PROGRAM GUIDE

PATHOLOGY AND LABORATORY MEDICINE **RESEARCH DAY 2018**









PATHOLOGY AND LABORATORY MEDICINE **RESEARCH DAY 2018**



Our Pathology and Laboratory Medicine Research Day is one of the most rewarding days of the academic year. We dedicate this day to celebrating our research accomplishments and our people. All of our trainees in various programs including undergraduate programs, thesis-based graduate studies, professional graduate program, dual Oral and Maxillofacial Surgery MSc program, and Pathology postgraduate programs are presenting their research work. I would like to congratulate all the talented presenters for a fantastic job in representing the Department of Pathology and Laboratory Medicine. We have set yet another record with 84 research presentations this year. This is certainly an amazing achievement for our small but mighty department. I hope that you realize our truly multidisciplinary approach to studying health and disease as you listen to the platform presentations and view the posters.

We welcome Dr. Harold Atkins to our Research Day as the Keynote speaker. Dr. Atkins is a physician of the Ottawa Hospital Blood and Marrow Transplant Program, an Associate Professor of Medicine at the University of Ottawa, a scientist in the Center for Innovative Cancer Research and the medical director of the Regenerative Medicine Program at the Ottawa Hospital Research Institute. He specializes in hematopoietic stem cell transplantation and has spearheaded the use of stem cell transplantation for immune repair to treat patients with severe autoimmune diseases of the nervous system focusing on Multiple Sclerosis. Dr. Atkins has received numerous awards for his pioneering work, including the 2016 Chrétien Researcher of the Year Award and the 2017 Till & McCulloch Award.

The organizing committee and 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, Tracey Koning, Cheryl Campbell, Jina Kum, Vy Ngo, Sandy Rattana, and Mellonie Carnahan. Lastly, I would like to thank the judges for interacting with our presenters, sharing their valuable experience, and offering insights. I hope you enjoy the day and learn about the fantastic research being carried out in our department.

Subrata Chakrabarti, MBBS, PhD, FRCP(C)

Chair, Department of Pathology and Laboratory Medicine, Schulich Medicine & Dentistry, Western University Chief, Department of Pathology and Laboratory Medicine, London Health Sciences Centre and St. Joseph's Health Care

9:00 - 9:15 am	Welcome and Opening Remarks Dr. Subrata Chakrabarti Chair/Chief, Pathology and Laboratory Medicine, Schulich School of Medicine & Dentistry, Western University and London Health Sciences Centre	
9:15 - 10:15 am	Keynote Address Dr. Harold Atkins Scientist, Cancer Therapeutics Program, Ottawa Hospital Research Institute	
10:15 – 10:30	Nutritional Break	
10:30 - 12:00	Platform Presentations	
12:00 - 12:15	Group Photo	
12:15 - 1:00	Lunch The Great Hall, Somerville House	
1:00 – 2:00	Poster Presentation - Session 1	
2:30 – 3:30	Poster Presentation - Session 2	
3:30 - 3:45	CME Evaluation	
3:45 - 4:00	Travel to Windermere Manor	
4:00 - 7:00	Awards Ceremony Windermere Manor	
Overarching Learning Objectiv	es:	
Pathology and Laboratory Me	athology research conducted in the Department of dicine at Western University and the London Health les and translate knowledge into practice, teaching,	
and clinical science research	tools and expertise are available to facilitate basic at the Department of Pathology and Laboratory and the London Health Sciences Centre.	
	in the pathogenesis of acute and chronic diseases.	
Maintenance of Certification	roup Learning Activity (Section 1) as defined by the program of The Royal College of Physicians and d by Continuing Professional Development, Schulich	

School of Medicine & Dentistry, Western University (4.5 hours). Each participant should claim only those hours of credit that he/she actually spent participating in the education program. 25% of this program is dedicated to participant interaction.

This program has no commercial support.

NOTES

Time	Last Name	First Name	Title
10:30 am	Langdon	Kristopher	Executive dysfunction and altered cerebrovascular activity in a rodent model of vascular cognitive impairment
10:45 am	Ruicci	Kara	TAM receptors activate P90RSK/mTORC1 signalling to mediate acquired resistance to PI3K inhibition in head and neck squamous cell carcinoma
11:00 am	Cecchini	Matthew	Identification of Unique Gene Expression Profiles in Usual Interstitial Pneumonia and Non- specific Interstitial Pneumonia
11:15 am	Shin	Alice	Role of inflammation in Dclk1+ cell-derived colitis-associated colon cancer
11:30 am	Kum	Jina	Glucose Modulates Transforming Growth Factor-β Signalling in Bone Marrow-Derived Progenitor Cells to Enhance Adipogenic Differentiation
11:45 am	Tran	Christopher	Assessing the Characteristics and Turnaround Times for "Rush" Pathology Specimens: A Descriptive Analysis

Executive dysfunction and altered cerebrovascular activity in a rodent model of vascular cognitive impairment

Kristopher D. Langdon^a, Chris Cordova^b, Shirley Granter-Button^b, Jamie Boyd^c, James Peeling^d, Timothy Murphy^c, Dale Corbett^e

¹Department of Pathology and Laboratory Medicine, Western University/ London Health Sciences Centre

- ² Memorial University, Faculty of Medicine
- ³ University of British Columbia, Psychiatry

⁴ University of Manitoba College of Medicine, Radiology

⁵ University of Ottawa, Department of Cellular and Molecular Medicine

Abstract: Most basic science research has focused on overt stroke caused by blockage of major blood vessels. Less attention has been paid to small vessel disease giving rise to covert stroke that often leads to vascular cognitive impairment (VCI). One reason for this may be the relative lack of relevant animal models. This talk will describe a model of VCI induced in middleaged Sprague-Dawley rats exposed to a diet high in saturated fats, salt and refined sugar (HFSS). In Experiment 1, rats fed HFSS and subjected to a small mediodorsal (MD) thalamic stroke with or without concomitant cerebral hypoperfusion experienced significant executive dysfunction. In Experiment 2, dietary influences on functional, physiological and anatomical parameters were assessed. We found significant hypertension, blockage of brain microvessels (2-photon microscopy) and white matter atrophy in HFSS diet animals. As in Experiment 1, profound, specific set-shifting executive dysfunction was noted following both small MD infarcts (0.332 mm³) and the HFSS diet. In summary, these data describe a middle-aged animal model of VCI that includes clinicallyrelevant metabolic disturbances and small vessel disease and as such may be helpful in developing new cognitive therapies.

TAM receptors activate P90RSK/mTORC1 signalling to mediate acquired resistance to PI3Ka inhibition in head and neck squamous cell carcinoma

Ruicci KM^{1,2}, Meens J³, Plantinga P¹, Howlett CJ^{1,5}, Ailles L³, Boutros PC⁴, Barrett JW² & Nichols AC^{1,2}

¹Dept. of Pathology & Laboratory Medicine, Western University, London ON ²Dept. of Otolaryngology – Head & Neck Surgery, Western University, London ON

- ³ Division of Stem Cell and Developmental Biology, Ontario Institute for Cancer Research, Toronto ON
- ⁴ Informatics & Biocomputing Platform, Ontario Institute for Cancer Research, Toronto ON

Introduction: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, with an incidence of >600 000 cases per year and a 50% mortality rate. PIK3CA—which encodes the alpha (α) isoform of PI3-kinase—is the most frequently altered actionable target in HNSCC. Small-molecule PI3K α inhibitors are under active investigation and have shown promise in clinical trials; however, the development of resistance limits the utility of these agents over time. In the present study, we aimed to elucidate mechanisms of acquired resistance to the PI3K α inhibitor Alpelisib (BYL719) in HNSCC, in order to identify potential secondary therapeutic targets.

Methods: We developed multiple BYL719-resistant HNSCC cell lines by escalating drug treatment over time. Simultaneously, we established multiple patient derived xenograft (PDX) models of acquired drug resistance by treating xenografts with BYL719 (50mg/kg) for a prolonged (>100 days) period. Using reverse phase protein arrays (RPPA) we examined differentially-expressed proteins and phospho-proteins and validated these findings in vitro using immunoblotting, flow cytometry and quantitative real-time PCR (qRT-PCR).

Results: Over time, both cell line and PDX models acquired resistance to BYL719, highlighting the likelihood of this phenomenon when PI3K α inhibitors are used clinically. We identified TAM family receptor tyrosine kinases (RTKs) Tyro3, AxI and Mertk to be upregulated in BYL719-resistant models. Downstream, we observed hyper-activation of ERK/P90RSK signalling, leading to sustained, PI3K-indpendent mTORC1 activation and cell survival.

Discussion: Our findings highlight novel targets for either combinatorial, or second-line therapies that may prevent or delay resistance to PI3K inhibition. TAM and P90RSK inhibitors are available and some are used clinically. Examining the efficacy of such therapeutics pre-clinically may indicate their utility for HNSCC treatment.

Identification of Unique Gene Expression Profiles in Usual Interstitial Pneumonia and Non-specific Interstitial Pneumonia

Matthew J. Cecchini¹, Karishma Hosein², Christopher J. Howlett¹, Mariamma Joseph¹, Marco Mura^{2,3}

¹Department of Pathology, Western University, London, Canada ² Division of Respirology, Western University, London, Canada ³ Toronto Lung Transplant Program, University of Toronto, Toronto, Ontario

Introduction: Usual interstitial pneumonia (UIP) and non-specific interstitial pneumonia (NSIP) are common forms of interstitial lung disease (ILD). Recent studies have identified potential links between UIP and senescence. Senescence is a form of irreversible cell cycle arrest that can be caused by DNA damage, shortened telomeres or oncogene activation. Senescent fibroblasts express p16 and can develop a senescence-associated secretory phenotype (SASP) to produce profibrotic growth factors and matrix remodeling proteins.

Design: RNA was extracted and hybridized to the Human Gene 1.0 set array (Affymetrix) from explanted lungs in 22 patients with definitive UIP pattern in the native lungs and typical CT features of UIP, 10 subjects with histological pattern of NSIP, 5 with mixed UIP-NSIP pattern, and 11 normal controls. Differentially expressed genes were identified based on a fold change \geq 1.5 and a q value (false discovery ratio) <0.05. Immunohistochemistry for p16, p53, Periostin and IDO1 was performed on surgical lung biopsies from 22 UIP, 9 NSIP and 5 mixed cases.

Results: UIP cases showed a unique expression pattern with an increased expression of ACTA-2, MUC5B and IGFBP-5 compared to the NSIP cases. Immunohistochemistry identified p16 positive fibroblastic foci in 15 of 23 (65%) of UIP cases, 0 of 9 (0%) NSIP cases and 2 of 5 (40%) mixed cases. P16 fibroblastic foci were also positive for p53 by immunohistochemistry. No difference in Periostin or IDO1 was identified by immunohistochemistry.

Conclusion: UIP cases expressed a distinct subset of growth factors and matrix remodeling genes. P16 positive fibroblastic foci were identified in the majority of UIP cases and highlights a potential important etiological basis for senescence in UIP and supports the ongoing development of senolytic therapies to disrupt this process. Further, there is a potential use for p16 as an adjunct marker to distinguish UIP from NSIP.

PLATFORM PRESENTATION #2

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

Alice E. Shin¹, Elena N. Fazio¹, Hayley Good¹, Liyue Zhang¹, Philip M. Sherman^{2,3}, Timothy C. Wang⁴, Samuel Asfaha¹

- ¹Department of Medicine, Department of Pathology and Laboratory Medicine, Schulich School of Medicine & Dentistry, Western University; Lawson Health Research Institute, London Regional Cancer Program, London, ON, Canada
- ² Cell Biology Program, Research Institute, Division of Gastroenterology, Hepatology and Nutrition, Hospital for Sick Children, Toronto, ON, Canada
- ³ Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, ON, Canada
- ⁴ Division of Digestive and Liver Diseases, Department of Medicine, Columbia University Medical Center, New York, NY, United States

Introduction: Colorectal cancer (CRC) is the second leading cause of cancer death in Canada, with the major risk factor being chronic inflammation. However, how inflammation leads to cancer is not well understood. Our recent work has focused on a colonic epithelial cell known as the tuft cell demarked by doublecortin-like kinase 1 (Dclk1). We previously showed that Dclk1 labels long-lived quiescent cells in the colon that serve as a cellular origin of CRC upon dextran sulfate sodium (DSS)-induced inflammation-induced tumor promotion and explore the mechanism by which inflammation induces tuft cell cancer initiation. We hypothesize that colonic inflammatory insults lead to dedifferentiation of Dclk1+ tuft cells to a stem cell state susceptible to tumor initiation.

Methods: To investigate the various forms of injury or infection that can activate quiescent tuft cells, we generated tamoxifen-inducible Cre transgenic mice that allow for Dclk1+ cell lineage tracing and cell-specific knock-out of the tumor suppressor adenomatous polyposis coli (APC) (Dclk1/APC^{fl/fl}). Following tamoxifen induction, mice were administered colitis-inducing agents dextran sodium sulfate (DSS), trinitrobenzene sulfonic acid (TNBS), oxazolone or *Citrobacter rodentium*. To examine the role of dedifferentiation in colonic tumor initiation, we ablated intestinal stem cells (ISCs) post DSS-induced colitis in our Dclk1/APC^{fl/fl} tumor model.

Results: Treatment with DSS, TNBS, oxazolone, or *C. rodentium* induced colonic inflammation as detected by significantly increased myeloperoxidase (MPO) activity and histologic analysis. Surprisingly, development of colonic tumors was specific to DSS-induced colitis. Interestingly, ablation of ISCs post coli tis significantly reduced colonic tumors in Dclk1/APC^{11/1} mice.

Discussion: Our data suggests that an inflammatory response unique to DSS colitis results in transformation of Dclk1+ tuft cells and colonic tumor formation, and this appears to be mediated through Lgr5-expressing cells. These findings provide insight into the molecular mechanisms by which Dclk1-derived colonic tumors arise.

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

Jina J.Y. Kum¹, Christopher J. Howlett¹, and Zia A. Khan^{1,2}

- ¹Department of Pathology and Laboratory Medicine, Western University, London ON
- ² Metabolism and Diabetes Research Program, Lawson Health Research Institute, London ON

Introduction: Enhanced marrow adiposity and skeletal fragility are chronic complications of diabetes, a disease characterized by hyperglycemia. We have shown that high glucose conditions enhance adipogenic differentiation of bone marrow-derived progenitor cells (bm-MPCs) while impairing osteoblastogenesis. Additionally, our laboratory has shown that the vascular dysfunction and inadequate repair in diabetes entail vasculogenic impairment due to the depletion of regenerative stem cells in the marrow. These findings suggest that changes in the marrow composition depletes the regenerative stem cells, and may lead to organ dysfunction in diabetes.

Methods: bm-MPCs were induced to differentiate into adipocytes and osteoblasts to identify signaling pathways that drive differentiation. Our gene expression profiles led to the discovery of transforming growth factor- β (TGF- β) signalling pathway in bm-MPC differentiation. Hence, bm-MPCs were challenged with exogenous TGF- β 1 to assess for cellular and molecular alterations in adipogenic and osteogenic induction media.

Results: Primary human bm-MPCs that were induced to differentiate into osteoblast downregulated all ligand and receptors of the TGF- β pathway. However, exposure of bm-MPCs to high glucose levels prevented this suppression, implicating normalization of the TGF- β pathway as a mechanism of inhibited osteogenesis in diabetes. Interestingly, bm-MPCs that were challenged with TGF- β 1 inhibited both adipogenic and osteogenic differentiation. To dissect the intracellular proteins mediating the changes by TGF- β 1, various downstream protein inhibitors of this pathway were used to identify the involvement of the non-canonical TAK1-JNK axis in mediating the effect of TGF- β 1 in bm-MPC adipogenic differentiation.

Discussion: Our findings raise an interesting possibility that TGF- β 1 may prevent cell differentiation by maintaining a precursor phenotype in bm-MPCs. Specifically, high glucose levels may fine-tune TGF- β signalling to affect the canonical and non-canonical pathway balance in bm-MPCs to induce adipogenic differentiation. Future studies will be to determine whether altering this balance can inhibit enhanced adipogenesis and rescue the regenerative stem cells in the marrow.

PLATFORM PRESENTATION #6

Assessing the Characteristics and Turnaround Times for "Rush" Pathology Specimens: A Descriptive Analysis

Christopher Tran¹, Keith Kwan¹, Helen Ettler¹

¹Department of Pathology and Laboratory Medicine, London Health Sciences Centre

Introduction: The London Health Sciences Center (LHSC) Department of Pathology and Laboratory Medicine processes over 57 000 surgical and 14 000 cytology specimens per year. For any of these specimens, a "rush" or STAT request can be made, implying the need for urgent diagnosis. No previous studies have evaluated STAT or "rush" specimens, and the aim of this study was to better understand the characteristics and turnaround times for "rush" specimens.

