmetrical intrauterine growth restriction, placental insufficiency, glucose tolerance, pancreas morphology, beta-cell mass, sexual dimorphism.

RGNEF protein misfolding and toxicity in ALS

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Protein misfolding and impaired cellular stress responses are closely associated with neurodegeneration in ALS. Yet, it remains unclear how these complex processes contribute to ALS-specific pathogenesis. All eukaryotic cells are equipped with effective protein quality control mechanisms that are regulated by cellular stress response program. The heat shock response (HSR), the unfolded protein response (UPR), and the antioxidant response (AR) are essential cellular stress response programs that are induced by exposure to avert conditions and the presence of misfolded proteins. TDP-43, FUS/TLS, and RGNEF appear misfolded and often aggregated in ALS-affected neurons. Apparently, stress response programs fail to prevent the toxic consequences associated with TDP-43, FUS/TLS, and RGNEF misfolding in ALS. Also, recent genetic, biochemical, and pathological findings indicate a potential role of a protein called Rho Guanine nucleotide exchange factor, RGNEF, in ALS but its normal function and its specific contributions to ALS pathogenesis remain mostly elusive. I hypothesize that cellular stress response programs dysregulated by ALS-specific protein misfolding and the impaired function of misfolded RGNEF drive ALS-specific neurodegeneration.

Here, I seek to determine the mechanisms by which TDP-43, FUS/TLS, and RGNEF misfolding modifies the heat shock response, the unfolded protein response, and the antioxidant response and the ensuing effects on neuronal function and survival in ALS. Further, I aim to identify genetic and protein-protein interactions of RGNEF to elucidate its physiological and pathophysiological functions in ALS. To this end, I will employ a powerful combination of high through-put screens in yeast models, cell biological assays in mammalian cells, and pathological studies using postmortem specimen from ALS patients.

Keywords: protein misfolding, neurodegeneration, cell toxicity, gene expression, ALS

Identifying Wrong Blood in Tube in Hematology Samples Using Delta HMR on Sysmex XN Analyzer

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Introduction: Wrong Blood in Tube (WBIT) is a pre-analytical error that occurs when the blood in the tube does not match the blood of the patient identified on the label. Current analyzers for detecting WBIT employ a delta Mean Cell Volume (MCV) check but have been shown to have a low positive predictive value. Other methods of detecting WBIT implement delta mean cell hemoglobin (MCH) and Red Blood Cell Distribution Width (RDWCV) checks. This study aims to optimize WBIT detection by comparing delta Hemoglobin MCH RDWCV (HMR) and delta MCV. We hypothesize that HMR will more accurately identify WBIT compared to the delta MCV.

Methods: The study was performed in three sequential experiments using Sysmex XN 1000 analyzer. In experiment one, we ran 200 samples with 40 true positive "test" hematology samples to assess sensitivity and specificity of delta HMR and MCV. In the second experiment, we assessed the sensitivity of delta HMR and delta MCV in cohort of 100 patient samples with paired samples drawn within three days each other. Finally we will assess the accuracy of delta HMR and delta MCV in a core laboratory environment to detect WBIT in 300 hematology and chemistry samples.

Results: In experiment one we saw that delta MCV had a specificity of 100.0% and a sensitivity of 82.5%. Delta MCH and RDWCV had a specificity of 97.3% and 100% and a sensitivity of 85.0% and 65.0% respectively. In experiment two Receiver Operating Characteristic curves showed that delta MCV had an area under curve of 0.8981, delta MCH of 0.8987, and delta RDWCV of 0.8474.

Discussion: Although delta MCV was more sensitive than delta HMR, delta MCH is a potential standalone test for WBIT detection. Modifications to delta MCV, MCH, and RDWCV cutoffs should be considered before further comparing delta MCV and HMR.

Keywords: blood testing, delta check, mean cell volume, hemoglobin, transfusion

Comparison of Surgical Pathology Quality Assurance Measures at Five Ontario Hospitals

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Introduction: All pathology and laboratory medicine departments in Ontario are bound by a standardized quality assurance system that ensures high quality patient care and safety. There may be preferences and procedures that are site specific.

Aim: To compare and contrast quality assurance measures for surgical pathology specimens at five Ontario hospitals, ranging from tertiary care to community hospital settings.

Methods: Surgical specimens were followed from the time they were received in the pathology laboratory to the time glass slides were made. Procedures for specimen discard were also reviewed. Quality assurance measures and checkpoints were documented at each laboratory station including: specimen receiving, opening, grossing, tissue processing, embedding, cutting, staining, sorting, assigning, and storage.

Results: A comprehensive checklist table was created to compare and contrast surgical pathology quality assurance measures taken at five different hospitals, designated anonymously as "Hospital 1", "Hospital 2", etc. Results show that while there is a general consensus amongst the institutions for most quality assurance steps, there are a few distinctions. Some of these differences include: the amount and types of specimen identifiers used for cassettes and slides, tracking systems used, and dictation methods.

Discussion and Conclusion: While all pathology labs abide by standardized guidelines, site specific preferences in procedures are documented. Advantages and disadvantages to varying techniques at each site are assessed. By comparing hospital pathology departments, we hope to learn from each other and continue to improve on the quality assurance and safety of our individual departments.

Keywords: Quality Assurance, Surgical Pathology, Patient Care, Ontario

Discovery of Novel and Recurrent Structural Genomic Rearrangements Detected by Microarray Analysis in Patient Population Tested at London Health Sciences Centre

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Introduction: Chromosomal microarray analysis(CMA) is the first-tier test for identifying rearrangements in individuals with developmental delay/intellectual disability, autism spectrum disorders, or multiple congenital abnormalities. The London Health Sciences Centre Clinical Cytogenetics Laboratory has tested ~5000 probands using the ThermoFisher CytoScanHD SNP Array platform and compiled a database of reported copy number variants(CNVs). Although CMA was introduced >10 years ago, many novel genomic rearrangements continue to be discovered, the clinical significance of which remain unknown. We hypothesize that i) some genomic rearrangements identified at LHSC are novel; ii) candidate gene(s) for certain clinical phenotypes could be identified by comparing genomic overlap between different sized CNVs from the same chromosomal region.