Methods: The sample included all "rush" request cases between January 1, 2016 and December 31, 2016. Transplant-related, cancelled and duplicate requests were excluded. Temporal-, specimen-, and patient-specific variables were collected for all of the cases. Subgroup analyses for cases that requested a specific date for diagnosis were performed.

Results: A total of 826 cases were included in the analysis. Of these, 740 (89.6%) were surgical specimens, 83 were cytology samples (10.0%), and 3 (0.4%) were Pap smears. The average turnaround time from the date of collection was 3.36 days (SD = 2.75). The most common specimen specialties were GI (31.4%), cytology (10.0%), gynecology (10.1%), and otolaryngology (9.5%). At the time of specimen collection, 54.4% of patients were outpatients, and 45.6% were inpatients. Of the "rush" requests, 264 specified a date required for diagnosis, most of which were collected from outpatient visits (72.3%). The average turnaround time requested was 4.70 days (SD = 3.44), and 82.5% of the requests were signed out by the requested date.

Discussion: A significant proportion of "rush" requests may not impact immediate clinical management. Identifying specimens that require an immediate diagnosis, in contrast to specimens where a diagnosis may be required by a specific date or follow up appointment, may allow more effective triaging of cases. Defining benchmarks for "rush" specimens may also help in evaluating the effectiveness in handling time-sensitive cases.

Poster Session 1 1:00 - 2:00 pm

#	Last Name	First Name	Title
1	Gannavarapu	Srinitya	Targeting hARSA-corrected Autologous Mesenchymal Stem Cells to Treat Sulfatide Accumulation in Metachromatic Leukodystrophy
2	Tai	Felicia	Minimizing Overutilization of Vitamin D Serum Testing within London Health Sciences Centre
3	Aref-Eshghi	Erfan	Molecular Diagnosis of Hereditary Syndromes and Cancer Using Genomic DNA Methylation
4	Schroeder	Peyton	Real World Motor Vehicle Collision Head Injury Risk and Car Seat Use for Children under 8 Years Old
5	Xie	Mary	Evaluation of Blast Cell Percentage in Myelodysplastic Syndrome by Flow Cytometry
6	Di Gregorio	Sonja	Aging as a major modifier of polyglutamine aggregation and toxicity
7	Avino	Mariano	Detecting Low Frequency Variants in Non-Subtype B HIV- 1 Integrase Associated with Drug Resistance in Uganda
8	Ni	Rui	Deletion of CHOP prevents necroptosis in diabetic cardiomyopathy
9	Shi	Thomas	Rate of Atypical Urothelial Cells Diagnosis in 2017 After Implementation of The Paris System for Urinary Cytology

#	Last Name	First Name	Title	#	Last Name	First Name	Title
10	Gan	Ingrid	Mitochondrial membrane permeability plays a critical role for endothelial cell necroptosis and cardiac allograft rejection	19	Hill	Seana	Defining domains of chromatin accessibility in human homologous metaphase chromosomes
11	Liu	Daheng	Transcriptional Alterations Associated with Deteriorated CD8+ T Cell Function in	20	Solomon	Lauren	Estrogen Effects on Th2 cell phenotype: Key to Severe Asthma in Women?
			Cancer-Induced T Cell Exhaustion	21	Glover	Rebecca	Impulsivity in Indigenous Suicide in Ontario, Canada
12	Li	Chu Meng	Cardiac GHSR1a and Ghrelin Expression after Severe Sepsis and Correlation with IL-6, SERCA2a, and ERK1/2	22	Palmer	John	Comparison of gp120 Variable Region Indel Rates Between HIV-1 Subtypes
13	Rogala	Benjamin	Phenotype of granular cell tumors	23	Lenartowicz	Karina	Unfolded protein response induction in the presence of TDP-43 and different metabolic conditions in
14	Caron	Christine	An Investigation into T Cell Mediated Drug				Saccharomyces cerevisiae
15	Rollo	Emily	Hypersensitivity Reactions Best Practices in Online Pathology Education for Nursing Students: A Literature	24	McLean	Lachlan	Evaluating Utility of Protein S100A7 in Predicting Progression of Oral Epithelial Dysplasia
			Review	25	Phillips	Alyshia	Stem Cells in Odontogenic Tissues
16	Zheng	Dong	Prevention of doxorubicin- induced cardiotoxicity by nicotinamide riboside	26	Brockman	Joseph	Nicotinamide Riboside Improves Autophagic Flux in Doxorubicin-Treated
17	Tauro	Michael	Alpha-Synuclein Interferes with Mitochondrial Homeostasis to Cause Toxicity				Cardiomyocytes
18	Harnett	Amber	Identifying and Targeting Cancer Stem Cells in Colorectal Cancer	27	Qamar	Adnan	Altered Mitochondrial Permeability in Ischemia/ Reperfusion Injury Influences Apoptotic and Necroptotic Death Pathways in Endothelial Cells

#	Last Name	First Name	Title	#	L
28	Rumana	Rafiq	Self-Reported Cardiovascular Health, Knowledge, and Behaviours in Western Students	36	S
29	Cao	Ting	Up-regulation of mitochondria- targeted calpain-1 induces dilated heart failure in transgenic mice: an important role of mitochondrial reactive oxygen species	37 38	F
30	Li	Shao Shi	Curious case of Intravascular Large B-Cell lymphoma found on autopsy	39	S
31	Gomes	Janice	Development of a microparticle-based tool as an indicator of dialysis induced injury		
32	Kyle	Emily	Peripheral funisitis caused by Candida albicans: a case report		
33	Gaed	Mena	Non-Cancerous Abnormalities that Could Mimic Prostate Cancer in mpMR Images		
34	Sullivan	Rebecca	The Growth Hormone Secretagogue Receptor, Ghrelin, and Biochemical Signaling Molecules in Human Heart Disease		
35	Dhardwar	Avneet	Perceptions of pathologists, pathologist residents, and pathologist's assistants on non-forensic autopsies: a qualitative study		

#	Last Name	First Name	Title
36	Smith	Nicole	Testicular Hemangioma Mimicking Cystic Dysplasia of the Testis
37	Plantinga	Paul	Histological elucidation of tumour deposits in colorectal adenocarcinoma
38	Lee	Christine	Three case studies of hereditary breast cancers associated with variants of unknown significance: RNA analysis
39	Soparlo	Jeff	S100A7 as a quantitative screening tool for predicting transformation in potentially malignant oral lesions

Poster Session 2 2:30 - 3:30 pm

#	Last Name	First Name	Title
40	Hintermayer	Matthew	Tau protein pathology following traumatic brain injury – a longitudinal investigation
41	Shah	Sundip	The Impact of Surgical Resection Margins on Survival of Patients with Pancreatic Ductal Adenocarcinoma
42	Esmaeili	Mohammad	Two faces of Parkin oxidation and misfolding: gain- and loss-of-function in Parkinson's disease
43	Singh	Ranjit P.	Polysialic Acid Synthesis by Polysialyltransferase ST8SialV in Prostate Cancer: Using Glycans as Novel Anti-Cancer Target
44	Wang	Yi Xuan	Expanding the catalog of differentially compacted loci on homologous chromosomes in the human genome
45	Roes	Michael	Molecular Mechanisms of Resistance to Targeted Therapies in Prostate Cancer
46	Carere	Deanna Alexis	The LHSC Whole Exome Sequencing Study: A Progress Report
47	AlYamany	Basma	Neuropathological changes in a case of adult onset leukodystrophy with axonal spheroids and pigmented glia with CSF1R mutation

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48	Но	Jasper	Machine Learning Prediction of HIV-1 Integrase Drug Exposure History	5	6	Altesha	Ashraf	The Effect of Ischemia Reperfusion Injury on circRNA Expression in th Heart
49	Thomas	Anu	H19 regulates glucose- induced EndMT in chronic diabetic complications	5	7	Olabode	Abayomi	Evidence for a Fragmented Origin of HIV-1/M from a Window Analysis of Near Full-
50	Chadwick	Erika	The Use of CT in Forensic Post Mortem Examination					length Genomes
			to Facilitate Targeted Dissection Methods	5	8	Gong	Jieping	Molecular Interplay of Apoptosis and Necroptosis in
51	Morrison	Matthew	Human tissue kallikreins are aberrantly expressed					Mitochondrial Dysfunction
			in pleomorphic adenoma	5	9	Goebel	Emily	Perceptions of pimping
52	Asadi	Farzad	Heterogenous Glucagon- Immunoreactive Complexes as Novel Regulators of Insulin Secretion					in a pathology residency program – a study using interviews and rich- picture drawings
			500000	6	0	Nirmaiyan	Sahana	Transforming Growth
53	Vijeyakumaran	Meerah	Effect of Estrogen and Glucocorticoid Signaling on Th2 cells – Implications in Severe					Factor Beta 1 Signalling i Bone Marrow Precursor Cells
			Asthma	6	1	Naghibosadat	Maedeh	Characterization and the role of Ghrelin receptor
54	Han	Seongryoung	Wnt Pathway Activation in Development of Carboplatin Resistance in Ovarian Cancer					in the vasculature of Duchenne Muscular Dystrophy
				6	2	Krstic	Milica	The transcriptional
55	Cecchini	Matthew	The Utility of CDX2 Loss as a Prognostic Marker in Stage II Colon Cancer					regulator TBX3 promote progression from non- invasive to invasive brea cancer

#	Last Name	First Name	Title
	Armstrong	James	Acetylsalicylic acid reduces collagen contraction, remodelling and myofibroblast proliferation in subconjunctival tissue mimetic
54	Zhao	Guang-Ju	Partial depletion of regulatory T cells prevents secondary pseudomonas aeruginosa
ō	Peet	Rachel	infection post sepsis by enhancing host inflammatory response Comparing Bone Marrow
6	Ngo	Vy	Staging and Original Lymph Node Pathology in Non-Hodgkin's Lymphoma Nrf2 regulation in the
	Phillips	vy Alyshia	antioxidant response in cancer A Case of Hirschsprung Disease with Total Colonic
58	Kula	Freibe	Aganglionosis and Small Bowel Involvement
5	Kyle	Emily	Models and Peer Teaching as Pedagogical Methods in the Surgical Pathology Laboratory
69	Good	Hayley	The Role of Cyclooxygenase in Colitis- Associated Colon Cancer
0	Veramkovich	Vitali	Characterization of Effects of Growth Differentiation Factor 15 on T Cells

Targeting hARSA-corrected Autologous Mesenchymal Stem Cells to Treat Sulfatide Accumulation in Metachromatic Leukodystrophy

Srinitya Gannavarapu¹, Cathy Regan¹, Stephanie Newman¹ and Tony Rupar^{1, 2, 3}

¹ Department of Pathology and Laboratory Medicine, Western University

- ² Department of Pediatrics, Western University
- ³ Children's Health Research Institute Ontario, Western University

Introduction: Late-infantile metachromatic leukodystrophy (MLD), an inherited lysosomal storage disease characterized by deficient arylsulfatase A (ARSA) activity results in sulfatide accumulation in myelin-producing cells. Affected children present with progressive neurological symptoms such as deteriorating intellectual and motor skills around 2 years of age and usually do not survive beyond 5-10 years. Restricted transfer across the blood brain barrier has severely impacted success of enzyme replacement and gene therapies. We propose the use of autologous mesenchymal stem cells (MSCs) transduced with human hARSA-overexpressing viral vectors to cross-correct the enzyme deficiency. We hypothesize that transduced MSCs will adopt a region-specific cell phenotype to produce and secrete ARSA for uptake by distal cells, thereby effectively correcting the impaired sulfatide catabolic pathway in our MLD mouse.

Methods: We used lentiviral (LV-ARSA/LV-GFP) and adeno-associated viral vectors (AAVrh9-ARSA/AAVrh9-GFP, AAV10-ARSA/AAV10-GFP) to transduce disease-affected and/or disease-relevant (localized to brain) cell lines. Enzyme activity, qPCR vector copy number quantification and immunofluorescent imaging are used as indicators of *in vitro* transduction efficiency of vectors. To study the *in vivo* viability, migration, localization and efficacy differences among our vectors, we are transducing mouse MSCs exogenously and transplanting them intracerebroventricularly in our mouse model.

Results: Our *in vitro* results show LV-ARSA/LV-GFP vectors transduce all cell types with comparable efficiency. Whereas AAVrh9-ARSA/AAVrh9-GFP and AAV10-ARSA/AAV10-GFP display tropism differences, with cells from neuronal lineages exhibiting better transduction efficiencies than fibroblasts. We predict longer term monitoring will show little to no difference in amount of enzyme produced and secreted for LV-ARSA/LV-GFP while both AAVrh9-ARSA/AAVrh9-GFP and AAV10-ARSA/AAV10-GFP will likely exhibit a dilution effect because of their lack of integration into the host genome.

Discussion: Our findings will delineate the efficacy and feasibility of using autologous cell-based gene therapy for MLD, to mitigate potentially harmful immunological responses, allowing for better treatment outcomes. Studies of genotoxicity are warranted.

Minimizing Overutilization of Vitamin D Serum Testing within London Health Sciences Centre

Felicia Tai¹, Ian Chin-Yee^{1, 2}, Vipin Bhayana^{1, 2}, and Angela Rutledge^{1, 2}

¹ Department of Pathology and Laboratory Medicine, Western University ² London Health Sciences Centre and St. Joseph's Health Care London

Introduction: Vitamin D deficiency in the general Canadian population is reported to be greater than 60%. Because of this prevalence, national clinical guidelines recommend against routine testing of serum vitamin D. Ontario Health Insurance Plan (OHIP) has not covered vitamin D serum testing for the general population since 2010. Despite these measures, serum testing of vitamin D in the forms of 25-hydroxyvitamin D (25-OH) and 1,25 dihydroxyvitamin D (1,25 di-OH) have been prevalent at London Health Sciences Centre (LHSC). This study aims to develop a rational policy to reduce inappropriate vitamin D testing at LHSC.

Methods: By incorporating various Plan-Do-Study-Act (PDSA) cycles, guideline of appropriate clinical indications for 25-OH and 1,25 di-OH testing were created through reviewing existing guidelines and initiatives from other institutes and collecting feedback from local experts and clinicians frequently ordering the tests. A computerized physician order entry (CPOE) tool incorporating the guidelines was implemented within the LHSC laboratory information system (LIS), Cerner Millennium. This electronic testing restriction required physicians to select an appropriate reason for testing before the order would be permitted. Vitamin D testing trends before and after this utilization initiative were evaluated using Excel PivotTable and unpaired T-test.

Results: Audits of the LIS showed 1,722 1, 25 di-OH tests and 12,120 25-OH tests ordered within LHSC from January 1st, 2016 to December 31st, 2017. Testing frequency and reasons for testing did not always appear to be appropriate. Following implementation of the electronic ordering restrictions on January 15th, 2018, a significant decrease in both 25-OH and 1,25 di-OH test orders was observed.

Discussion: The interventions appear to have been successful in reducing overutilization of 25-OH and 1,25 di-OH tests. Test orders will be assessed again at six months post-intervention to determine success over a longer period of time.

Molecular Diagnosis of Hereditary Syndromes and Cancer Using Genomic DNA Methylation

Erfan Aref-Eshghi^{1,2}, Laila C. Schenkel^{1, 2}, Charles Schwartz³, Kym M. Boycott⁴, Jean-Claude Cutz⁵, Hanxin Lin^{1, 2}, Peter Ainsworth^{1, 2}, Bekim Sadikovic^{1, 2}

- ¹ Department of Pathology and Laboratory Medicine, Western University
- ² Molecular Genetics Laboratory, London Health Sciences
- ³ Greenwood Genetics Center, Greenwood, SC, USA
- ⁴ Children's Hospital of Eastern Ontario Research Institute, University of Ottawa
- ⁵ Department of Pathology and Laboratory Medicine, McMaster University

Introduction: DNA methylation of the CpGs plays an integral role in the regulation of the processes that control normal development. Aberrant DNA methylation in early development leads to neurodevelopmental syndromes, while its disruption in somatic tissues is associated with carcinogenesis. As a stable molecule, genomic DNA methylation has been the focus of biomarker discovery. Currently, the diagnosis of neurodevelopmental syndromes is challenging due to overlapping clinical presentations. Similarly, in prostate cancer the pathology examination of prostate needle biopsies have high false negative rates due to the molecular/morphological heterogeneity of adenocarcinoma. We hypothesize that both conditions generate DNA methylation epi-signatures which can be utilized in molecular diagnosis/ screening.

Methods: Peripheral blood samples from patients with neurodevelopmental syndromes and archival prostate tissues were assessed for genome-wide methylation changes. Supervised and unsupervised machine learning techniques were used to develop classification models for each disorder.