Methods: Reported CNVs from 2014-2018 were sorted into cluster groups using descriptive statistics generated by a java-based computer program. The CNVs were visualized using custom tracks created for the University of California Santa Cruz(UCSC) Genome Browser [hg19]. Retrospective analysis was then performed, highlighting CNVs of interest and assessing their genomic content/features with browser genomic tracks and literature search.

Results: Of 4,894 cases tested at LHSC, 1,106 CNVs and regions of homozygosity were reported. Of the 881 CNVs, 268(30.4%) were classified as pathogenic, 61(6.9%) as likely pathogenic, and 552(62.7%) as variants of unknown significance. 415 probands had parental follow-up testing, and CNVs were paternally inherited in 95(22.9%), maternally inherited in 128(30.8%), de novo in 82(19.8%) and uncertain in the remaining. The most common CNV deletions(del) and/or duplications(dup) include: chromosome regions 1q21.1(del/dup), 1q21.1q21.2(del/dup), 2p16.3(del), 15q11.2(del), 15q11.2q12(dup), 15q11.2q13.1(del/dup), 15q13.1q13.3(del/dup), 15q13.2q13.3(del/dup), 16p11.2(del/dup), 16p12.2(del), 16p13.12p13.13(del/dup), 17p13.3(del), 17p12(del/dup), 17p12p11.2(del/dup), 17q12(del/dup), 22q11.2(del/dup), 22q11.23(dup), Xp22.31(del/dup). A subset of unique CNVs that partially overlap common CNVs are being investigated for genotype/phenotype relationships.

Conclusions: Our results offer a better understanding of the variety and qualities of genomic rearrangements that exist within Southwestern Ontario. Candidate gene(s) are identified for certain phenotypes.

Keywords: Microarray, Chromosome, Rearrangements, Copy Number Variation, Novel, Intellectual Disability, Developmental Delay, Autism, Congenital Abnormalities, Southwestern Ontario, LHSC

Evidence of a phagocytic phenotype in granular cell tumors

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Introduction: Granular cell tumors (GCTs) are uncommon solitary soft tissue neoplasms. Clinically they present as nondescript, firm yellowish nodules 1-3 cm in size with a high incidence, 65-85%, in the dorsolateral tongue. Histological description is of polygonal cells with dense hyperchromatic nuclei and cytoplasmic eosinophilic granules arranged in sheets with pseudoepitheliomatous hyperplasia. GCTs are thought to have a neural crest origin due to expression of S100 and SOX10 proteins, but the phenotype has not been characterized in detail. Our lab has recently shown that GCTs, express HLA-DR an antigen presenting protein. 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 (APC) 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, and neural markers S100, NSE and SOX10. 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 6/11 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 were inconsistent with the immunohistochemistry complicating interpretation of the results.

Conclusions: Our results show evidence suggesting a phagocytic phenotype for the cells in GCTs, which has not been previously explored. Further work is required to determine the cause of the discrepancy between the immunohistochemistry and the RT-PCR findings.

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

Review of departmental usage of immunohistochemistry and special stains over a 3 year period.

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Introduction: Every year, the number of tissue biopsies and resection cases for surgical pathology evaluation are on the rise. In addition to it, the complexity of individual cases adds to the workload of a surgical pathologist. With increasing expectations to dissipate more information on smaller amounts of tissue, comes the need for increase in ancillary testing, particularly immunohistochemistry (IHC). IHC helps in the diagnosis and in prognostication of certain cancer cases. The process of immunohistochemistry involves selective identification of antigens (proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. We extracted data pertaining to our IHC and special stains utilization over a 3 year period from 2015 to 2017. We intend to review the trends within the same individual (intraobserver) and compare it to our colleagues with similar daily practice (interobserver).

Observation & Conclusion: There was variation among pathologists between 2015-2016, however, in 2017 it was found that in most pathologists practice, the amount of IHC and Special Stain utilization had increased. Through this data, we were able to identify each pathologist's ordering manner pertaining to the subspecialty they practice. Overall we were able to come away with two conclusions. The amount of IHC and Special Stains had increased over a 3 year period and that certain pathologists practice with the use of IHC and Special Stains more frequently than others.

Keywords: Immunohistochemistry, Special Stain

Viral Vector Therapy as a Therapeutic Option for Peripheral Nerve Disease Associated with Metachromatic Leukodystrophy.

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Introduction: Metachromatic leukodystrophy (MLD) is an autosomal recessive lipidosis caused by the deficiency of the lysosomal enzyme arylsulfatase A (ARSA) and can be considered a lysosomal storage disorder (LSD) with predominant neurological involvement. The enzymatic defect results in the accumulation of the ARSA substrate galactosylceramide I3-sulfate (sulfatide), a major sphingolipid of myelin. The disease is characterized by myelin degeneration in both the CNS and peripheral nervous system (PNS), associated with the accumulation of sulfatide in glial cells and neurons. Even though the enzymatic deficiency is systemic, disease manifestations are restricted to the nervous system. Children affected by MLD display progressive neurologic symptoms, including ataxia, seizures, and quadriplegia, culminating in decerebration and eventual death in early childhood. There are currently no treatments for MLD. Experimental treatments that have been tested such as HSCT and CNS gene therapy mainly target the CNS with no impact on the PNS.

Methods: ARSA^{-/-} and WT mice at 6 months of age were injected vial tail vein with AAV-9 or AAVrh10 viral vectors carrying either the ARSA or GFP transgene. Tissues were harvested from mice 3-6 months following injection. Histopathology and PCR analysis were performed on mice treated with GFP. Tissue collection and lipid analysis were performed on mice treated with ARSA.

Results: In the present study, we compared single stranded (ss) AAV serotypes- AAV-9 and AAVrh10, packaged with a codon-optimized ARSA or eGFP. The vectors were delivered via tail vein injection to assess access to the PNS in ARSA^{-/-} mice at 6 months of age at a dose of 2x10E10 vector copies / grams per mouse. The gene transfer facilitated both GFP bio distribution (measured via PCR and histopathology) and ARSA bio distribution (measured via HPLC) along with detectable enzymatic activity throughout the sciatic nerve, spinal cord and liver.

Discussion and Conclusion: In summary, we demonstrate for the first time that tail vein injection of AAV-9 and AAVrh10 to an ARSA^{-/-} mouse as a treatment strategy for MLD is successfully able to target the PNS and significantly decrease sulfatide accumulation indicating potential therapeutic benefit.

Keywords: metachromatic leukodystrophy, gene therapy, arylsulfatase A, viral vector, AAV, peripheral nervous system

Characterization of microvasculature in Duchenne Muscular Dystrophy

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Background: Duchenne muscular dystrophy (DMD) is a fatal x-linked recessive neuromuscular disease characterized by progressive muscular degeneration caused by loss-of-function mutation in the protein dystrophin. Angiogenesis, the process of new blood vessel formation from preexisting ones, has been shown to be dysfunctional in DMD, leading to ischemia, inflammation, and fibrosis. Our lab has shown that angiopoietin-1 (Ang-1), a pericyte angiogenic factor, alone is capable of restoring angiogenic function. However, endogenous levels of Ang-1 has been shown to be significantly lower in severely fibrotic tissue than in healthy wild type mice. Moreover, the expression of Tie 2, the receptor tyrosine kinase for Ang-1, has not been characterized in DMD. Lastly, while the interaction between Ang-1/Tie-2 interaction is shown to be angiogenic, the Ang-2/Tie-2 antagonistic interaction is pro-inflammatory.

Hypothesis: We hypothesize elevated pro-inflammatory factors in DMD skeletal muscle results in abnormal Angiopoietin/Tie-2 signaling, shifting from an Ang-1/Tie-2 interaction to Ang-2/Tie-2 interaction as disease severity progresses.

Materials and Methods: Muscle tissues (gastrocnemius and diaphragm) from each of our 3 DMD mouse models that display weak, intermediate, and severe disease phenotypes, will be collected prior to and after their development of overt fibrosis. Immunoblots will be used to assess the expression of Angiopoietin/Tie-2 factors, pericyte markers, secreted proinflammatory markers, and endothelial inflammatory markers. H&E and Masson's Trichrome staining will be performed to assess the extent of muscle degeneration/regeneration and fibrosis. Primary fibroblast and endothelial cell cultures from previously mentioned muscle types will be subjected to exogenous proinflammatory cytokine mixes. Western blots and immunocytochemistry will be used to assess gene expression alterations within the cell cultures.

Discussion: Characterization of the vasculature will allow us to evaluate an optimal window of opportunity to introduce vascular-targeted therapies, thus, correcting vascular deficiencies. We are currently working with Akkrivis to develop a vascular delivery platform.

Glucose Modulates Transforming Growth Factor- β Signalling in Bone Marrow-derived Progenitor Cells to Enhance Adipogenic Differentiation

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Introduction: Enhanced marrow adiposity and skeletal fragility are common consequences of diabetes mellitus, a prevalent disease characterized by hyperglycemia. We have shown that high glucose conditions enhance adipogenic differentiation of bone marrow-derived progenitor cells while impairing osteogenic differentiation. To understand this mechanism of skewed differentiation towards adipocytes, I screened various signaling pathways and identified transforming growth factor- β 1 (TGF- β 1) pathway as a potential regulator. Moreover, we have shown that vascular dysfunction and inadequate repair in diabetes entail vasculogenic impairment due to the depletion of regenerative stem cells in the bone marrow. Hence, I hypothesize that hyperglycemia alters TGF- β 1 signalling to favour adipogenesis and suppress osteogenesis in the bone marrow, which alters the stem cell niche, ultimately depleting regenerative stem cells.

Methods: I challenged primary human bone marrow-derived cells with TGF- β 1 in adipogenic induction media to assess for cellular and molecular alterations. To further dissect the intracellular signalling proteins mediating the changes by TGF- β 1, I exposed the cells with various inhibitors of downstream proteins of the TGF- β pathway.

Results: My results show that marrow cells directed to differentiate into adipocytes activate canonical SMAD1/5. Exposure to TGF- β 1 normalizes SMAD1/5 activation and suppresses adipogenic differentiation of marrow cells. Interestingly, TAK1 has been previously reported to negatively regulate SMAD1/5 and inhibit precocious differentiation. In support of these findings, I show that the inhibition of non-canonical TAK1-JNK axis reverses the effect of TGF- β 1 and normalizes adipogenic differentiation.

Discussion: My findings show that restoring TGF- β 1 signalling activates TAK1-JNK axis to possibly maintain a precursor phenotype in marrow cells. In contrast, the induction of SMAD1/5 may play a role in adipogenic differentiation. Our studies suggest that diabetes may fine-tune the balance between canonical and non-canonical TGF- β pathways in marrow cells to favour adipogenic differentiation.