Results: We identified highly-sensitive/specific peripheral-blood epi-signatures in multiple conditions including Floating-Harbor, DNMT1 neuropathy, ATRX, Kabuki, Sotos, CHARGE, Claes-Jensen, Genitopattelar, and Coffin-Siris syndromes. Using ~1,000 selected CpGs we trained a multi-class prediction model, enabling concurrent classification of the mentioned disorders, with 100% accuracy as determined using multiple validating cohorts. We demonstrated the ability of the algorithm to identify undiagnosed cases in screening, resolve ambiguous cases carrying variants of unknown significance, or to assign a new diagnosis to patients with an initially different diagnosis. Similarly, in prostate cancer, using four CpG loci, we achieved 96% sensitivity and 98% specificity in differentiationg the tumor form benign prostate as confirmed using an external cohort of 234 tumors and 92 benign samples. We also showed that this method can sensitively detect metastatic lesions in bone, lymph node, and soft tissue.

Discussion: This study describes unique, machine learning-derived DNA methylation signatures, enabling highly sensitive and specific molecular diagnosis in both hereditary genetic syndromes and somatic/acquired disorders.

Real World Motor Vehicle Collision Head Injury Risk and Car Seat Use for Children under 8 Years Old

Peyton Schroeder¹, Kevin McClafferty ², Douglas Fraser ^{3, 4}, Michael J. Shkrum^{1, 4}

- ¹ Motor Vehicle Safety Research Team, Department of Pathology and Laboratory Medicine, Western University
- ² Southwestern Collision Analysis
- ³ Departments of Paediatrics, Physiology & Pharmacology, and Clinical Neurological Sciences, Western University
- ⁴ London Health Sciences Centre

Background: Motor vehicle collisions (MVCs) are the leading cause of death for people under the age of 17 years. Almost 80% of rear seat motor vehicle passengers are children. Previous studies have shown that many injuries to children in MVCs involved the head and chest. Child restraint systems (CRS) are designed to address the morphological differences between adults and youth in the rear seat and provide adequate injury protection. In this study, the hypothesis that head injuries in children under eight years old who are in the rear seats of motor vehicles involved in collisions will be influenced by the types of specialized restraint system used was tested.

Methods: Several datasets obtained from Transport Canada and Level 1 Pediatric Trauma Centre emergency and admission reports containing collision, occupant, and injury information were combined and the trends were analyzed. Collision investigation cases selected for this study contained at least one pediatric occupant seated in the rear rows, whether they were injured or not.

Results: Investigations for 41 cases were analyzed (6 involving fatalities). These 41 cases involved 61 child occupants. There was CRS information for 51 children, six using rear-facing CRS, 17 using an forward-facing child restraint (FFCRS), and 18 using a booster seat. Ten children were unrestrained, or their CRS was unknown. Sixteen children had severe head injuries, and five of those were fatal. Eight of sixteen FFCRS were incorrectly used or installed, or not used. Fatal and non-fatal severe head injuries were caused by contact with the seatback (5), interior surface (5), exterior of other vehicle (3), unrestrained cargo (1), and other occupants (2).

Conclusions: FFCRS misuse for child passengers was a factor leading to more severe head injuries. Head injuries seen in the present study were caused by contacting the objects within the rear seat compartment. The findings from this study indicate that head injuries are related to factors other than improper use of a restraint system.

Evaluation of Blast Cell Percentage in Myelodysplastic Syndrome by Flow Cytometry

Mary Xie¹, Ben Hedley², Nikhil Sangle², Cyrus Hsia³, and Ian Chin-Yee³

- ¹ Department of Pathology and Laboratory Medicine, Western University
- ² Department of Pathology and Laboratory Medicine, London Health Sciences Centre
- ³ Department of Medicine, Division of Hematology, London Health Sciences Centre

Introduction: Percentage of bone marrow (BM) blasts is a critical prognostic factor in the International Prognostic Scoring System (IPSS) for myelodysplastic syndrome (MDS) and helps determine management of the disease. Morphologic evaluation is the established method of determining blast count, but it is subject to high interobserver variance. Flow cytometry (FCM) has been proposed as a useful tool to enumerate blast cells in MDS. We hypothesize that flow cytometry is a more reproducible method of determining blast cell percentage in MDS compared to morphology.

Methods: BM aspirates from 39 MDS patients at the London Health Sciences Centre underwent morphologic review by 3 independent hematopathologists to determine blast percentage based on a 500 cell differential count. The same BM aspirates underwent FCM analysis by 3 independent operators to evaluate blasts as a percentage of total nucleated cells. In addition, we collected patient information including age, sex, cytogenetic features, and blood cell counts in order to stratify patients into their IPSS risk categories. Next, we will calculate the correlation coefficient (r) for the average blast percentages obtained by FCM and morphology. Finally, we will calculate the interobserver agreement for each method as measured by Cohen's kappa statistic (κ).

Expected Findings: Our expected findings are that a) average blast cell percentages determined by flow cytometry will have a positive linear correlation to those obtained by morphology; and b) blast cell percentages determined by flow cytometry will have higher interobserver agreement than those obtained by morphology.

Significance: Our expected findings will support the use of flow cytometry as a supplementary tool to determine blast percentage for MDS prognosis. Clinical use of flow cytometry in addition to morphology will allow for more reliable prognosis and improved management of MDS.

Aging as a major modifier of polyglutamine aggregation and toxicity

Sonja E. Di Gregorio and Martin L. Duennwald¹

¹ Department of Pathology and Laboratory Medicine, Western

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

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

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

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

Detecting Low Frequency Variants in Non-Subtype B HIV-1 Integrase Associated with Drug Resistance in Uganda

M Avino1, E Ndashimye^{1, 2}, Daniel J. Lizotte¹, F Kyeyune², I Nankya², RM Gibson¹, E Nabulime², C Kityo², P Mugyenyi², ME Quiñones-Mateu^{2,3}, EJ Arts¹, and AFY Poon¹

- ¹ Western University
- $^{\rm 2}$ Joint Clinical Research Centre/Case Western Reserve University Center for AIDS Research, Kampala, Uganda

³ Case Western Reserve University, Cleveland, Ohio, USA

Introduction: Next-generation sequencing provides a sensitive and costeffective assay for low-frequency variants in diverse HIV-1 infections, but historically has been underutilized for non-subtype B HIV-1 infections in resource-limited settings. Here, we use deep sequencing to analyze samples from treatment-naïve individuals and individuals experiencing virological failure on combination antiretroviral treatment in Uganda. Our objective was to detect associations between low-frequency mutations in HIV-1 integrase and treatment outcomes in Uganda.

Methods: We retrieved a total of 362 archived plasma samples from patients at the Joint Clinical Research Centre (Kampala) with non-B infections, of which 85 were treatment-naive and 277 had experienced virological failure (VF) on first- (N=129), second-line (N=116) or raltegravir (RAL)-based (N=32) regimens. For each sample, we extracted HIV-1 plasma RNA and generated amplicon libraries for two overlapping regions spanning HIV-1 integrase for sequencing on an Illumina MiSeq. Sequencing reads were iteratively aligned with bowtie2 and subtypes were classified with SCUEAL. Amino acid presence/absence matrices were generated at a 1% frequency cutoff and multiple imputations (n=50) were analyzed by L1-norm support vector machine (SVM) classification with 5-fold cross-validation.

Results: Overall, HIV-1 subtype A (47%) was the most frequent, followed by D (21%). More importantly, we detected several polymorphisms associated with integrase inhibitor resistance (e.g., E138K, G140A, Y143R, S147G, Q148K) in a small number of VF samples, although none of these polymorphisms were significantly associated with treatment outcomes. Our SVM analysis determined that the mutations T93A and V126M were the most strongly associated with first-line VF; T174A and K211T with second-line VF; and V165I and V151I with RAL-based VF.

Conclusions: Detecting minority HIV-1 variants with deep sequencing is important in settings where patients frequently discontinue treatment following VF, often leading to reversion to wild-type genotype by the follow-up visit. Our method describes a general strategy for detecting potential associations between the residual polymorphisms and treatment outcomes.

Deletion of CHOP prevents necroptosis in diabetic cardiomyopathy

Rui Ni^{1, 3}, Dong Zheng^{1, 2, 3}, Tianqing Peng^{1, 2, 3}

- ¹ Departments of Pathology and Laboratory,
- ² Medicine Departments of Medicine, Schulich School of Medicine & Dentistry, Western University;
- ³ Critical Illness Research, Lawson Health Research Institute, London Health Sciences Centre

Introduction: Endoplasmic reticulum (ER) stress has been implicated in promoting diabetic cardiomyopathy. ER stress induces CCAAT/-enhancerbinding protein homologous protein (CHOP) expression. Thus, the protein levels of CHOP are elevated in diabetic hearts. CHOP is a transcription factor that mediates pro-apoptotic gene expression and represses anti-apoptotic gene expression during ER stress. Recently studies have also implicated CHOP in inducing pyroptosis through inflammasome activation. Both apoptosis and pyroptosis contribute to the loss of cardiomyocytes in development of diabetic cardiomyopathy. However, it remains to be determined whether CHOP plays a role in necroptosis in diabetic cardiomyopathy.

Methods: Type-1 diabetes was induced in CHOP^{-/-} mice and their wild-type littermates by multiple injections with streptozotocin (STZ, 50 mg/kg/day for 5 days, i.p.). Two months after STZ injection, myocardial function was assessed by echocardiography. Cardiac necrosis, hypertrophy, fibrosis and proinflammatory response were analyzed. Western blot analysis was performed to determine the protein levels of phosphorylated mixed lineage kinase domain like pseudokinase (MLKL), CHOP, Bcl-2, ikB and phosphorylated p65 in hearts. Adult mouse cardiomyocytes were isolated and incubated with high glucose in combination of inhibitors of necroptosis. CHOP was knocked down by using siRNA. Cell death was assessed by measuring LDH release and annexin V staining.

Results: Type-1 diabetic mice displayed a significant elevation of CHOP protein levels in hearts, which correlated with increases in caspase-3 activity, cardiac hypertrophy and fibrosis, and myocardial dysfunction. These effects of diabetes were attenuated by CHOP deletion in CHOP^{-/-} mice. Diabetes induced less inflammatory cytokine expression (TNF- α and IL-1 β) in CHOP^{-/-} compared with wild-type mouse hearts. The anti-inflammatory effects of CHOP deficiency were associated with decreased p65 phosphorylation and increased increased in diabetic hearts. Intriguingly, diabetes induced a significant necrotic cell death and increased the protein levels of phosphorylated MLKL in hearts, indicative of necroptosis. Deletion of CHOP reduced the number of necrotic cells and the protein levels of phosphorylated MLKL in diabetic CHOP^{-/-} mouse hearts, suggesting a role of CHOP induction in cardiac necroptosis. [abstract truncated].

Discussion and Conclusion: CHOP contributes to necroptosis in cardiomyocytes under diabetic conditions. Deletion of CHOP reduces diabetic cardiomyopathy at least in part by inhibiting necroptosis, apoptosis and proinflammatory response. Further investigation is warranted to determine the molecular mechanisms by which CHOP promotes necroptosis in diabetic cardiomyopathy.

POSTER ABSTRACT #9

Rate of Atypical Urothelial Cells Diagnosis in 2017 After Implementation of The Paris System for Urinary Cytology

Thomas Shi¹, Matt Kubica¹, Catherine M McLachlin¹, Alison Nell¹, Mariamma G Joseph¹

¹Department of Pathology and Laboratory Medicine, London Health Sciences Center

Introduction: Urine cytology is an efficient way to detect high grade urothelial carcinoma. The Paris System for Reporting Urine Cytology (TPS) was implemented in 2016 to standardize the terminology and diagnostic categories. Past research has shown the Atypical Urothelial Cells (AUC) rate significantly decreased after implementation of TPS at LHSC. We wish to investigate to see if this drop in AUC rate is sustained in 2017.

Methods: Collect urine cytology data for 2017, analyze rate of each diagnostic category. Use chi-squared test to assess for any statistically significant difference.

Results: No statistically significant difference was detected amongst all 2017 quarters.

Discussion: Drop in AUC rate is sustained in 2017 at LHSC. AUC rate for urine cytology could become a performance indicator in the future akin to thyroid cytology.

Mitochondrial membrane permeability plays a critical role for endothelial cell necroptosis and cardiac allograft rejection

Ingrid Gan^{1, 2}, Jifu Jiang^{1, 2}, Dameng Lian^{1, 2}, Xuyan Huang^{1, 2}, Winnie Liu³, Aaron Haig³, Anthony M. Jevnikar^{1, 3}, Zhu-Xu Zhang^{1, 2, 3}

- ¹ Matthew Mailing Centre for Translational Transplant Studies
- ² Multi Organ Transplant Program, London Health Sciences Centre
- ³ Department of Pathology and Laboratory Medicine
- ⁴ Department of Medicine, Western University

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

Methods: *In vitro*, we induced necroptosis in murine microvascular endothelial cells (MVECs) with TNF α and caspase-8 inhibitor. Necrotic cell death was measured using Sytox Green nucleic acid staining and quantified with the Essen Bioscience Incucyte Zoom live cell imaging. *In vivo*, cardiac grafts from wildtype C57BL/6 and mitochondrial permeability transition (MPT) deficient (CypD^{-/-}) mice were heterotopically transplanted into allogeneic BALB/c mice followed by rapamycin treatment.

Results: TNF α triggered cells to undergo RIPK1- and RIPK3-dependent necroptosis under caspase-8 inhibition. Interestingly, inhibition of MPT could also inhibit cell necroptotic death. MPT is largely regulated by Cyclophilin-D (Cyp-D). Cyp-D deficiency or RNA silencing protected MVEC from necroptosis. In vivo, CypD-deficient cardiac allografts showed prolonged survival in allogeneic BALB/c mice compared to wild type C57BL/6 grafts post transplantation.

Conclusions: Our studies show that MPT may be an important mechanistic mediator of necroptosis in MVECs, and targeting mitochondria-mediated cell death to reduce cardiac graft rejection has therapeutic potential.

Transcriptional Alterations Associated with Deteriorated CD8+ T Cell Function in Cancer-Induced T Cell Exhaustion

Daheng Liu¹, Rakesh Joshi^{1, 2}, Meng Zhang², Weiping Min^{1, 2}

 ¹ Department of Pathology and Laboratory Medicine, Western University
² Department of Surgery, Matthew Mailing Transplant Centre, Lawson Health Research Institute

Introduction: In cancer and chronic infections, T cells are observed to undergo a gradual decline in function, in a process known as T cell exhaustion. Exhausted T cells fail to adequately perform their effector functions, and are characterized by the expression of inhibitory receptors (*iRs*) on their surfaces. In the present study, we identified eight transcription factors involved in T cell maturation and proliferation, including EOMES, BATF, NFATc1, FOXP1, IRF4, T-bet, BLIMP-1 and VHL, and sought to determine how their expression levels correlate with the exhaustion phenotype in CD8 T cells.

Methods: We induced exhaustion in Jurkat cells, our in vitro model of human CD8 T cells, by coculturing them with HepG2 tumor cells. We then confirmed the exhaustion status of the Jurkat cells by evaluating their surface expression of the *iRs* PD-1, TIM-3, BTLA and TIGIT. We will compare the expression levels of the eight transcription factors in functional and exhausted Jurkat cells, and determine the effect of PD-1 blockade on the expression of these transcription factors.

Expected Results: Our expected findings are that T cell exhaustion is associated with altered expressions of select transcription factors, and modulating the expression of these transcription factors result in changes in T cell function. In addition, we expect PD-1 blockade to decrease the expression of the transcription factors implicated in promoting T cell exhaustion, and increase the expression of the transcription factors involved in T cell activation and proliferation.

Discussion: This will be the first study to compare expression levels of multiple transcription factors between functional and exhausted CD8 T cells. In addition, the results from our experiments will potentially confirm HepG2 co-culture as a valid method of inducing exhaustion in Jurkat cell lines. Furthermore, our study will reveal transcription factors that are affected by PD-1 activation; this is crucial in determining signaling pathways downstream of PD-1.

Cardiac GHSR1a and Ghrelin Expression after Severe Sepsis and Correlation with IL-6, SERCA2a, and ERK1/2

Chu Meng Li^a, Rebecca Sullivan^{a, c}, Reza Khazaee^b, Ruud Veldhuizen^b, Savita Dhanvantari^{a, c}

^a Department of Pathology and Laboratory Medicine, Western University
^b Department of Physiology and Pharmacology, Western University
^c Molecular Imaging Program, Lawson Health Research Institute

Introduction: Sepsis is a growing medical problem typically accompanied by cardiac dysfunction. Despite medical advancement, it is still both poorly diagnosed and treated, highlighting the importance of discovering signaling pathways involved in its pathogenesis that could be potential biomarkers or therapeutic targets. Ghrelin and its receptor, growth hormone secretagogue receptor 1a (GHSR1a), form a recently identified signaling system that has been shown to be important in cardiac physiology and pathologies. These findings suggest that ghrelin-GHSR1a may likewise be a major factor in sepsis-induced cardiac dysfunction.