Keywords: TGF- β 1, adipogenesis, differentiation, bone marrow-derived progenitor cells, diabetes, high glucose

Reconstitution of Human Mitochondrial Calcium Uniporter (MCU) Activity in Bacteria to Evaluate Cancer-Linked Mutations

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Introduction: The mitochondrial calcium uniporter (MCU) is the core protein component that mediates calcium uptake into mitochondria. Functional MCU requires association with essential MCU regulator (EMRE), a protein whose topology in the mitochondrial membrane has yet to be resolved. Previous work has attributed aberrant MCU function to apoptosis-resistance and the development of cancer. Specifically, amino acid substitutions D147N and D148Y in the matrix-oriented N-terminal domain of MCU have been linked to squamous cell carcinoma and adenocarcinoma respectively. We hypothesize that EMRE is oriented with the N- and C-termini in the matrix and intermembrane space respectively, and cancer-associated mutations at the D147 and D148 residues of the MCU N-terminal domain inhibit MCU function.

Methods: To resolve EMRE's membrane topology, we engineered two MCU/EMRE fusion constructs into a pET-28a-MCU bacterial protein expression vector and expressed these fusions in *Escherichia coli*. We then assessed calcium uptake in *E. coli* separately expressing the two MCU/EMRE fusions using Fura-2-acetoxymethyl ester (Fura-2-AM) ratiometric calcium spectroscopy. PCR-mediated site-directed mutagenesis will be used to introduce cancer-related D147N and D148Y mutations into the functional MCU/EMRE fusion construct.

Results: We have established the Fura-2 loading and calcium uptake assessment protocol for *E. coli*. We found that cells expressing the EMRE-MCU fusion were highly sensitive to MCU-specific inhibition by the small molecule ruthenium-360. We anticipate that the introduction of D147N and D148Y mutations into EMRE-MCU will inhibit MCU channel function, thereby decreasing calcium uptake into *E. coli*.

Discussion: We have established a simple system for the measurement of human MCU activity in the absence of confounding post-translational modifications and expression of negative MCU regulators. Our data suggests that EMRE is topologically oriented with the C-terminus in the matrix. Our future results will reveal whether the D147 and D148 cancer-related mutations can contribute to cancer by altering MCU-mediated calcium uptake.

Keywords: mitochondria, calcium, MCU, cancer, apoptosis, EMRE, *Escherichia coli*

Meeting cytogenetic biodosimetry capacity requirements of population-scale radiation exposures with geostatistical sampling

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Introduction: Automated biodosimetry can facilitate accurate and timely triaging of exposed individuals after large scale radiation events. The Automated Dicentric Chromosome Identification and Dose Estimation (ADCI) system determines radiation exposures from cytogenetic data from multiple samples. High throughput ADCI processes 40,000 samples in parallel, comprising 46,196,000 metaphase images, in ~25 hours on a supercomputer. The logistics of acquiring these samples and generating these data far exceeds the capacities of first responders and testing laboratories.

Aim: We sought to reduce sampling and testing requirements while still accurately identifying those eligible for cytokine-based therapies.

Methods: The spatial boundaries of graduated radiation exposures were determined by targeted, multistep geostatistical analysis of small populations. Physical radiation plumes modelled nuclear detonation scenarios of simulated exposures at multiple North American locations. Models assumed only ground-zero locations, historical prevailing wind direction and speeds. Initially, locations proximate to these sites were randomly sampled (0.01% of population). Empirical Bayesian Kriging established radiation dose contour levels circumscribing these sites. Densification of each plume identified critical locations for additional sampling. After repeated Kriging, overlapping grids between each pair of contours of successive plumes were compared based on their diagonal Bray-Curtis distances and root-mean-square deviations, which provided the criterion (<10% difference) to discontinue sampling.

Results/Conclusions: We modeled 46 urban scenarios, including 24 high density/city and 4 rural/low density scenarios under various weather conditions. Multiple (3-7) rounds of sampling and Kriging were required for the biodosimetry maps to converge, requiring testing of between 128 and 417 samples for different scenarios. On average, 74±9% of those eligible for cytokine therapy (with ≥2 Gy exposures) were accurately localized by biodosimetry mapping. Geostatistical sampling limits the number of radiation-exposed individuals requiring laboratory testing, the time required for triage, and radiation exposures of first responders. Treatment for acute radiation exposure will be expedited in population-scale nuclear events.

Keywords: population-scale radiation exposure, individual triage, automated biodosimetry, geostatistical sampling, Kriging, radiation plumes

Regulation of T helper 2 cell Function by Glucocorticoid and Estrogen Receptor Signaling

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Introduction: Allergic asthma is mediated by IL-4, IL-5 and IL-13 and Th2 cells, CD4+ T helper cells expressing chemoattractant receptor-homologous molecule (CRTh2). This type 2 inflammatory response is usually controlled by glucocorticoids (GC), but in women with severe asthma, administration of GC is insufficient to control symptoms. Our lab showed that women with severe asthma have a higher percentage of circulating Th2 cells, compared to women with mild asthma as well as men of either asthma phenotype. Recently, Th2 cells with CD161, and hPGDS, have been shown to be pathogenic Th2 cells (pTh2) and correlate with severity of atopic dermatitis. However, no link between pTh2 cells and asthma has been established. We hypothesize that Th2 cells (CD4+CRTh2+) treated with GC and estrogen, will exhibit increased expression of markers of the pTh2 subset (CD161, hPGDS) as well as other genes that may influence Th2 cell phenotype and/or function.

Methods: Primary Th2 cells were treated with the GC dexamethasone (Dex), an ER alpha agonist (PPT) or both (24 hours). Staining for CRTh2, CD161 and hPGDS was assessed by flow cytometry. Whole genome analysis of the influence of these treatments on Th2 cells was performed by RNA sequencing by genome Quebec, and gene expression was validated by qRT-PCR. Pathway analysis was performed using DAVID.

Results: Th2 cells exposed to Dex/PPT showed an increase in CRTh2 and CD161, at both the mRNA and protein level. RNA sequencing showed this treatment affecting the expression of over 1000 genes, with over 10 pathways altered. A major pathway affected was cytokine-cytokine-receptors.

Discussion: Th2 cells treated with a combination of GC and ER agonist showed an increase in genes of the pTh2 cell subset, as well as other cytokine receptors. This data could suggest that in women, GC treatment may influence Th2 cell function and asthma severity.

Keywords: CRTh2, Glucocorticoids, Estrogen Receptor alpha, Asthma, pathogenic Th2, CD161

A Case of Sudden Unexpected Death in Epilepsy with Diffuse Leptomenigeal Glioneuronal Tumour.

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Diffuse leptomenigeal glioneuronal tumour (DLGT) is a rare neoplasm that occurs mainly in children. Patients more commonly present with signs and symptoms of increased intracranial pressure, owing to the obstructive nature of the tumour. A less common presentation is seizures. We describe a case of a 41-year-old gentleman that presented as a sudden unexpected death in epilepsy (SUDEP). He was known to have a seizure disorder, on medication. Post mortem histological examination confirmed the presence of oligodendroglial-like cells, diffusely infiltrating the leptomeninges and invading the brain parenchyma, with both neuronal and glial immunohistochemical features. The histological, immunohistochemical and molecular features supporting this diagnosis have been reviewed.

Keywords: diffuse leptomenigeal glioneuronal tumour, SUDEP, epilepsy

Development and Implementation of a Mobile Assessment Tool for Pathologists' Assistant Grossing Competency

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Introduction: In recent years, the competency-based medical education (CBME) model has become the popular approach to medical education. At the Schulich School of Medicine and Dentistry, however, the grossing competency assessments of Pathologists' Assistant (PA) students do not align with CBME as they are infrequent, conducted on paper, do not provide feedback, and do not allow for student access. The development of a mobile tool for PA grossing assessment has not been previously described in literature, and such a tool could be utilized to assess the competency of any learner regardless of field. Because of this, we sought to develop and implement a mobile evaluation tool for the assessment of PA student grossing competency.

Methods: To develop our assessment tool, we utilized a mobile tool already in use for the evaluation of pathology residents and adapted the criteria for the assessment of PA students. The tool was then tested by three members of the pathology department to determine speed and ease-of-use, and Dropbox was utilized to share completed assessments.

Results: We have successfully developed and implemented a mobile tool for PA grossing assessment. We were unable to directly alter the resident tool and had to generate a similar tool using alternative software. Because evaluations could not be exported off the iPad, Dropbox was utilized to save and share completed assessments. The tool was determined to be easy to use and only required three minutes to complete an assessment. A set of instructions indicating how to use the tool was also generated to aid in implementation.

Conclusion: Our mobile tool is functional on an iPad, aligns with CBME, and gives students and supervisors the ability to access previous assessments. The tool is efficient and easy to use as it requires approximately three minutes to complete an assessment.

Keywords: competency-based medical education, mobile device, iPad, assessment, pathology, Pathologists' Assistant, grossing

Effects of Impaired Fetal Development on Sepsis: Sex-Differences in Kidneys

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Introduction: Sepsis is a life-threatening condition which stems from a dysregulated host immune response to bacterial infection. Sepsis causes multi-organ dysfunction and is the most common cause of acute kidney injury (AKI). The impact of different host-associated factors on sepsis severity has been investigated, however, the effects of intrauterine growth restriction (IUGR) and gender has not yet been explored. In this study, histologic abnormalities of both females and males in a murine IUGR early AKI sepsis model are characterised.

Methods: Pregnant rats were randomized to one of two isocaloric diets which contained 8% or 20% protein. After weaning, all rats received a 20% protein diet until 130-150 days of age, at which point they received either an intraperitoneal fecal slurry injection to induce sepsis or a sham saline injection. Rats were monitored for 6 hours prior to euthanasia. Blood from the ascending vena cava was obtained and cultured to verify sepsis. Kidneys were weighed before formalin fixation and processed for histologic assessment. H&E-stained tissue sections were scored semi-quantitatively for distribution, location and intensity of histological changes that characterise inflammation, apoptosis and necrosis. Gram stain highlights bacterial burden.

Results: We anticipate that IUGR will lead to an enhanced inflammatory response, but the magnitude will vary between sexes, which will be histologically observable in the kidney.

Discussion: In murine models of IUGR, neonatal kidneys are underdeveloped with fewer nephrons, which could result in long term functional restrictions. Since AKI is a sequelae of sepsis, abnormal renal morphology and/or function could exacerbate disease. Furthermore, sexual dimorphism has been associated with sepsis-associated morbidity and mortality, with improved survival for females. Interestingly, in research, male mouse models are predominant. IUGR as a risk factor for sepsis and its associated sexual dimorphisms has not been explored in this context.

Keywords: sepsis, sexual dimorphisms, intrauterine growth restriction (IUGR), impaired fetal development, acute kidney injury (AKI), histopathology, murine model

The Neuronal Protein Stathmin-2 Regulates Glucagon Secretion from α -Cells

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Introduction: In people with diabetes, dysfunction of pancreatic alpha cells results in hyperglucagonemia which exacerbates hyperglycemia. Thus, inhibition of glucagon hypersecretion from α -cells is an appealing strategy for the treatment of diabetes. Recently, we identified a glucagon interactome within the secretory granules of alpha cell and showed that proteins within the interactome could modulate glucagon secretion. In the present study, we sought to determine key protein(s) within the interactome that modulate glucagon secretion.

Methods: Secreted glucagon interactome from α TC1-6 cells was shown by immunoblotting. Then, protein components of the interactome were determined by liquid chromatography-mass spectrometry. Immunoprecipitation coupled with immunoblotting was used to show interaction between glucagon and stathmin-2 and confocal immunofluorescence microscopy was used to show colocalization. Studies were done in COS-7, PC12, N2a and α TC1-6 cells. Glucagon measurement was done by ELISA.