Methods: The aim of this study is to investigate the expression of ghrelin, GHSR1a, SERCA2a, IL-6 and ERK1/2 in cardiac tissues of healthy or septic, male or female rats via fluorescence microscopy. Immunofluorescence and a custom-made fluorescent ghrelin analog will be used to tag proteins of interest. Fluorescence will be quantified and the data obtained will be statistically analyzed to evaluate whether protein expressions are affected by sepsis and sex and whether correlations exist between expressions of proteins.

Results: We expect that the cardiac expressions of GHSR1a and ghrelin will be altered sex-dependently in severe sepsis and will correlate with the expressions of IL-6, SERCA2a, and ERK1/2.

Conclusion: Findings are important for determining role of ghrelin-GHSR1a system in sepsis-induced cardiac dysfunction and whether SERCA2a, IL-6, and ERK1/2 are possible components in this cardiac regulation network.

POSTER ABSTRACT #11

Phenotype of granular cell tumors

Benjamin Rogala^{1, 2}, Meera Shah¹, Linda Jackson-Boeters^{1, 2}, Zia Khan¹, Mark Darling^{1, 2}

¹ Department of Pathology and Laboratory Medicine, Western University ² Division of Oral Pathology, Department of Pathology,

London Health Sciences Centre

Introduction: Granular cell tumours (GCTs) are relatively uncommon solitary benign subepithelial lesions with an incidence of 65-85% in the dorsolateral tongue, and a female to male ratio of 2:1. Histologically, these lesions appear as sheets or ribbons of large polygonal cells with prominent central nuclei and cytoplasmic eosinophilic granules. First described in 1926 as Abrikosoff's tumor, they were thought to be of myogenic origin; but subsequently proposed to be of neural crest origin. Recently, our laboratory has shown that GCTs are immunoreactive to HLA-DR suggesting an antigen presenting cell (APC)-phenotype. The aim of this study is to further characterize the phagocytic phenotype of GCTs using immunohistochemistry in support of a possible antigen presenting cell origin.

Methods: Twenty-two cases of GCTs and 10 control cases of schwannomas from the oral cavity were assessed immunohistochemically for protein expression of APC-phenotype associated antigens CD68, HLA-DR, CD163, CD40, and CD11c. They were also stained for protein expression of neural crest associated antigen SOX10 and neural associated antigens S100 and NSE. Tumours were scored for intensity and number of reactive cells.

Results: Our results show that 22/22 granular cell tumours stained densely positive for HLA-DR, CD68, S100, NSE and Sox10, while CD163, was negative. All schwannoma cases stained positive for HLA-DR, CD163, S100, NSE and Sox10 while CD68 stained with low intensity. CD40 and CD11c immunoreactivity was not detected in GCTs or schwannomas.

Conclusions: Our results show evidence suggesting a phagocytic phenotype of GCT cells, which has not been previously explored. We plan to build on these findings by extracting RNA and profiling APC phenotype-associated genes by quantitative PCR.

An Investigation into T Cell Mediated Drug Hypersensitivity Reactions

Christine C Caron^{1, 4}, Abdelbaset A Elzagallaai^{2, 3, 4}, Michael J Rieder^{1, 2, 3, 4, 5}

- ¹ Department of Pathology and Laboratory Medicine, Western University ² Department of Pediatrics, Western University
- ³ Department of Physiology and Pharmacology, Western University
- ⁴ Robarts Research Institute, Western University
- ⁵ Division of Clinical Pharmacology, Department of Medicine

Introduction: Adverse drug reactions affect hundreds of thousands of people and cost billions of dollars annually. Delayed drug hypersensitivity reactions (DHRs) are T-cell-mediated idiosyncratic reactions that can occur days to weeks after initial exposure to the culprit drug. Many different drugs, such as NSAIDs and antibiotics, can cause mild to severe reactions, but much of the pathophysiology remains unknown. Previous research into the pathophysiology groups clinical presentations together, without accounting for the type or severity of the reactions. There is some evidence that suggests that different reactions have different cytokine profiles and T-cell subset involvement, and therefore different underlying mechanisms. This project will address this issue, and determine pathophysiology in context of both the drug and resulting clinical presentation.

Methods: Patient peripheral blood mononuclear cells (PBMCs) will be isolated from peripheral blood, stained for proliferation, stimulated with the culprit drug, incubated, stained for key T cell surface markers with fluorescent antibodies, and analyzed by flow cytometry. The cytokine profiles of each patient will be analyzed by ELISpot and ELISA. This will determine the T cell subsets involved in each clinical presentation of delayed drug hypersensitivity. In addition, a biobank of the isolated PMBCs will be generated for future research on these samples.

Results: Anticipated results are that each clinical presentation will have different activation of T cell subsets and cytokine profiles as determined by flow cytometry analysis, and ELISpot.

Discussion: This study is investigating T-cell involvement in different clinical presentations of DHRs, which will provide new insight into their underlying pathophysiology while aiming at developing reliable tests for prediction and diagnosis of DHRs. Hopefully we can contribute to lessening both patient suffering and associated healthcare costs, by improving accurate prediction and early diagnosis.

Best Practices in Online Pathology Education for Nursing Students: A Literature Review

Emily Rollo¹ and Candace Gibson¹

¹ Department of Pathology and Laboratory Medicine, Western University

Introduction: The teaching of pathology to second year nursing students in the 4-year collaborative Fanshawe College-UWO program is accomplished via an online course developed by the Pathology department at Western University. This study aims to determine best practices in teaching pathology in an online format to nursing students by obtaining student feedback through the implementation of an online survey and conducting a comprehensive review of existing literature on the subject.

Methods: Information on specific aspects of course satisfaction from the 2017 Pathology 2420A class of nursing students will be obtained via anonymous survey delivered through Qualtrics survey tool (https://uwo.eu.qualtrics. com/jfe/form/SV_73tiEC8v7yiX6Kx?Q_DL=26udmoC3iNfTfhP_73tiE C8v 7 yiX6Kx_MLRP_0URpNSYGo3L1FD7&Q_CHL=gl). All members of the class will be contacted through email with an attached link to the survey. The information obtained from the survey will be analyzed and quantified based on the individual responses provided and subsequently correlated with trends and results of the reviewed literature.

Results: Data collection is ongoing and more responses are required to establish statistically significant results. Research into current literature on the topic of online pathology education of nursing students is a unique field, however, most studies acknowledge the complexities involved in the teaching of a hard science to a more applied science based group and the importance of interactive lectures, activities, quizzes and discussions.

Conclusions: This study will serve to provide direction for the future modification of pathology teaching materials and hence benefit future nursing students directly. In addition, it will provide the broader community of nurses with a more well-rounded background education in and understanding of the pathological basis of disease that has been specifically tailored to their needs.

Prevention of doxorubicin-induced cardiotoxicity by nicotinamide riboside

Dong Zheng^{1, 2, 3}, Yi Zhang⁴, and Tianqing Peng^{1, 2, 3, 4}

- ¹ Department of Pathology and Laboratory Medicine, Western University
- ² Department of Medicine, Western University
- ³ Critical Illness Research, Lawson Health Research Institute, London Health Sciences Centre
- ⁴ Institutes of Biology and Medical Sciences, Soochow University

Introduction: Doxorubicin is widely used as a first-line chemotherapeutic drug for various malignancies. However, doxorubicin causes severe cardiotoxicity and effective therapies are limited. Recent studies showed that autophagic flux is impaired and contributes to doxorubicin-induced cardiac injury. Nicotinamide riboside (NR) is a precursor of NAD+, which has been implicated in various bio-physiological processes. Of note, NAD+ is a co-factor required for Sirt1 activity. Activation of Sirt1 improves autophagic flux. Thus, this study aimed to investigate whether NR protects against doxorubicin-induced cardiotoxicity by improving autophagic flux.

Methods: Cardiac injury was induced in mice by injection of doxorubicin (20 mg/kg, i.p.). NR (100-500 mg/kg, i.p.) was given 30 min before doxorubicin injection. Myocardial function was assessed by echocardiography and myocardial injury analyzed 5 days after doxorubicin injection. Cultured cardiomyocytes were incubated with NR followed by doxorubicin (1 µmol/L) for 24 hours. Apoptosis, necroptosis, and oxidant stress were determined. Autophagy and autophagic flux were analyzed in cardiomyocytes in vitro and mouse hearts in vivo.

Results: Administration of NR elevated NAD+ levels, reduced oxidant stress, inhibited cardiac cell death and attenuated myocardial dysfunction in doxorubicin-injected mice in a dose-dependent manner. These protective effects of NR were associated with improved autophagic flux and offset by inhibition of autophagic flux with chloroquine. In cultured cardiomyocytes, NR improved autophagic flux, inhibited oxidant stress and prevented apoptosis and necroptosis induced by doxorubicin-induced cardiomyocytes. Furthermore, inhibition of Sirt1 activity with EX-527 reversed the effects of NR on oxidant stress and cell death as well as myocardial dysfunction in doxorubicin-induced cardiotoxicity.

Conclusions: NR prevents doxorubicin-induced cardiac injury by improving autophagic flux via the NAD⁺/Sirt1 signaling. Thus, NR may be a potentially useful drug to prevent doxorubicin-induced cardiotoxicity.

POSTER ABSTRACT #15

Alpha-Synuclein Interferes with Mitochondrial Homeostasis to Cause Toxicity

Michael Tauro¹, Martin Duennwald¹

¹ Department of Pathology and Laboratory Medicine, Western University

Introduction: Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide. PD is characterised by a loss of dopaminergic neurons of the substantia nigra, leading to loss of voluntary motor control and regulation. While various genetic mutations have causal roles in PD, the exact mechanisms underlying the neurodegeneration remain unknown. Recently, much evidence has linked mitochondrial dysfunction as a key player in PD pathology, with mutations in mitochondrial-related genes such as PINK1, parkin, and DJ-1 all causing familial PD. Curiously, alpha-synuclein (aSyn), the most notorious of PD causing proteins has not been found to have a direct link connecting mitochondrial dysfunction to toxicity/neurodegeneration. Yet many in-vitro studies investigating aSyn toxicity have not utilized a model which sufficiently relies on mitochondrial ATP production to the same extent as neurons do. This may explain the lack of data connecting aSyn pathology to mitochondrial dysfunction.

Hypothesis: aSyn causes toxicity by interfering with mitochondrial homeostasis and function.

Materials and Methods: We have modified the previously established aSyn yeast model in various fundamental ways which necessitate mitochondrial function for survival.

Results: Under low expression levels, aSyn is not toxic when grown with carbon sources that allow for high levels of glycolysis and fermentation. By contrast, under these same expression levels, aSyn becomes highly toxic when cells are grown under conditions which necessitate mitochondria for ATP production (i.e. oxidative phosphorylation). aSyn also has a strong genetic interaction with genes responsible for mitochondrial fusion, a necessary process for mitochondrial homeostasis.

Discussion and Summary: While many studies have previously shown that aSyn interferes with mitochondrial processes, none have shown a resulting toxic phenotype. Our studies are the first to show aSyn's interference with mitochondrial function directly resulting in toxicity. This not only elucidates the role of aSyn in PD, but also bridges the gap between many other PD causing proteins and aSyn, with mitochondrial homeostasis as the converging point.

Identifying and Targeting Cancer Stem Cells in Colorectal Cancer

Amber Harnett^{1, 2}, Alice E. Shin^{1,2}, Hayley J. Good^{1,2}, and Samuel Asfaha^{1,2}

- ¹ Department of Medicine, Department of Pathology and Laboratory Medicine, Department of Oncology, Western University
- ² London Regional Cancer Program, Lawson Health Research Institute

Introduction: Colorectal cancer (CRC) is the second leading cause of cancer death in Canada. Advanced CRC is associated with tumours that are resistant to conventional therapy. These resistant tumours are believed to arise from cells known as cancer stem cells (CSCs). CSCs sustain tumour growth and tumour progression. In 2012, Clevers and colleagues identified Lgr5 as a marker of CSCs. However, it is not known whether more than one CSC population exists and this may influence treatment modalities for cancer patients. Our lab recently identified keratin-19 (Krt19) and HOP homeobox (HOPX) as potential CSC markers. However, it is not known what role Krt19+ and HOPX+ versus Lgr5+ CSCs play in tumor maintenance and growth. In the present study, I will determine if Krt19 and HOPX label CSC populations and assess the effects of ablating a known Lgr5+ CSC ablation on intestinal tumour growth to determine which cancer stem cells are the ideal targets to halt tumour growth in cancer therapy.

Methods: To determine if Krt19 and HOPX label CSC populations, cohorts of tumour bearing AOM/DSS treated Krt19-CreERT;TdTomato and HOPX-CreERT;TdTomato mice were administered tamoxifen to induce lineage tracing of Krt19 and HOPX derived cells within established tumours. 8 weeks post tamoxifen treatment, adenomas were analyzed using fluorescence microscopy for RFP+ cells. To assess the role of Lgr5+ CSCs in tumour initiation, cohorts of AOM/DSS treated Lgr5-DTR-eGFP mice were administered diphtheria toxin (DT) or saline throughout tumourigenesis. At 28 weeks of age, the groups were compared with respect to tumour burden (number and size) and histology. Subsequently, to determine the role of Lgr5+ CSCs within established tumours in vitro, tumour organoid size upon DT mediated ablation of Lgr5+ CSCs was monitored using three different in vitro tumour organoid models.

Results: Our results indicate that Krt19 labels a CSC population and ablation of Lgr5+ CSCs cells has no effect on tumour initiation or growth in vitro or in vivo.

Conclusion: These results suggest additional CSC populations exist besides Lgr5, which are important for tumour initiation and growth. Consequently, these findings have important clinical implications for the development of new therapies.

Defining domains of chromatin accessibility in human homologous metaphase chromosomes.

Seana L Hill¹, Yi Xuan Wang¹, Peter K Rogan² and Joan H Knoll¹

¹ Dept of Pathology & Laboratory Medicine, Western University ² Dept of Biochemistry and Dept of Computer Science, Western University

Introduction: Proper segregation of chromosomes requires extensive condensation during mitosis to ensure diploid daughter cells. The mechanism behind this process, however, is not well understood. We previously reported a stable localized difference in chromosome homologue condensation (termed differential accessibility or DA) detected by fluorescence in situ hybridization (FISH) using short, single copy (sc)DNA probes in ~10% of scFISH probes mapped in the human genome. Epigenetic marks correlated with DA are currently insufficient to identify DA regions without FISH. To better understand genomic representation of DA, we are expanding the catalogue of scFISH probes exhibiting DA through determining the size of DA domains. We hypothesize analyzing intervals within and adjacent to DA regions will allow determination of their extent within the genome.

Methods: New DA regions were identified by PubMed literature search of FISH images from historical gene mapping studies, using large cloned probes of several 100kb. To define DA domains, scFISH probes were developed for multiple sc intervals (1.7-4.0kb) within each region. Probes were labeled and hybridized to metaphase chromosomes using our previous protocols and analyzed with epifluorescence microscopy.

Results: Eighteen suspected regions of DA were identified by PubMed literature review. Two regions, XDH(1p23) (CytogenCellGen;68:61) and COX5A(15q25) (CytogenCellGen;83:226), have been investigated at 2 different sc probe regions to examine DA domain size. All probe regions (1725-3002bp) showed evidence of DA, with XDH and COX5A spanning minimum genomic regions of 19kb and 110kb, respectively. Additional probes of these and other DA regions are being validated and will be reported.

Discussion: These results demonstrate that DA regions extend beyond defined sc probe intervals within the deduced genomic regions from the original publications. Establishing the extent of these domains is valuable to further characterize DA regions by size and potential unique epigenetic features that would advance identification and understanding of genomic DA representation.

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

Lauren A. Solomon¹, Tharsan Kanagalingam¹, Meerah Vijeyakumaran¹, Lisa Cameron¹

¹Department of Pathology and Laboratory Medicine, Western University

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 (PGD)2 that influences Th2 cytokine production, inhibition of apoptosis, and chemotaxis. Inhaled glucocorticosteroids (GCs) are the primary treatment of allergic asthma and improve asthma symptoms by inhibiting Th2 cytokine production and at high levels by killing Th2 cells. Women are more likely than men to have severe asthma and to 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 inhaled GC. These findings lead us to consider whether female sex hormones could influence Th2 cell response to GC. The mechanism(s) underlying this steroid insensitivity is not well understood and may differ amongst asthma phenotypes.