Results: Two distinct glucagon-immunoreactive bands were secreted from α TC1-6 cells as low molecular weight complex (LMWC) and high molecular weight complex (HMWC). Proteomic analysis of these bands revealed the presence of stathmin-2 in LMWC. Interaction and colocalization of glucagon with stathmin-2 were shown in transfected COS-7, PC12, N2a and α TC1-6 cells as well as native α TC1-6 cells. Depletion of stathmin-2 in α TC1-6 cells drastically reduced both basal and K⁺-stimulated secretion of the LMWC and HMWC. As well, depletion of stathmin-2 in α TC1-6 cells increased secretion of free glucagon and decreased bound glucagon secretion without affecting total glucagon secretion. In contrast, overexpression of stathmin-2 dramatically suppressed secretion of both bound and free glucagon. Over-expression of stathmin-2 increased colocalization of glucagon with early endosomal and late endosomal markers, and depletion of stathmin-2 drastically reduced colocalization.

Conclusions: Stathmin-2 acts as a unique transporter protein for glucagon and modulates glucagon secretion through the endosomal-lysosomal system. We suggest that stathmin-2 is a novel target for the treatment of hyperglucagonemia of diabetes.

Keywords: Alpha cell, glucagon, glucagon hypersecretion, glucagon interactome, stathmin-2, glucagon transport, diabetes

The role of pThr175tau and the N-terminal phosphatase activating domain in tau cytoplasmic inclusion formation

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Introduction: Traumatic brain injury (TBI) is a significant risk factor for the development of various neurodegenerative diseases which share the neuropathological hallmarks of neuritic tangles of the microtubule-associated protein tau, and an elevated activity of glycogen synthase kinase 3 β (GSK3 β) within neurons. Aberrantly phosphorylated pThr175 tau is present in various tauopathies and pseudophosphorylating tau at this residue initiates the formation of cytoplasmic inclusions and an upregulated activity of GSK3 β in vitro. One postulate regarding the mechanism by which pThr175 tau induces increased GSK3 β activity involves the opening of tau protein's native hairpin conformation and exposure of the N-terminal phosphatase activating domain (PAD). Once exposed, the PAD interacts with protein phosphatase 1, which in turn activates GSK3 β . We hypothesize that TBI in rats is associated with changes in kinase activity and the aberrant phosphorylation of tau protein at Thr175, which leads to GSK3 β activation via PAD exposure.

Methods: Sprague Dawley rats were exposed to TBI or sham surgery and were sacrificed at various timepoints following procedure (1d-120d). Tissue was analyzed by immunohistochemistry or kinase analysis. Mutant pseudophosphorylated tau protein constructs lacking the PAD or possessing mutations were utilized to elucidate the role of the PAD in GSK3 β activation.

Results: TBI is associated with the early phosphorylation of tau at Thr175. PAD exposure colocalizes with pThr175 tau, although regions of pThr175 tau without PAD exposure were observed at early timepoints. These epitopes fully colocalized at 60 days post TBI, suggesting that pThr175 tau formation might precede PAD exposure in this model. In vitro, pseudophosphorylated tau protein is associated with increases in PAD exposure and active GSK3 β . PAD deletion resulted in normalization of active GSK3 β levels.

Discussion: Investigating the pathogenesis of tauopathy in these models will identify clinically relevant pharmacological targets, which may be utilized to prevent disability from concussion and TBI.

Keywords: Amyotrophic Lateral Sclerosis with Cognitive Impairment (ALSci), Chronic Traumatic Encephalopathy (CTE), Traumatic Brain Injury (TBI), Tauopathy, GSK3 β

Dysregulation of circRNAs in Amyotrophic Lateral Sclerosis

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease caused by death of motor neurons. Increasing evidence suggests that widespread alterations in RNA metabolism play a critical role in ALS. Circular RNAs (circRNAs) are a class of RNAs highly expressed in animal brains whose role at different cellular levels is starting to be elucidated. The participation of circRNAs in ALS pathogenesis is currently unknown.

Material and methods: We performed whole transcriptome RNA-seq (Illumina, Partek/GSA analysis) of libraries prepared from ribosome-depleted RNA from human spinal cord tissues to investigate alteration of circRNAs in sporadic ALS patients (sALS) compared to matched controls. Further experiments including quantitative real-time PCR, fluorescence in situ hybridization and northern blotting to validate RNA-seq data and study cell distribution of circRNAs, as well as functional analysis using in vivo and in vitro models will be performed to investigate the role of specific circRNAs in ALS disease.

Result: Using RNA-seq and improved bioinformatic stratification, a total of 52,130 circRNAs were detected in human spinal cord, 25.7% of which were dysregulated in sALS patients. Differential expression analysis revealed that 23.5% of total circRNAs were upregulated while only 2.3% were downregulated. Applying selection based on fold change ± 3 lead to 30 circRNAs, some of which are encoded in genes associated with ALS pathogenesis. Further bioinformatic analysis of these dysregulated circRNAs indicated they potentially have interactions with important RNA-binding proteins linked to ALS.

Conclusion: In summary, our data suggest the importance of extending the knowledge on transcriptome alterations in ALS, specifically of circRNAs, to increase our understanding of ALS disease.

Keywords: Amyotrophic lateral sclerosis, spinal cord, circular RNAs, transcriptome RNA-sequencing

Temperature-Specific Surfactin Production and Biocontrol Potential of *Bacillus methylotrophicus* 1B-23

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Introduction: Biological control (Biocontrol) represents an ecologically-friendly alternative to chemical pesticides to prevent agricultural disease. Species of the *Bacillus* genus are commonly touted for their biocontrol potential, as they produce a wide range of antimicrobial products, including the secondary metabolite, lipopeptide biosurfactant, surfactin. We hypothesize that Canadian strain, *Bacillus methylotrophicus* 1B-23 will effectively inhibit selected plant pathogens and that the temperature of *B. methylotrophicus* 1B-23 growth conditions will alter surfactin production.

Methods: Here, we investigated *B. methylotrophicus* 1B-23 for its biocontrol capability against several bacterial and fungal plant pathogens qualitatively, in vitro, and determined surfactin's relative contribution. We tested the effect of temperature on surfactin production by *B. methylotrophicus* 1B-23, semi-quantitatively, using HPLC-MS. Lastly, to explain temperature differential surfactin production, we modeled growth and surfactin kinetics by measuring cell density and surfactin production each day, over a seven-day period, at selected temperatures.

Results: Our results show that *B. methylotrophicus* 1B-23 effectively inhibited the majority of the tested plant pathogens. We confirmed surfactin's strong antibacterial effect, with modest antifungal activity. We showed that this strain optimally produces surfactin at lower temperatures than many other characterized *Bacillus* strains. Lastly, we demonstrated how this strain's growth kinetics and surfactin production over time differ by growth temperature.

Discussion: These findings suggest that this unique, Canadian strain of *Bacillus* could be used as a biocontrol agent. We, for the first time in *B. methylotrophicus*, showed that surfactin production was optimal at lower temperatures and independent of cell culture growth. This work gives researchers insight on optimal incubation temperature for surfactin production and purification in *B. methylotrophicus*. Given surfactin's role in biofilm formation and biocontrol, taken together with these data, we suggest potential use for *B. methylotrophicus* 1B-23 as biocontrol that can be applied earlier in season or in colder regions, than other biocontrol strains.

Keywords: Biocontrol, Biological control, *Bacillus methylotrophicus*, surfactin, biosurfactant, surfactin production

Safety and effectiveness of cardiac rehabilitation delivered in non-hospital settings by non-physician healthcare professionals

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Introduction: Cardiac rehabilitation is designed to improve cardiovascular health following a cardiac event through behavioral changes, improved cardiorespiratory fitness, and cardiovascular disease risk factor control. Despite the well-established benefits, including cost-effectiveness, participation in cardiac rehabilitation programs is very low. Current cardiac rehabilitation programs tend to be located in hospitals where there is direct supervision by a physician. This hospital-based delivery of care is seen by some as contributing to barriers to care and have resulted in recommendations, by some, for community-based care models. The purpose of this study is to conduct a scoping review to assess the literature for the safety and effectiveness of cardiac rehabilitation delivered in non-hospital settings by non-physician healthcare professionals.

Methods: The search was executed in three databases: PubMed, CINAHL, and Scopus. The search strategy was designed to be comprehensive, and it consisted of a range of different MeSH terms and keywords. The initial database search without any limits returned 455 studies in PubMed, 203 studies in CINAHL, and 271 studies in Scopus. Once the limits of English language as well as studies published after January 1st, 1995 were implemented in the search, the results were narrowed down to 281 in PubMed, 183 in CINAHL, and 216 in Scopus.

Results: Once the duplicates between the 3 databases were removed using Zotero, the remaining 522 studies were imported into Abtrackr to begin the screening process. It is anticipated that the results will show that there is no difference in the safety and effectiveness of cardiac rehabilitation delivered in non-hospital settings by non-physician healthcare professionals compared to cardiac rehabilitation delivered in hospital settings by physicians.

Discussion: The findings of this review will help determine if non-physician supervised cardiac rehabilitation can be safely implemented in communities to help remove participation barriers and ultimately attempt to increase participation rates.

Keywords: cardiac rehabilitation, safety, effectiveness, non-physician, risk, non-hospital setting

Post-Mortem Microbiological Analysis of Sudden Infant Death Syndrome

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Background: Despite medical and diagnostic advances in the past 20 years, clinical patterns of Sudden Unexpected Death in Infancy (SUDI) remain relatively unknown and the death rate of SIDS globally has remained constant. Many theories, such as the “Triple-Risk Hypothesis” do not explain all cases of SUDI and fail to incorporate pathogenic exposure, whereas the validity of other theories, such as the “Common Bacterial Hypothesis” are hotly debated. Examining autopsies of SUDI cases may be beneficial in understanding epidemiological “risk-factors” as well as develop a better understanding of the etiology and sequelae of SUDI. It is hypothesized that pathogenic exposure are significant contributors to death in SUDI.

Methods and Design: A retrospective analysis of 150 cases (from the period of 2011-2018) of SUDI patients will be conducted. Autopsy reports are strictly from the Southwestern-Ontario region, and data collection will be completed in the Department of Pathology at the London Health Sciences Centre. Demographic information (gender, age, height/weight), medical history (non-specific/specific symptoms), co-morbid conditions, histopathological findings, and ancillary microbiological findings are the measures in question and will be analyzed qualitatively.

Predicted Results: It is anticipated that infections play a significant role in SUDI, with viral infections being statistically more significant with bacteria. Furthermore, it is anticipated that samples from respiratory pathways (tracheal, nasopharyngeal, lung) will be statistically significant compared to other samples (cerebrospinal fluid, blood, heart). It is also predicted that there will be a pattern of demographic trends, histopathological findings and other clinical epidemiological factors amongst SUDI cases.

Significance: It is the goal of this study to narrow future avenues of research with the hopes of eventually improving detection of at-risk infants. In doing so, new diagnostic techniques and treatment plans may be developed in the future to decrease the global rate of SUDI, as well as updating old theories.