Methods: Using RNA sequencing, we examined gene expression in primary Th2 cells following exposure to GCs in the presence or absence of an estrogen mimic, PPT (10mM).

Results While GCs repressed Th2 cytokines, regardless of addition of PPT, many GC-mediated effects were suppressed or even counter-acted by PPT. These included reducing expression of pro-apoptotic genes, increasing anti-apoptotic genes well as genes mediating PGD2 signaling such as PGD synthase (PGDS), and its receptor CRTh2. Further sequence analysis of gene promoters revealed both ERE and GRE sites within the promoters of genes suppressed or counter-acted by PPT such as hPGDS.

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

POSTER ABSTRACT #19

Impulsivity in Indigenous Suicide in Ontario, Canada

Rebecca Glover¹, Gerald McKinley¹

¹ Department of Pathology and Laboratory Medicine, Western University

Introduction: Suicide is the leading cause of death for people aged 15-34 in Canada, representing 23% of all deaths in the age group. Suicide among Indigenous youth is five to six times higher than non-Indigenous youth in Canada. This project explores impulsivity and triggering events, including domestic abuse and substance abuse, as well as previous self-harm, that move a person from ideation to suicidal behaviour.

Methods: This project uses a secondary analysis from a file review of 151 cases completed at the Office of the Chief Coroner, Ontario. The interval between last contact and the time of death of each deceased was measured, and the triggering events and social circumstances were recorded. The results were analyzed to assess a correlation between triggering events and impulsivity.

Results and Discussion: The data show a negative correlation with time since last contact and suicidal act. There is prevalent abuse, CAS involvement, and other trigger events in these cases, which have been shown to increase impulsivity in persons.

Conclusions: Further analysis will relate these triggering factors to the impulsive state. These correlations can be used to coordinate prevention and intervention methods designed specifically for at-risk populations.

Comparison of gp120 Variable Region Indel Rates Between HIV-1 Subtypes

John Palmer¹, Abayomi Olabode¹, Art Poon¹

¹ Department of Pathology and Laboratory Medicine, Western University

Introduction: Human immunodeficiency virus (HIV) can effectively evade the human immune system due in part to the high genetic variability exhibited by the five disordered loop structures on the gp120 envelope glycoprotein. This genetic variation has been reported to correlate with HIV pathogenicity as it drives the accumulation of diverse antigenic variants that enable immune system elusion. There are two mechanisms that contribute to this genetic variation and deletion events (indels) have not, despite these events being a powerful source of mutations. Given the lack of understanding on the role of indel evolution, the existing correlation between gp120 variation and HIV-1 pathogenicity, and the differences in pathogenicity between HIV-1 subtypes, this study seeks to determine gp120 variable region indel rates and compare them between HIV-1 subtypes.

Methods: To accomplish this, we obtained patient-derived gp120 sequences from the Los Alamos National Laboratory HIV Database from all the major HIV subtypes and recombinants. We processed and aligned the resulting sequences and used maximum likelihood methods to reconstruct a time-scaled phylogenetic tree for each HIV-1 subtype and recombinant form. Sets of adjacent tree tips will be scanned for length discrepancies and statistically modeled with a Poisson distribution to determine subtype indel rates.

Expected Results: We hypothesize that indel rates will significantly differ among HIV-1 subtypes and recombinants. Further, we expect to find a correlation between the pathogenicity and indel rates of HIV-1 subtypes considering that quickly-mutating subtypes are more effective at eluding host immune systems and establishing infection.

Significance: Determination of indel rates will characterize a previouslyundocumented genetic parameter of gp120, the elucidation of which may improve therapeutic targeting of this glycoprotein. Comparing gp120 indel rates between HIV-1 subtypes will also provide insight into the role of indels in immune evasion based on correlations with subtype pathogenicity.

POSTER ABSTRACT #21

POSTER ABSTRACT #23

Unfolded protein response induction in the presence of TDP-43 and different metabolic conditions in *Saccharomyces cerevisiae*

Karina A. Lenartowicz¹, Sonja E. Di Gregorio¹, Martin L. Duennwald¹

¹ Department of Pathology and Laboratory Medicine, Western University

Introduction: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by protein aggregation and subsequent death of motor neurons. TAR-DNA binding protein (TDP-43) is the most commonly observed protein in aggregates in ALS, which are associated with cellular toxicity and death. The unfolded protein response (UPR) is a cellular stress pathway that ameliorates protein damage and misfolding in the endoplasmic reticulum (ER). In this study, we aim to establish the baseline levels of UPR activation under different metabolic conditions, and characterize the effect of TDP-43 on the UPR in Saccharomyces cerevisiae.

Methods: Yeast cells were transformed with a UPRE-mCherry reporter system and were grown under different metabolic conditions using glucose, galactose, glycerol, oleic acid, and myristic acid as carbon sources. In addition, yeast cells were stressed with tumicamycin (TM) or dithiothreitol (DTT) to establish UPR activation under varied metabolic conditions. Cells were then subsequently transformed with a TDP-43 plasmid, and exposed to the above metabolic and cellular stress conditions.

Results: Our results show an increased activation of the UPR in yeast cells grown in glycerol. This mostly likely occurs in response to increased reactive oxygen species (ROS) created by the respiratory activity of mitochondria that is caused by using glycerol as a carbon source. When TDP-43 is expressed in yeast cells, we see hyper-activation of the unfolded protein response in yeast models of ALS.

Conclusions: Our results will systemically determine UPR activity and induction in *Saccharomyces cerevisiae* under different metabolic and growth conditions. In addition, we seek to establish a link between TDP-43 overexpression and hyper-activation of the UPR, serving as a mechanistic link between the UPR and ALS pathogenesis - thus providing a potential therapeutic target in for ALS treatment.

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

L. McLean^{1, 2}, L. Jackson-Boeters¹, J. Armstrong², A. Poon¹, M. R. Darling¹

- ¹ Department of Pathology and Laboratory Medicine, Division of Oral Pathology, Western University
- ² Division of Oral and Maxillofacial Surgery, London Health Sciences Centre

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 will correlate with the histologic grade and can be used as a reliable marker for the progression of dysplastic lesions.

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

Results: Preliminary analysis suggests S100A7 has increased expression in higher risk lesions, however, S100A7 staining of dysplastic lesions does not appear to predict transformation to malignancy.

Conclusion: 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.

Stem Cells in Odontogenic Tissues

Alyshia Phillips¹, Linda Jackson-Boeters¹, Zia Khan¹, Mark Darling^{1, 2}

¹ Department of Pathology and Laboratory Medicine, Western University

² Department of Pathology and Laboratory Medicine, London Health Sciences Centre

Introduction: Odontogenic lesions often have varied clinical presentations and outcomes. Tumours and cysts range from hamartoma-like lesions to malignant neoplasms. Histologically, odontogenic lesions contain primitive and developing tissues, such as enamel epithelium, dental papilla, enamel protein and fibrous connective tissue. This prompts the question whether these lesions originate from an atypical expansion of stem cells. In this study, this possibility was explored by profiling stem cell antigens in odontomas and hyperplastic dental follicles.

Methods: Paraffin-embedded odontogenic tissue samples were obtained from Pathology Tissue Archives at Western University. RNA was isolated and subjected to quantitative PCR. Transcript levels of *OCT4*, *SOX2*, *NANOG* and *CD133* were measured and the data was normalized to β -actin as the reference gene. A melting curve analysis was performed to determine specificity. Stem cell antigen expression was confirmed using immunohistochemistry.

Results: *OCT4, SOX2* and *CD133* were detected in 14, 10 and 3 (N = 19) odontogenic tissue samples, respectively. All tissue samples showed presence of NANOG mRNA. *CD133* illustrated a different expression pattern from the other three pluripotent transcription factors. Immunohistochemistry revealed presence of *CD133* protein in odontogenic tissues. Assessment of other stem cell antigens by immunohistochemistry is pending.

Conclusions: Based on the different stem cell antigen pattern, our results point to the potential presence of two different stem cell populations in odontogenic lesions. We plan to continue assaying other known stem cell antigens to gain an understanding of the various stem cell phenotypes. To achieve our goals, we will co-label stem cell antigens to delineate the cell phenotype. Future studies will provide a greater understanding of the potential role of stem cells and the origin of odontogenic lesions, and may lead to the development of specific treatment options.

Nicotinamide Riboside Improves Autophagic Flux in Doxorubicin-Treated Cardiomyocytes

Joseph Brockman¹, Dong Zheng^{1,2}, Tianqing Peng^{1,2}

¹ Department of Pathology and Laboratory Medicine, Western University ² Center for Critical Illness Research, Lawson Health Research Institute

Introduction: Doxorubicin (DOX) is an efficacious chemotherapeutic agent whose clinical use is limited by dose-dependent cardiotoxicity. DOX has been shown to impair the cardioprotective effects of autophagy by inhibiting autophagic flux. Preliminary studies by our research group have discovered that nicotinamide riboside (NR), a precursor of NAD+, reduces DOX-induced cardiac injury *in vitro* and *in vivo*. NAD+ activates sirtuin-1 (SIRT1), a deacetylase that activates transcription factors involved in autophagosome formation including Transcription Factor EB (TFEB). We hypothesize that NR pretreatment in adult mice exposed to DOX will increase cardiomyocyte autophagic flux, thereby attenuating DOX-induced cardiotoxicity.

Methods: To measure autophagic flux, we have obtained a transgenic mouse model containing a RFP-GFR-LC3 transgene under regulation of the CAG promoter. Following genotyping to confirm transgenicity, we will pretreat mice with NR or sham then expose them to DOX. The hearts will be collected and cryo-sectioned for fluorescent microscopic analysis. Fluorescent punctate per high-power field (HPF) will be counted to asses autophagic flux *in vivo*. One-way ANOVA followed by Tukey *post hoc* test will be used to analyze autophagosome numbers and autolysosome numbers respectively, among different groups. A *p* value of less th an 0.05 will be considered significant.

Results: Our results to show decreased numbers of red punctate and increased numbers of yellow punctate in NR-pretreated groups exposed to DOX, which suggests decreased autolysosome aggregation and increased autophagosome formation, respectively.

Conclusion: These data suggest that pretreatment with NR can promote autophagic flux in DOX-treated adult mice cardiomyocytes and attenuate DOX-mediated cardiotoxicity.

Altered Mitochondrial Permeability in Ischemia/Reperfusion Injury Influences Apoptotic and Necroptotic Death Pathways in Endothelial Cells

Adnan Qamar^{1, 2}, Ingrid Gan^{1, 2}, Jieping Gong^{1, 2}, Xuyan Huang^{1, 2}, Anthony Jevnikar^{1, 2}, Zhu-Xu Zhang^{1, 2, 3}

- ¹ Matthew Mailing Center for Translational Transplant Studies, Lawson Health Research Institute
- ² Multi Organ Transplant Program, Lawson Health Research Institute
- ³ Department of Pathology and Laboratory Medicine, Western University

Introduction: Inhibition of cell death has tremendous potential for the management of diseases characterized by ischemia/reperfusion injury (IRI) and in organ transplantation. However, effective clinical management strategies are lacking due to inadequate understanding of the cell death mechanisms. Endothelial cells (ECs) are critical mediators of organ dysfunction in IRI. Our data indicates that inhibition of caspases in ECs during hypoxia decreases apoptosis but increases necroptosis – a programmed form of necrosis. The major death effector complex of necroptosis, the necrosome, translocates to the mitochondria and induces the formation of the mitochondrial permeability transition pore (MPTP) which results in loss of mitochondrial transmembrane potential and generation of ROS that ultimately lead to cellular demise. IRI significantly alters mitochondrial metabolism and permeability. In the present study, we wish to explore how mitochondrial dysfunction in IRI influences cell death pathways.

Methods: To evaluate the effects of IRI, EC cultures will be incubated under various hypoxic conditions. TNF α and IFN γ will be added to induce cell death. Various inhibitors will be used to suppress the activity of key molecules involved in the downstream pathways of necroptosis and apoptosis. Cell death will be quantified using the Essen BioScience Live Cell Analysis System.

Results: Our data indicates that necroptosis plays a significant role in hypoxia induced EC death. We have previously shown that inhibition of CypD, a critical regulator of MPTP, decreases TNF α -mediated cell death under normoxia. We will be investigating how altered mitochondrial permeability during IRI influences cell death pathways and the specific roles of cytochrome C, ROS, AIF and Endo G in mediating death in ECs exposed to hypoxia.

Conclusions: Altered mitochondrial metabolism and permeability in ECs exposed to IRI may influence cell death pathways. Understanding of these cell death pathways is critical in formulating clinically applicable therapeutic and preventative strategies aimed at reducing cell death in diseases characterized by IRI.

Self-Reported Cardiovascular Health, Knowledge, and Behaviours in Western Students

Rumana Rafiq, Stephanie Frisbee

¹ Department of Pathology and Laboratory Medicine, Western University

Introduction: Cardiovascular disease (CVD) is the leading cause of death globally, and the second leading cause of death in Canada. It is known that CVD in adults begins in early adulthood; therefore, young adults are a priority age group for CVD prevention. The present study is aimed at measuring cardiovascular health and health beliefs in undergraduate students at Western University. We hypothesized that students will be able to identify "generic" risk factors for cardiovascular disease but will be unable to identify their own level of risk, will have poor health behaviors, and will perceive a low susceptibility to CVD.

Methods: An online survey tool was adapted from the American Heart Association's (AHA's) comprehensive Cardiovascular Health Index (CVHI) to measure undergraduate cardiovascular health. Students were recruited through flyers, in person communication, and online methods (Facebook and email).

Expected Results: Our expected findings are that a) students will be unaware of their biological risk factor values (b) health behaviours such as exercise and diet will be a concern, and c) there will be an inconsistency between health beliefs and health behaviors.

Significance: University is a critical time for young adults to develop healthy lifestyle habits to last throughout life (Fernandes & Lofgren, 2011). Our results will use a CVHI based survey on undergraduates for the first time. These findings may provide novel information regarding young adult CVD risk factors that can be used in university health promotion and CVD primordial and primary prevention.

Up-regulat dilated hea mitochono *Ting Cao, D*

POSTER ABSTRACT #29

Up-regulation of mitochondria-targeted calpain-1 induces dilated heart failure in transgenic mice: an important role of mitochondrial reactive oxygen species

Ting Cao, Dong Zheng, Lulu Zhang, Rui Ni, Tianqing Peng

¹ Lawson Health Research Institute, Departments of Medicine and Pathology and Laboratory Medicine, Western University

Introduction: Calpain-1 has been shown to increase in mitochondria of the heart under pathological conditions including ischemia/reperfusion, diabetes and sepsis. Our recent study reported that increased calpain-1 in mitochondria is associated with myocardial injury and dysfunction. This study was to investigate whether forced up-regulation of calpain-1 restricted to mitochondria induced myocardial injury and heart failure.

Methods: Novel lines of transgenic mice over-expressing cardiomyocytespecific and mitochondria-targeted calpain-1 was generated (Tg-mtcalpain1/ tTA). Both Tg-mtcalpain1/tTA mice and their wild-type littermates received a daily injection of mitochondrial-targeted antioxidant mito-TEMPO or TEMPO as a control starting at age of 8 weeks for one month. Myocardial function, mitochondrial ROS generation, oxidative damage, ATP synthesis activity and expression, myocardial hypertrophy and fibrosis were analyzed.

Results: Two lines of Tg-mtcalpain1/tTA mice were generated with low and high levels of transgenic human calpain-1 expression (Tg-mtcalpain1/tTA^{high}), respectively. Transgenic up-regulation of human calpain-1 protein restricted to mitochondria was verified in Tg-mtcalpain1/tTA mouse hearts. Transgenic up-regulation of mitochondrial-targeted calpain-1 dose-dependently decreased the protein levels of ATP synthase alpha-subunit (ATP5A1) and ATP synthase activity, increased mitochondrial superoxide generation, and induced oxidative damage and cell death in hearts. As a consequence, Tg-mtcalpain1/tTA mice developed cardiac hypertrophy, fibrosis and myocardial dysfunction, with ventricular chamber dilation, heart failure and early death in Tg-mtcalpain1/tTA^{high} mice. However, administration of mito-TEMPO but not TEMPO attenuated the progression of cardiac hypertrophy, fibrosis and myocardial dysfunction in both lines of Tg-mtcalpain1/tTA mice. Relevant to clinical settings, the protein levels of ATP5A1 were significantly reduced in explanted hearts from patients with heart failure.

Conclusions: Transgenic up-regulation of mitochondrial calpain-1 induces myocardial injury and dysfunction at least in part by impairing ATP synthase activity and promoting mitochondrial superoxide generation. Thus, increased mitochondrial calpain-1 may represent a novel mechanism underlying heart failure.

Curious case of Intravascular Large B-Cell lymphoma found on autopsy

Shao Shi Li¹, Edward Tweedie¹, and Chris Howlett¹

¹ Department of Pathology and Laboratory Medicine, Western University

A 75 year old women presented to Emergency Department in August, 2017 after one year history of increased leg swelling, difficult mobilization, as well as pain and numbness. The etiology of her bilateral leg swelling was unclear. Previous bone marrow biopsy to rule out multiple myeloma as well as amyloidosis, was negative. Skin biopsy was negative for vasculitis and panniculitis. Echocardiogram of heart was normal. CT chest, abdomen, and pelvis did not show any evidence of malignancy. After admission, her renal function worsened, and there was an increase in her white blood cell count. She died days later.

Microscopic examination from autopsy revealed a diagnosis of intravascular large B-cell Lymphoma, diffusely involving multiple organs including heart, lungs, skin, pancreas, liver, thyroid, peri-adrenal fat, bladder, kidneys, thyroid, and pituitary. This multi-organ involvement accounts for the variety of signs and symptoms, including the skin changes. No lymphadenopathy was identified and the bone marrow did not appear to be involved by this malignancy.

Intravascular diffuse large B-cell lymphoma is a rare extra-nodal lymphoma where growth is restricted to the lumina of vessels, particularly capillaries. The cells are large, with 1 or more prominent nucleoli, scant cytoplasm, and frequent mitotic figures. Its estimated frequency is estimated at <1 per 1 million persons.

Reference: Orwat DE, Batalis NI. Intravascular Barge B-cell Lymphoma. Arch Pathol Lab Med. 2012; 136: 333-338.

Development of a microparticle-based tool as an indicator of dialysis induced injury

Janice Gomes¹, Fabrice Lucien², Thamara Dayarathna³, Tyler Cooper⁴, Stephen Pasternak^{1,4}, Hon S. Leong², and Christopher W. McIntyre^{1,3}

- ¹ Department of Pathology and Laboratory Medicine, University of Western Ontario
- ² Department of Urology, Mayo Clinic, Rochester, MN
- ³ Lawson Health Research Institute
- ⁴ Robarts Research Institute

Introduction: Of the approximately 35,000 Canadians who have Kidney Failure, hemodialysis(HD) is a treatment option, but is unfortunately iatrogenic. Some of the complications include hemodynamic instability and microvascular dysfunction. This leads to ischemia and abnormal perfusion to vital organs. Previous studies have not found a blood-based biomarker that is indicative of this HD induced vascular damage. However, a biomarker that has gained interest and has been identified as being a predictive and prognostic marker of vascular injury are microparticles (MPs). We hypothesize that a MP based assay has the potential to indicate HD induced damage.

Methods: We used Nanoscale Flow Cytometry to build a standard operating procedure for MP analysis. MPs were assessed and enumerated by staining with endothelial, leukocyte, platelet, and erythrocyte fluorophore-conjugated antibodies. An endothelial cell line (HUVEC) was used to optimize antibodies and assess the effects of dialysate on microparticle release. Moreover, we analyzed MP levels from dialysis patient samples collected pre, during, and post HD treatment from a clinical trial that assessed the effects of standard dialysis treatment and an interventional treatment (cooled dialysate).

Results: Through the creation of a standard operating procedure, we determined an overall protocol for sample analysis. We enumerated endothelial(HUVEC) derived MPS in cell culture media from different treatment conditions. Furthermore, when utilizing the assay on HD patient samples, we observed differences in MP levels from patient plasma samples taken pre, during and post HD. We also found that activated endothelial MP levels and activated platelet MP levels were positively correlated with HD ultrafiltration rate.

Discussion: Creation of a blood-based MP assay has the potential to be used as an indicator for dialysis induced injury. This assay might allow us to determine which patients are susceptible to dialysis-induced cardiac injury and might help stratify patients based upon risk of additional morbidity.

Peripheral funisitis caused by Candida albicans: a case report

Emily Kyle¹, Jordan Schmidt², Helen C. Ettler^{1, 3}

- ¹ Department of Pathology and Laboratory Medicine, Western University
- ² Division of Maternal Fetal Medicine. Western University
- ³ London Health Sciences Centre

Chorioamnionitis, an inflammation of the fetal membranes of the placental disk, often results from an ascending infection from bacterial or fungal organisms present in the vagina. Peripheral funisitis, a subset of chorioamnionitis, refers to an inflammatory reaction at the peripheral edge of the umbilical cord. Peripheral funisitis is a rare but important cause of preterm labour during pregnancy and can be associated with greater mortality rates in these preterm infants. If the infection eventually ascends to the level of the umbilical cord, the formation of abscesses can occur. These abscesses can be identified grossly on the umbilical cord as multiple small white or yellow plaques, most often as a result of infection specifically due to Candida albicans.

Here, we describe the case of a 37-year-old G3P2 woman pregnant with dichorionic diamnionic twins, presenting to obstetrics at 36+0 weeks with preterm labour, with eventual vaginal delivery of both twins. A diagnosis of peripheral funisitis was eventually confirmed in only one of the umbilical cords through the discovery of fungal Candida organisms in wedge-shaped microabscesses along the periphery of the cord after silver staining of the sections.

Non-Cancerous Abnormalities that Could Mimic Prostate Cancer in mpMR Images

Mena Gaed^{1, 2}, Eli Gibson², Cathie Crukley², Jose A Gomez¹, Glenn Bauman³, Aaron Ward², Madeleine Moussa¹, Aaron Fenster²

- ¹ Department of Pathology and Laboratory Medicine, London Health Science Center, Western University
- ² Robarts Research Institute, University of Western Ontario
- ³ Department of Oncology, University of Western Ontario, Lawson Health Research Institute

Prostate Cancer (PCa) is the most common non-cutaneous cancer in North American men. Multi-parametric MR imaging (mp-MRI) has the potential to be used as a non-invasive procedure to predict locations and prognosis of PCa. The study aim is to examine non-cancerous pathology lesions and normal histology that could mimic cancer in mpMRI signals. The study includes 19 radical prostatectomy specimens from LHSC were marked with 10 strand-shaped fiducials per specimen which were used as landmarks in histology processing and ex vivo MRI. Initial registration between fiducials on histology and MR images was performed followed by development of interactive digital technique for deformable registration of in vivo to ex vivo MRI with digital histopathology images. The relationship between MRI signals and non-cancerous abnormalities that could mimic PCa has not been tested previously in correlation with digital histopathology imaging. unregisted mp-MRI images contored by 4 individual radiology observer according to Prostate Imaging Reporting and Data System (PI-RADS), Analysis of the radiology data showed prostatic intraepithelial neoplasia (PIN), atrophy and benign prostatic hyperplasia (BPH) as a main non-cancerous abnormalities responsible for cancer like signals on mp-MRI. This will help increase the accuracy of detecting PCa and play a role in the diagnosis and classifying the confounders that mimic cancer in MR images.

The Growth Hormone Secretagogue Receptor, Ghrelin, and Biochemical Signaling Molecules in Human Heart Disease

Rebecca Sullivan¹, Varinder Randhawa⁶, Anne Stokes², Derek Wu1, Carlie Charron³, Tyler Lalonde³, Leonard Luyt^{3, 5}, Bob Kiaii⁶, Gerald Wisenberg^{4, 7}, and Savita Dhanvantari^{1, 4, 7, 8}

- ¹ Pathology and Laboratory Medicine, Western University
- ² Medical Sciences, University of Ottawa
- ³ Chemistry, Western University
- ⁴ Imaging Program, Lawson Health Research Institute
- ⁵ Department of Oncology, London Regional Cancer Program, Western University
- ⁶ Cardiac Surgery Unit, London Health Science Centre
- ⁷ Medical Biophysics, Western University
- ⁸ Metabolism and Diabetes, Lawson Health Research Institute

Introduction: Heart disease (HD) refers to a group of conditions that affect the structure and function of the heart. Currently, diagnosis of HD is made based on clinical features and circulating biomarkers, notably B-type natriuretic peptide (BNP). However, a biomarker specific to cardiac tissue that can detect heart dysfunction at early stages is lacking. Our group is characterizing the growth hormone secretagogue receptor (GHSR) and its ligand ghrelin as possible specific biomarkers. We have previously developed and characterized a fluorescent analog of ghrelin, Cy5-ghrelin(1-19), and used it to establish GHSR as a possible biomarker in human heart failure. We are now using this imaging tool to map changes in the regional and cellular distribution of GHSR in human HD.

Methods: Samples from various myocardial regions were obtained from 30 cardiac surgery patients with varying degrees of HD. and samples were obtained from various myocardial regions. Cy5-ghrelin(1-19) was used to detect GHSR, and fluorescent antibodies were used to measure ghrelin, BNP, and markers of biochemical signaling. Images were acquired using fluorescence microscopy and analyzed using ImageJ FIJI.

Results: GHSR had both higher abundance and variability in all regions when compared to ghrelin and BNP. There was a strong positive correlation between ghrelin and GHSR (p<0.0001) in agreement with our previous results. Ghrelin and BNP also had a strong positive correlation (p<0.0001) with a weaker correlation between GHSR and BNP, again in agreement with previous findings.

Conclusion: In all cardiac regions GHSR was present in greater abundance than ghrelin and BNP. There was high between-subject variability of GHSR and ghrelin within the right atrium, which may indicate the severity of HD. Ongoing work is examining the biochemical signaling molecules involved in HD. We suggest that the distribution of ghrelin and GHSR may help to elucidate the biology of HD.

POSTER ABSTRACT #33

Perceptions of pathologists, pathologist residents, and pathologist's assistants on non-forensic autopsies: a qualitative study

Avneet K. Dhardwar¹, Bertha Garcia¹

¹ Department of Pathology and Laboratory Medicine, Western University

The rate of non-forensic autopsies has been declining drastically in most developed countries since the 1960s (1). Previous studies have described the autopsy as the "golden standard" procedure not only because of its utility as a quality control measure to determine the quality and/or effectiveness of treatment but also because of its role in helping to understand diseases and providing information that is not detected to the same degree or at all by other imaging techniques, even with today's technological advancements (2,3). This study's aim was to gather information from pathologists, pathologist residents, and pathologist's assistants working at University Hospital in London, Ontario, to learn more about why the non-forensic autopsy rates are declining and how this trend can be reversed.

A qualitative, phenomenological approach was taken where participants answered open-ended questions in semi-structured, one-on-one interviews. Each group of professionals provided unique perspectives on autopsies based on their varying extent of work experiences and diverse personal attitudes towards autopsies.

Almost all of the professionals discussed the difficulties they encountered as a result of clinicians filling out consent forms for autopsies incorrectly. The majority of professionals also described how in general, clinicians and the public lack knowledge on autopsies, what they consist of, and their purposes. This explains why many pathologists and pathologist's assistants felt that sometimes non-forensic autopsies are performed when the pathologists would agree it is not the best use of resources. The information retrieved from these interviews could be used to influence specific interventions designed to improve the usefulness of autopsies as well as their rates, when necessary.

Testicular Hemangioma Mimicking Cystic Dysplasia of the Testis

Nicole Smith¹, Emily Goebel², Bret Wehrli², Jose A. Gomez², Nancy G. Chan²

¹ Department of Pathology and Laboratory Medicine, Western University

² Department of Pathology and Laboratory Medicine,

London Health Sciences Centre

Introduction: Testicular hemangiomas are very rare, benign tumours resulting from blood vessel proliferation within the testes. They occur predominantly in infants and young adults. Cystic dysplasia is another rare, benign condition affecting the testes which arises due to cystic dilation of the rete testis. Ultrasound has been shown to be the most reliable and accurate method for analysing scrotal abnormalities in children—cystic dysplasia often demonstrates cystic lesions within the upper pole of the testis and testicular hemangiomas often show hypoechoic and hypervascular lesions. However, our case demonstrates that final diagnosis relies on histopathological evaluation.

Case: A 3 week old male presented with a right scrotal mass and hydrocele. Ultrasound showed a mildly enlarged testicle with heterogeneous echotexture and multiple areas of anechoic cysts replacing the testicular tissue. Bilateral vascular flow was demonstrated with Doppler ultrasound. Testicular tumour markers were within the normal range. Based on these findings, it was believed that the enlargement was cystic dysplasia of the testis. An orchiectomy was performed at 8 months. On pathological examination, the testicle contained an ill-defined pale tan lesion. Histologically, the sections showed a vascular proliferation within the spermatic cord as well as a wedge infarct replacing approximately 75% of the testicle.

Discussion: Ultrasound is a good method for analysing scrotal masses in children and can help differentiate between benign and malignant conditions. However, histopathology may be required for definitive diagnosis.

POSTER ABSTRACT #37

Histological elucidation of tumour deposits in colorectal adenocarcinoma

Paul Plantinga, David Driman

¹ Department of Pathology and Laboratory Medicine, London Health Sciences Centre

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

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

Results: Preliminary results of 58 TDs reveal 5 with perineural and 9 with venous invasion, with 1 additional TD showing both. The remaining 43 TDs remain classified as TDs after further examination.

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

Three case studies of hereditary breast cancers associated with variants of unknown significance: RNA analysis

Christine Lee¹, Hanxin Lin²

- ¹ Department of Microbiology and Immunology, Western University
- ² Division of Molecular Diagnostics, London Health Sciences Centre -Victoria Hospital

Introduction: Next generation sequencing has greatly improved clinical practices in the surveillance and treatment of prevalent human diseases such as breast cancer. Presently, a molecular diagnostic approach is employed to detect hereditary breast and ovarian cancer risk by using gene panels which detect disease-associated mutations, called pathogenic variants (PVs). Patient's genomes may be screened for an ever-expanding range of cancer susceptibility genes to elucidate prognosis, appropriate prophylaxes, and increase surveillance within the family to assess familial recurrence risk. Accompanying the provisions of whole-genome interrogations are increased findings of variants of unknown significance (VUS). These are genetic variants whose pathogenicity remains unconfirmed, limiting directional conclusions. The prospective clinical utility of understanding the role of VUSs establishes the need for further investigation.

Methods: RNA analysis for each case was completed which consisted of PCR amplification of the regions of interest using patient cDNA, followed by sequence validation by Sanger Sequencing. This confirmed the variants' locations, leading to better characterization of the variants' clinical effects.

Cases: All three cases relate to hereditary breast cancer. The first case was a 48-year-old woman diagnosed at the current age and presented a checkpoint kinase 2 (CHEK2) exon duplication. The second case was a 62-year-old woman, diagnosed at age 61. She presented a possible splice-site mutation in the RAD51C gene, which may cause increased alternative splicing of the exon 3. The third case was a 49-year-old woman who was diagnosed with breast cancer at age 37 and ovarian cancer at 41 years. She presented a splice-site mutation in the ataxia telangiectasia mutated (ATM) gene, which resulted in exon skipping.

Discussion: The findings contribute to the clinical characterisation of VUSs to improve the scope of gene panels and ultimately patient care. Further functional investigations, such as kinase functional testing, are required.

S100A7 as a quantitative screening tool for predicting transformation in potentially malignant oral lesions

J. Soparlo^{1, 2}, L. Jackson-Boeters¹, Z. A. Khan¹, M. R. Darling¹

¹ Department of Pathology and Laboratory Medicine, Division of Oral Pathology, Western University

² Division of Oral and Maxillofacial Surgery, London Health Sciences Centre

Objectives: 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.

Methods: Formalin fixed paraffin embedded specimens from 31 patients with oral squamous cell carcinoma, including an initial biopsy of a lesion from the same site were included, with 10 samples of dysplasia which had not advanced to squamous cell carcinoma, and 10 samples of hyperkeratosis as control groups. Specimens were stained for S100A7 protein using a standard immunohistochemistry protocol. Expression of S100A7 will be assessed semiquantitatively, using an intensity and proportion scale, as well as by image analysis. As S100A7 is active in the MAPK signaling pathway, other proteins in the pathway will also be evaluated to assess functional activity.

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

Conclusion: 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.

Tau protein pathology following traumatic brain injury – a longitudinal investigation

Matthew A. Hintermayer¹, Alexander J. Moszczynski¹, Michael J. Strong^{1, 2}

- ¹ Molecular Brain Research Group, Robarts Research Institute, Western University
- ² Department of Clinical Neurological Sciences, Western University

Introduction: Traumatic brain injury (TBI) is a significant risk factor for the development of various neurodegenerative diseases, including: Chronic Traumatic Encephalopathy (CTE), Alzheimer's Disease (AD), and Amyotrophic Lateral Sclerosis (ALS) with cognitive impairment (ALSci). Research has identified a phospho-epitope of tau protein (tau phosphorylated at Threonine-175; 'pThr¹⁷⁵) which has been identified in a variety of neurodegenerative conditions – including ALS, CTE, and AD – and is absent in age-matched controls. Controlled TBI can initiate the formation of pThr¹⁷⁵ *in vivo*, developing a tauopathy identical to that seen in CTE and ALSci. Investigating the changes in kinase activity and modifications to tau protein in the acute and chronic phases following TBI would allow for the mechanisms of tauopathy initiation and propagation to be elucidated. We hypothesize that: 1) oxidative stress *in vitro* initiates pThr¹⁷⁵ tau formation; and 2) TBI has both acute and behavioural disturbances.