Keywords: Sudden Unexpected Death in Infancy (SUDI), Sudden Infant Death Syndrome (SIDS), Sudden Unexpected Death in Childhood (SUDC), Infection, Bacteriology, Virology, Autopsy, Epidemiology, Post-mortem analysis

The Impact of Intraoperative Consultation on Patient Management at London Health Sciences Centre

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Introduction: Intraoperative consultations (IOCs) aim to answer specific questions in order to direct surgery. Depending on IOC results, surgery may be continued, modified or terminated. Communication errors are a recognised pitfall of IOC. This study analyzes the rationale for, communication of, and impact on patient management of intraoperative consultation (IOC) at our institution.

Methods: The electronic database at London Health Sciences Centre (LHSC) was searched for IOCs performed during the first half of 2016. Operative and pathology reports were compared to determine the reason for IOC, who received the report, whether the pathologist and the surgeon were face to face, whether the pathology was accurately recorded in the operative report and whether IOC impacted patient management.

Results: A total of 1186 IOCs were performed. The most common reason was margin assessment (54.6%), followed by tissue diagnosis (23.7%). The pathology report was communicated to the surgeon in 87.2% of cases; the remainder to residents/fellows and nurses. The surgeon was present in the IOC room for only 0.7% of cases. The pathology was not mentioned in the operative report in 10.9% of cases and, when mentioned, was inaccurate in 13% of cases. There was an identifiable impact of IOC on patient management in 86% of cases.

Discussion: This study demonstrates that IOCs had a direct impact on 86% of cases; however, impact could not be determined in 10.9% as there was no mention of the pathology in the operative notes, and the pathology was misinterpreted in 13% of cases. This prompts a recommendation for improved documentation of the reason for IOC, and how it would (and did) affect patient management. If the surgeon is not present in the IOC room, getting them to read back the report and outline their management plan may reduce misunderstanding. Setting expectations for frozen section communication and documentation may improve transparency and reduce communication errors.

Keywords: frozen section, intraoperative consultation, patient management, communication, report, surgery, pathology, recommendations

CRISPR-based Approach for Identification of Novel Genes Essential for Spheroid Dormancy in Epithelial Ovarian Cancer

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Introduction: Epithelial ovarian cancer (EOC) forms multicellular clusters called spheroids that exfoliate from the primary tumor and disseminate via ascites fluid to seed secondary tumor deposits in the abdominal cavity. Withdrawal from cell cycle and dormancy are central to the nature of EOC spheroid cells and confer resistance to standard chemotherapy resulting in high treatment failure rate. Previously, we conducted a genome-wide CRISPR knockout screen in two ovarian cancer cell lines, iOvCa147 and OVCAR8, to identify genes required for EOC spheroid dormancy. Our screen revealed 2739 genes that are commonly essential for spheroid viability in both cell lines. Gene ontology analysis of these identified genes showed enrichment of Wnt/ β -catenin pathway. Given the role of Wnt/ β -catenin pathway in cell proliferation and apoptosis, we hypothesize that it can be implicated in ovarian cancer carcinogenesis.

Methods: To test this hypothesis, individual components of Wnt/ β -catenin pathway enriched in the genome-wide CRISPR screen will be knocked-out by sgRNAs in a panel of ovarian cancer cell lines. The effect of a specific gene knockout on spheroid cell survival in these cell lines will be assessed using cell viability assays. Additionally, a CRISPR mini-screen will be conducted to validate and narrow the list of 2739 genes found to be essential for EOC spheroids in the primary genome-wide screen.

Expected Results: Our preliminary analysis showed that Wnt/ β -catenin pathway is implicated in dormancy and survival of EOC spheroids. Using CRISPR/Cas9 based approach we expect to establish the role of individual components of this pathway in EOC carcinogenesis.

Discussion: Understanding the mechanisms involved in spheroid dormancy, with the goal of inhibiting this process, may offer more efficient therapeutic targets for EOC treatment. In this study we seek to establish the significance of Wnt/ β -catenin pathway in ovarian cancer using CRISPR/Cas9 based approach.

Keywords: EOC, spheroid, dormancy, CRISPR/Cas9, β -catenin dependent Wnt pathway

Retrospective review of CSF White Blood Cell Count and Viral PCR Results. A Laboratory Utilization Initiative.

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Introduction: Herpes simplex virus (HSV), varicella zoster virus (VZV) and enterovirus are common causes of meningitis and encephalitis. PCR testing for these viruses is commonly performed for patients presenting with headache/neck stiffness or decreased level of consciousness. However, when the cerebral spinal fluid (CSF) white blood cell (WBC) count is normal ($\leq 5 \times 10^6/L$) an infectious etiology is unlikely. Our objective was to determine that if the CSF WBC is normal the viral PCR result will be negative, except in those individuals which are immunocompromised.

Methods: LHSC CSF PCR tests for HSV/VZV/enterovirus from June to November, 2018 were included. Patient charts were reviewed to collect age, gender, immunocompromised status (HIV, transplant), imaging results and CSF WBC. This data was analyzed to determine whether normal CSF WBC corresponded to negative viral PCR results.

Results: A total of 233 patients were identified, with 254 viral PCR tests performed (21 repeat samples). There were 123 males and 110 female patients. A total of 67 patients were <18 years of age. Adult patients had an average age of 56 years. Viral PCR testing results were positive for: Enterovirus (8/101), HSV (4/220) and VZV (8/220). All patients positive for enterovirus had an abnormal WBC. All adult immunocompetent patients with a positive HSV/VZV result had an abnormal CSF, with the exception of one patient positive for VZV, with an active shingles outbreak.

Conclusion: Our study findings support that routine viral PCR testing is not indicated for adult immunocompetent patients with a normal CSF WBC. A laboratory utilization initiative to eliminate this testing would result in up to 80 fewer HSV/VZV tests and 85 fewer enterovirus tests, during this 5 month study period.

Keywords: PCR, meningitis, encephalitis, Herpes simplex virus, quality improvement, cerebral spinal fluid

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