Methods: Using an *in vitro* model of hydrogen peroxide stress, tau transfected Neuro2A cells will be examined for the formation of pThr¹⁷⁵ tau after acute stress exposure. Utilizing a rat model of TBI-induced tauopathy, a longitudinal analysis of stress kinase activity and neuropathology will be conducted to map the molecular and histological consequences of TBI. Behavioural analyses will be conducted on injured and non-injured rats to correlate the development of behavioural disturbances with neuropathological changes.

Results: Optimized stress conditions for transfected and untransfected Neuro2A cells have been determined. Lysates are being analyzed for the presence of pThr175 tau formation and the upregulation of stress-activated kinases.

Discussion and Conclusions: The data obtained from these experiments will contribute to our understanding of the mechanisms underlying the initiation and propagation of tauopathy, and will lead to the development of useful models for studying the neurodegenerative process.

The Impact of Surgical Resection Margins on Survival of Patients with Pancreatic Ductal Adenocarcinoma

Sundip Shah¹, Tanya Kruper², David Driman¹, Ken Leslie²

¹ Department of Pathology and Laboratory Medicine, Western University ² Department of Surgery, Western University

Pancreaticoduodenectomy also known as a Whipple's procedure is a complex operation that involves the removal of the head of pancreas, duodenum. common bile duct, gallbladder and sometimes a portion of stomach. It is used as a treatment option for patients with biliary, pancreatic and duodenal malignancies. For pancreatic ductal adenocarcinoma (PDAC), positive resections margins (R0) following a Whipple's procedure have traditionally been defined as 0 mm (tumour – margin) in North American centres. At the London Health Sciences Centre, only the closest margin/surface distance is consistently reported and used for post-operative prognostication. However, there are multiple surgical resections margins and anatomical surfaces on the resection specimen, including the anterior pancreatic surface, posterior pancreatic surface, pancreatic neck margin, superior mesenteric artery (SMA)/ uncinate, superior mesenteric vein (SMV), common bile duct (CBD), proximal luminal and distal luminal margins that may or may not be commented on. Prognosis may vary, depending on involved margin type, which may have implications for adjuvant therapy. We have decided to complete a retrospective cohort study of adult patients undergoing pancreaticoduodenectomy for PDAC to evaluate the effect of resection margin or surface distances on overall and disease - free survival. We are now embarking on a review of pathology slides to complete the objective of measuring margin and surface distances to tumour for all Whipple's resection specimens.

Two faces of Parkin oxidation and misfolding: gain- and loss-offunction in Parkinson's disease

Mohammad A Esmaeili¹ and Martin L. Duennwald¹

¹ Department of Pathology and Laboratory Medicine, Western University

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.

POSTER ABSTRACT #43

Polysialic Acid Synthesis by Polysialyltransferase ST8SialV in Prostate Cancer: Using Glycans as Novel Anti-Cancer Target

Ranjit Singh Padda^{1, 3}, Karla Williams^{1, 2}, Thamara Dayarathna¹, Chandan Chakraborty³, Zia Khan³, Hon Leong^{1, 3, 4}

- ¹ Lawson Health Research Institute
- ² Faculty of Pharmaceutical Sciences, University of British Columbia
- ³ Department of Pathology and Laboratory Medicine,
- University of Western Ontario
- ⁴ Department of Urology, Mayo clinic, Rochester Minnesota

Introduction: Aberrant glycosylation allows the subset of neoplastic cells to gain metastatic transformation. Polysialylation is a specific type of glycosylation in which polysialic acid (polySia) is introduced onto the cell surface with the help of two polysialyltransferase enzymes (polySTs), ST8SialI and ST8SiaIV. PolySia commonly serves as a surface moiety for neural cell adhesion molecule (NCAM), resulting in the formation of a polySia-NCAM complex. Overexpression of polySia is occasionally found in several types of human cancers, with a strong correlation of polySia expression to cancer staging, as well as poor clinical prognosis in patients. The expression, functional roles of the polySia in prostate cancer (PCa) have not been previously studied. Hence the aim of this current research is to elucidate the mechanisms and functional roles of polySia in PCa. We hypothesize that overexpression of polySia in PCa enhances cancer cell migration, proliferation, invasion and metastasis.

Methods: Expression of NCAM and polySTs (ST8sialI, ST8SialV) were determined in PCa cell lines (PC3, LNCaP, DU145) and compared with a benign prostate cell line (BPH). Levels of NCAM, polySTs were evaluated using qPCR, immunofluorescence and western blotting. PCa cells with or without polySia were used to study the functional role of polySia in PCa metastasis using CAM extravasation assay.

Results: There was a significant upregulation of polySia in PCa, with concomitant increase of ST8SiaIV expression. Further, we found minimal level of NCAM in PCa cells. In addition, in vivo cancer cell extravasation assay showed no difference in metastatic efficiency of clones with or without polySia expression.

Conclusions: Abnormal polysialylation of proteins occurs in PCa, shown by upregulation of polySia and polySTs. Loss of function for ST8SialV was sufficient for depleting polySia in PCa cells suggesting that polySia is biosynthesized solely by ST8SialV. Further, the negligible level of NCAM indicates that NCAM is not a major carrier protein for polySia in PCa. As of yet unidentified proteins are suspected to be polysialylated in the PCa, whose modification does not significantly alter cancer cell metastasis. Further investigations regarding the role of polySia in enhancing cell migration and survival of metastatic colonies are currently underway.

Expanding the catalog of differentially compacted loci on homologous chromosomes in the human genome

Yi Xuan Wang¹, Seana L Hill¹, Peter K Rogan² and Joan H Knoll¹

 ¹ Department of Pathology and Laboratory Medicine, Western University
² Department of Biochemistry and Dept of Computer Science, Western University

Introduction: Although differences in chromatin structure along individual chromosomes have been well established, variation in chromatin accessibility at the same locus between homologs is less understood. Previous research in our laboratory has shown that metaphase chromosome compaction differences (termed differential accessibility or DA) occurred between homologs in 10% of 500 single copy genomic loci tested (MolCytogen 2014;7:70), with an emphasis on regions from clinically relevant cytogenetic disorders (AmJMedGenet 2003;121A:245). The current study examined legacy gene mapping publications to identify novel regions of DA; and individually confirmed the findings in the laboratory. We hypothesize that legacy gene mapping fluorescent in situ hybridization (FISH) data reveals previously unrecognized regions of DA.

Methods: Through a literature search of past FISH gene mapping publications, candidate genomic regions were selected based on differences in fluorescence intensities of mapped DNA probes. For each candidate, the location of unsequenced FISH probes were anchored onto the human genome sequence v19 through bioinformatics gene analysis. Subsequently, intervals were identified for single copy FISH (scFISH) probe construction. Intervals were then amplified by long PCR, labeled with modified nucleotides, hybridized to human metaphase chromosomes, and analyzed with epifluorescence microscopy to determine DA.

Results: Eighteen candidate regions were selected from over 110 legacy FISH studies. To date, five probes from different regions have been successfully hybridized and analyzed. DA was present at all sites. Work on the remaining regions is ongoing.

Discussion: These findings suggest that legacy gene mapping data is a simple and effective strategy to identify DA. Results also indicate that DA regions in mitotic chromosomes are not restricted to clinically relevant loci; and are likely more widespread than currently recognized. A more comprehensive catalog of DA regions should advance understanding of the mechanisms leading to DA and provide more insight into the condensation of human mitotic chromosomes.

Molecular Mechanisms of Resistance to Targeted Therapies in Prostate Cancer

Michael Roes¹ and Fred Dick^{2, 3}

- ¹ Department of Pathology and Laboratory Medicine, Western University
- ² Department of Biochemistry, Western University
- ³ Department of Oncology, Western University

Introduction: Molecularly targeted cancer therapies are effective in improving patient outcomes while reducing harmful side effects and act by inhibiting molecules that cancer cells depend on for proliferation. However, resistance to these therapies is common. Prostate cancer cells can acquire resistance by switching lineages to a cell type that is no longer dependent on the molecular target. Recent studies have identified the retinoblastoma (RB) protein as a key player in this mechanism. RB is commonly known to function in cell cycle regulation, but new studies have identified roles in maintaining chromatin structure and integrity, which may be important in resistance to targeted therapies. However, a molecular mechanism of how this occurs has yet to be determined. In this study, I hypothesize that a genome wide screen will reveal genes in addition to RB that function in cell lineage transformation and resistance to targeted therapies in a prostate cancer cell line.

Methods: LNCaP prostate cancer cells will be infected with a library of lentiviral constructs that express Cas9 and guide RNAs that target all human genes to create a pool of cells where each bears a unique gene disruption. Following drug selection with the anti-androgen enzalutamide, high-throughput sequencing of the gRNA sequences will identify drug resistant clones.

Results: Preliminary experiments have determined the growth rate of LNCaP cells and IC80 of enzalutamide in these cells. Virus titration experiments also determined the volume of library-packaged lentivirus to achieve an MOI of 0.3 for infection. Current work includes performing the actual CRISPR screen.

Conclusions: Overall, this study addresses a major issue in current cancer therapies. The elucidation of molecular mechanisms of resistance can identify new drug targets to antagonize resistant cells or even prevent the emergence of resistance.

The LHSC Whole Exome Sequencing Study: A Progress Report

Carere, Deanna Alexis¹; Aref-Eshghi, Erfan²; Kerkhof, Jennifer¹; Stuart, Alan¹; Lin, Hanxin¹; Ainsworth, Peter¹; Rupar, Tony³; Siu, Victoria⁴; Prasad, Chitra⁴; Saleh, Maha⁴; Karp, Natalya⁴; Chakrabarti, Rana⁴; Campbell, Craig⁵; Zapata-Aldana, Eugenio⁵; Sadikovic, Bekim^{1, 2}

- ¹ Molecular Genetics Laboratory, Molecular Diagnostics Division, London Health Sciences Centre
- ² Department of Pathology and Laboratory Medicine, Western University
- ³ Biochemical Genetics Laboratory, Molecular Diagnostics Division, London Health Sciences Centre
- ⁴ Department of Paediatrics, Division of Genetics and Metabolism, London Health Sciences Centre
- ⁵ Department of Paediatric Neurology, Children's Hospital, London Health Sciences Centre

Introduction: Whole exome sequencing (WES) enables concurrent analysis of thousands of genes, and may be appropriate when a clinical diagnosis is uncertain or prior targeted testing has not identified a diagnosis. The LHSC WES Study was designed to pilot-test the technical protocols necessary to launch clinical WES.

Methods: LHSC physicians invited patients to the study, and submitted phenotype data, consent form, and DNA sample. Coding regions were enriched with the Roche SeqCap EZ MedExome Target Enrichment Kit (targeting >4600 clinically relevant genes), followed by high-throughput sequencing (Illumina NextSeq). Variants were filtered for quality and frequency with an in-house algorithm, and for phenotype using PhenoTips software. Variants were manually assessed for reporting by a genome analyst and molecular geneticist.

Results: By February 2018, WES was complete in 92 probands (86 families), across 7 diagnostic categories: developmental/intellectual delay plus other features (33%); specified pediatric syndrome or isolated features (e.g., Wilms' tumor; 19%); epilepsy (17%); multiple congenital anomalies (15%); non-epileptic neurological disorder (8%); metabolic disorder (4%); and adult-onset disorder (4%). WES identified ~10,000 variants per case; reduced to ~500 per case by quality/frequency filtering, and <200 per case by punotype filtering. To date, 44 cases have been assessed, encompassing 38 unique, indication-related reportable variants (14 Pathogenic/Likely Pathogenic; 24 Variants of Uncertain Significance). A molecular diagnosis consistent with referral indication was suggested in 9 of the 44 cases (20%). Pathogenic variants unrelated to indication, but in genes on the American College of Medical Genetics "medically actionable" list, were identified in 7 of 92 cases (8%); of note, these incidental findings are not currently being reported within the study protocol.

Discussion: WES can aid in the diagnosis of a range of conditions of suspected genetic etiology; however, many cases remain unsolved by sequencing of known genes, and unanticipated findings may present challenges to genetic counselling.

Neuropathological changes in a case of adult onset leukodystrophy with axonal spheroids and pigmented glia with CSF1R mutation

Basma AlYamany¹, Ryan Taylor², Elizabeth Finger^{2, 3}, Lee-Cyn Ang^{1, 4}

¹ Department of Pathology and Laboratory Medicine, London Health Sciences Centre

- ² Department of Clinical Neurological Sciences, Western University
- ³ Department of Neurology, London Health Sciences Centre
- ⁴ Department of Pathology and Laboratory Medicine, Western University

Adult onset leukodystrophy with axonal spheroids and pigmented glia (ALSP) is a white matter degenerative disorder. Mutations in the tyrosine kinase domain of the colony-stimulating factor 1 Receptor gene (CSF1R) have been associated with ALSP. The condition can present with a frontal lobe syndrome, which may include lack of social inhibition, poor judgment, memory impairment, personality changes, motor impairment and seizures. We describe a case of a 57-year-old lady that presented with acute cognitive decline and brain atrophy. Post mortem examination revealed large ventricles and bilateral abnormal discoloration of white matter with sparing of U-fibers. Histological examination confirmed the loss of myelin and axons, with axonal spheroids and pigmented microglia. The histological and molecular features supporting this diagnosis have been reviewed, along with the function and pathogenesis of CSF1R.

Machine Learning Prediction of HIV-1 Integrase Drug Exposure History

Jasper Ho¹ and Art Poon¹

¹ Department of Pathology and Laboratory Medicine, Western University

Introduction: The treatment exposures of the HIV-1 populations infecting patients are clinically significant information that guides subsequent treatment decisions, i.e., antiretroviral drug regimens. In this study, our objective is to develop a machine learning classifier to predict HIV-1 resistance to integrase inhibitors using treatment exposure and sequencing as a proxy of resistance, using HIV-1 integrase nucleotide sequence data. We hypothesize that this model predicts antiretroviral drug exposures from this genetic variation. Elucidating the contributions of both missense and silent mutations toward resistance using a naïve data-driven model will further our understanding of HIV-1 drug resistance for this relatively new class of antiretroviral drugs.

Methods: We obtained 12332 integrase nucleotide sequences from the Stanford University HIV drug resistance database (HIVdb) and supplemented these data with more recent sequences from the GenBank database. Next, we filtered incomplete sequence records and then annotated the sequences as being either drug-naïve or drug-exposed. We used pairwise alignment in R to align the sequences to the HXB2 integrase reference sequence. Missing nucleotides were imputed using the mice package in R. Finally, we trained and evaluated a support vector machine (SVM) classifier on these imputed data with cross-validation.

Results: Our preliminary results show that the time complexity of multiple imputations scales unfavourably with respect to the number of missing data per sequence and the number of sequences in our database. Optimization is this regard is ongoing. Supplementation of data and careful feature selection to maximize signal should improve the classification ability of our SVM.

Discussion: Developing an approach to quantify the contributions of nucleotide-level mutations toward viral resistance is applicable in both clinical and basic research contexts. Using data-driven approaches like support vector machines provides an empirical model that is more reproducible and potentially more sensitive than rules-based algorithms derived from expert knowledge, such as the HIVdb algorithm.

H19 regulates glucose-induced EndMT in chronic diabetic complications

Anu A. Thomas*, Linda Fei, Subrata Chakrabarti

¹ Department of Pathology and Laboratory Medicine, Western University

Background: Long non-coding RNAs (IncRNAs) influence the pathophysiology of various diseases. Here, we examined IncRNA H19, to elucidate its specific role in cellular changes in chronic diabetic complications. We have shown that glucose-induced endothelial-mesenchymal transition (EndMT) is mediated by mir-200b and p300 in heart and retina during diabetes. H19 regulates members of miR-200 family in other systems, through histone acetylation. Here we propose that H19 regulates EndMT in various diabetic complications, directly or through miR200b and p300.

Methods: Endothelial cells (ECs) were treated with high (25mM) and normal glucose (5mM) for various durations. Cell transfection with H19 siRNA and H19 overexpressing vector was followed by RNA analyses. In parallel, we investigated tissues from retina, kidney and heart from streptozotocin-induced diabetic mice.

Results: High-glucose treatment downregulated H19 RNA levels and expression of endothelial markers, namely VE-Cadherin and CD-31 and upregulated mesenchymal markers, eg SM22 and FSP1. Such EndMT related changes were enhanced by H19 knockdown while H19 overexpression prevented such changes. In keeping with in vitro data, the tissues showed changes consistent with EndMT.

Conclusions: This study showed that glucose-induced downregulation of H19 is causally related to EndMT, as evidenced by reduced expression of endothelial markers and increased production of mesenchymal markers in ECs and possibly in the organs affected by chronic diabetic complications. Understanding H19-mediated regulation of EndMT may have potential implications in development of RNA-based therapies.

The Use of CT in Forensic Post Mortem Examination to Facilitate Targeted Dissection Methods

Erika Chadwick¹, Art Poon¹, Michael Pickup²

¹ Department of Pathology and Laboratory Medicine, Western University ² Ontario Forensic Pathology Services, Toronto, ON

Introduction: Computed Tomography (CT) is a non-invasive diagnostic imaging technique that is frequently being used in forensic post-mortem examination to aid pathologists in determination of cause of death. CT is a tool that can be used to supplement autopsy by creating detailed cross-sectional images of the body through associated x-ray measurements. CT imaging can be a useful method in identification of individuals, preservation of evidence and identifying hazards present in a post-mortem exam. This retrospective study will investigate a randomly selected population of cases from 2015 and 2017 to evaluate the usefulness of CT to motivate the pathologist to perform a targeted dissection or an external exam. It is hypothesized that post-mortem imaging is a less-invasive method that can be overall beneficial in the workplace.

Methods: We randomly selected 100 cases; 50 cases from both 2017 and 50 cases from 2015; to analyze. The selected cases excluded scenarios in which a full, routine autopsy was required by standard operating procedures. The statistics from each case were compared to evaluate if CT a reliable non-invasive technique to encourage more targeted dissections, and in what situation is CT the most effective.

Results: Between both sets of data, it was proven that the most common death causes were drug toxicity, cardiovascular disease and severe trauma. The time difference between full autopsies and targeted autopsies was significant (P <0.001). It was shown that CT is most useful for deaths caused by severe trauma and cardiovascular disease, whereas forensic chemistry was most useful in determining the cause of death for drug toxicity.

Discussion: Although full post-mortem examinations are the most reliable method, it can be shown that CT is a supplementary tool that can be beneficial for decreasing workload and exposure, as well as for religious observances and providing comfort to family and friends of the deceased.

Human tissue kallikreins are aberrantly expressed in pleomorphic adenoma

Matthew D. Morrison¹, Linda Jackson-Boeters², Zia A. Khan², Mark R. Darling²

- ¹ Resident (PGY5), Division of Oral and Maxillofacial Surgery,
- Department of Dentistry, University of Western Ontario
- ² Department of Pathology and Laboratory Medicine, University of Western Ontario

Objectives: Pleomorphic adenoma (PA) is the most common benign salivary gland tumor. Human tissue kallikreins (KLKs) have been identified as biomarkers in many human tumors and may influence tumor behavior. We investigated *KLK1-15* messenger ribonucleic acid (mRNA) and proteins in PA specimens to determine a KLK expression profile for this tumor.

Methods: Fresh frozen PA tissue specimens (n = 26) and matched controls were subjected to RT-qPCR to detect *KLK1-15* mRNA. Expression of KLK1, KLK12, KLK13 and KLK8 proteins were then evaluated via immunostaining techniques. Statistical analyses were performed with the level of significance set at P < .05.

Results: We observed downregulation of mRNA expression levels for *KLK1*, *KLK12* and *KLK13*, and immunostaining studies revealed downregulation of the corresponding proteins. Capsular perforation was associated with increased KLK1 protein expression. Tumor size was not associated with capsular invasion and/or perforation.

Conclusions: This study is the first to detail a KLK expression profile for PA at both the transcriptional level and the protein level. Future work is required to develop clinical applications of these findings.

Heterogenous Glucagon-Immunoreactive Complexes as Novel Regulators of Insulin Secretion

Farzad Asadi¹ and Savita Dhanvantari^{1,2,3}

¹ Department of Pathology and Laboratory Medicine, Western University

² Departments of Pathology and Medical Biophysics, Western University ³ Lawson Health Research Institute

Introduction: Pancreatic alpha cells secrete glucagon, the glucose counterregulatory hormone. Our previous work has identified several proteins that interact with glucagon within the secretory granule, and are therefore potentially co-secreted with glucagon. The aim of the present study was to identify the proteins within glucagon immunoreactive complexes secreted from pancreatic alpha cells, and to determine the effects of these complexes on beta cell function.

Methods: To identify heterogenous secreted glucagon immunoreactive complexes, the pancreatic alpha cell line, alpha TC1-6, was treated with paracrine effectors, GABA and insulin, alone or in combination in the context of high glucose (25 mM) or low glucose (5.5 mM) for 24h or 72h. Samples of crude media were subjected to immunoblotting for glucagon. Media were then subjected to acid-ethanol extraction and protein bands were identified by LC-MS/MS. To examine the effect of secreted glucagon immunoreactive complexes on insulin secretion, alpha TC1-6 cells were treated as described above, and the conditioned media were collected and added to INS-1E cells. Glucose-stimulated insulin secretion was measured using ELISA.

Results: Three glucagon immunoreactive bands were identified in crude media from all treatment groups. Electrophoresis of the acid- ethanol soluble fraction revealed unique profiles of bands for each treatment group in terms of number and relative intensities. Proteomic analysis showed that each band is a complex of glucagon and secretory granule proteins that we have previously identified as a "glucagon interactome", the most novel of which were histones H2A and H2B. Conditioned medium from GABA-treated cells increased basal insulin secretion (p<0.05) with no effect on glucose-stimulated insulin secretion.

Conclusion: Pancreatic alpha cells secrete glucagon-immunoreactive complexes containing novel glucagon-interacting proteins we have previously identified. The heterogeneity of these complexes is determined by the microenvironment. We propose that this heterogeneity modulates beta cell function.

POSTER ABSTRACT #53

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

Meerah Vijeyakumaran¹, Tharsan Kanagalingam¹, Lauren Solomon¹, Teah Jazey¹, Lisa Cameron¹

¹ Department of Pathology and Laboratory Medicine, Western University

Introduction: The incidence and severity of asthma is greater in women than men and women are more susceptible to developing corticosteroid-resistant asthma. Despite this, the underlying mechanism(s) for the sex-specific prevalence of severe asthma is still poorly understood. Asthma is a T helper (Th) 2 cell-associated inflammatory disease where Th2 cytokines (IL-4, -5 and -13) influence asthma pathogenesis. CRTh2 (chemoattractant-homologous receptor expressed on Th2 cells) is a marker for Th2 cells and its activation by the pro-inflammatory lipid PGD2 mediates many aspects of their function. Inhaled glucocorticosteroids (GC) are the main treatment for asthma and in most patients reduce inflammatory cells and symptoms. However, in severe asthmatics taking high dose GC, women had persistently elevated levels of Th2 cells. Furthermore, a positive correlation was seen between Th2 cells and the dose of inhaled GC in women but not in men. This suggests a potential interaction between sex hormones and GC enhancing Th2 cell responses in women.

Hypothesis: The effects of GCs on Th2 cells are influenced by estrogen signaling

Methods: The potential cross-talk between estrogen and glucocorticoid signaling and its effect on CRTh2 expression and Th2 cell function was assessed by treating a Th2 model cell line (CCRF CEM) and/or primary Th2 cells with an ER α -selective agonist propylpyrazole-triol (PPT) and dexamethasone (Dex), a synthetic GC (0.1-1M). CRTh2 protein expression was assessed using flow cytometry and western blot analysis. Cytokine production by Th2 cells was measured using qRT PCR after PGD2 administration. Th2 cell death was assessed using flow cytometry for detection of Annexin V and 7-AAD.

Results: We found no change in surface expression of CRTh2 in Th2 cells treated with PPT, Estradiol or Dex alone. However, when Th2 cells were cultured with both PPT+Dex, this combination significantly increased surface and total CRTh2 expression and enhanced IL-13 production. Apoptosis induced by Dex was also reduced after PPT+Dex co-treatment.

Discussion and conclusion: These data show that the combination of GC and ER α signaling enhances CRTh2 expression, Th2 cytokine production and Th2 cell survival. *In vivo* this interaction may represent a mechanism by which Th2 cells are sustained in women, but not men, with severe asthma.

Wnt Pathway Activation in Development of Carboplatin Resistance in Ovarian Cancer

Seongryoung Han¹, Michele Weir^{1,3}, and Zia A. Khan^{1,2}

¹ Department of Pathology and Laboratory Medicine, Western University ² Diabetes Research Program, Lawson Health Research Institute

³ Pathology and Laboratory Medicine, London Health Sciences Centre

Introduction: Ovarian cancer (OC) is the most fatal gynecological malignancy due to limitations in current treatment options including carboplatin chemotherapy. Carboplatin exerts cytotoxicity through DNA damage response. Yet, many patients develop carboplatin resistance and cancer recurrence. For more effective OC treatments, the underlying mechanism of carboplatin resistance is needed to be elucidated. Our lab has previously suggested potential involvement of Wnt pathway and upregulated expression of placental growth factor (PGF) and interleukin 1-beta (IL1B) in carboplatin resistance. I hypothesize that OC mediates carboplatin resistance through activation of Wnt pathway leading to induced expression of angiogenic factors and escape from cell death.

Methods: OC cell line COV362 was cultured in varying concentrations of carboplatin to assess gene expression of angiogenic factors (PGF, IL1B) and Wnt-associated genes (AXIN2, WISP1, and CCND1). Next, I identified which stress-toxicity pathway regulates activation of Wnt/ β -catenin signaling using RT-PCR profiling. In addition, whether inhibition of these suggested pathways results in greater cell death was explored at high-dose carboplatin.

Results: My results show gene upregulation of PGF, IL1B and AXIN2 and downregulation of WISP1 in dose-response to increasing carboplatin. PCR profile revealed altered expression of genes involved in oxidative stress, DNA damage and inflammatory response at low-dose carboplatin which indicates resistance-inducing changes. Moreover, combined use of Wnt inhibitor and carboplatin failed to decrease cell viability in reference to cells exposed to carboplatin only.

Conclusion: The results provide a better insight into the mechanism by which carboplatin-induced stress and toxicity response activates Wnt pathway leading to angiogenesis and cell survival in OC. These findings will potentially provide additional targets to enhance chemotherapeutic efficacy in OC treatment.

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

Matthew J. Cecchini¹, Joanna C. Walsh¹, Jeremy Parfitt¹, Subrata Chakrabarti¹, Rohann Correa², Mary J. MacKenzie³, David K. Driman¹

¹ Departments of Pathology and Laboratory Medicine, and Oncology (Divisions of ²Radiation Oncology, ³Medical Oncology), London Regional Cancer Program London, London Health Science Centre

Background: The treatment for colorectal cancer (CRC) is largely surgical followed by adjuvant chemotherapy in high risk cases. In patients with stage II cancer, there is no clear benefit for chemotherapy and the current tools for assessment of risk of relapse and benefit of chemotherapy are inadequate. A recent study identified with tissue microarrays, that loss of CDX2 by immunohistochemistry (IHC) resulted in a worse prognosis and that this could be utilized to predict patients that would benefit from chemotherapy.

Design: Having observed that CDX2 expression can be patchy, which could affect tissue microarray evaluation, we elected to validate these prior results for clinical practice by using archival cases of stage II colon cancer. The pathology of all cases was reviewed, and three blocks were selected for CDX2 IHC. Related markers of colonic differentiation including CDX1, Muc2, GPX2 and villin were also assessed by IHC on a subset of cases.

Results: We studied 122 cases. CDX2 expression was diffusely lost in 11% and focally lost in 29% of cases. There was significant variation in CDX2 expression in a given tissue section in 53% of cases. We did not identify a difference in survival based on CDX2 expression. Expanding on our results from last year we identified in a subset of cases that Muc2 loss resulted in a reduced survival (HR 4.41; 95% CI 1.36–14.22).

Conclusion: Our results with whole slide IHC are different from the previous study, which used tissue microarrays in which only small parts of the tumor were assessed. This suggests that CDX2 may not be a useful prognostic marker in clinical practice. We have identified that loss of Muc2, a downstream transcriptional target of CDX2, is associated with reduced overall survival. This supports the use of colonic differentiation to identify high risk patients.

The Effect of Ischemia Reperfusion Injury on circRNA Expression in the Heart

Ashraf Altesha¹ and Xiufen Zheng^{1, 2}

¹ Department of Pathology and Laboratory Medicine, Western University

² Diabetes Research Program, Lawson Health Research Institute

³ Pathology and Laboratory Medicine, London Health Sciences Centre

Introduction: Circular RNA are endogenous molecules that display differential expression across species, developmental stages, and pathologies. Increasing evidence has implicated circRNA in the pathogenesis of multiple cardiac diseases including cardiac hypertrophy, myocardial infarction and fibrosis. In this study, we attempt to identify the effect of ischemia reperfusion (I/R) injury on the expression of circRNA in vivo and in vitro. We hypothesize that I/R injury increases circRNA levels in the HL-1 cardiomyocyte cell line in vitro, and in mouse heart tissue in vivo.

Methods: We will simulate the conditions for clinical organ preservation by culturing HL-1 cells in hypoxic conditions (0.5% O2) at 10°C in UW preservation solution for 4h, 8h, 12h, and 24h followed by reperfusion in full medium and normal oxygen conditions. RNA will be extracted, and the expression of select circRNA and their host genes will be measured. We will also demonstrate the effect of I/R injury on circRNA expression in vivo by excising mouse donor hearts, incubating them in UW solution for 24h, and transplanting the hearts into recipient mice. Hearts will then be harvested after 3 days for circRNA measurement.

Results: RnaseR treatment was used to validate the circularity of select circRNA transcripts. I/R injury increased the expression of multiple circRNA, including circFOXO3 and circHIPK3 in the HL-1 cardiomyocyte cell line. Multiple circRNA were heavily upregulated in mouse cardiac tissue after I/R injury including circZSWIM, circZmiz, circCRIM1, and circSULF.

Conclusion: Here we provide evidence that ischemia reperfusion injury modulates the expression of circular RNA in the heart. We demonstrate upregulation of circRNA both in vivo and in vitro. Host genes of the select circRNA were not significantly altered, indicating specificity of I/R injury on the alteration of circular transcripts in particular.

Evidence for a Fragmented Origin of HIV-1/M from a Window Analysis of Near Full-length Genomes

Abayomi S. Olabode¹, Mariano Avino¹, Tammy Ng¹, Faisal Abu-Sardanah¹, David W Dick¹, Art FY Poon¹

¹ Department of Pathology and Laboratory Medicine, Western University

Introduction: Reconstructing the early dynamics of HIV-1 can provide important insights into the initial drivers of the pandemic. Current estimates based on a segment of HIV-1 *env* date the origin of group M to ~1920. However, this estimate is assumed to apply uniformly across the virus genome. Recombination may cause different fragments to have integrated into the founding variant at different times. We tested this hypothesis with a phylogenetic analysis of near full-length HIV-1 genome sequences.

Methods: A total of n=7,816 HIV-1 GenBank records with a minimum of 8,000bps were manually screened for laboratory clones, multiply-sampled individuals and missing collection dates, resulting in 3,908 records. We generated a consensus from a multiple alignment of 100 genomes selected by clustering and network centrality. Next, we used pairwise alignment against the consensus to yield a draft alignment, filtered indel-rich regions and then generated a final alignment using MAFFT. Windows of 500bp were extracted at steps of 100bp, and trees were reconstructed using FastTree2 under the GTR model. Finally, we used the least-squares dating program (http://www.atgc-montpellier.fr/LSD/) to rescale each tree in time under a relaxed clock model.

Results: We found evidence of substantial variation in estimated root dates among windows, with estimates ranging from the early 1900s to the 1950s. Estimates were significantly autocorrelated, which was more consistent with a genome-level effect than uncertainty in phylogenetic reconstruction and dating. We show that the most recent estimates were obtained from windows within the *env* gene region, which has predominantly been targeted in studies of HIV-1 origins.

Conclusion: Our results are consistent with recent studies postulating abundant diversification and recombination of group M in the human population for decades before rapid expansion in the 1950s. The sliding window approach may enable us to accommodate early recombination events by relinquishing the framework of defined subtypes, although we cannot utilize the earliest available samples due to their limited genome coverage.

Molecular Interplay of Apoptosis and Necroptosis in Mitochondrial Dysfunction

Jieping Gong¹, Ingrid Gan^{1, 2}, and Zhu-Xu Zhang^{1, 2}

 Department of Pathology and Laboratory Medicine, Western University
Matthew Mailing Centre for Translational Transplant Studies, London Health Sciences Centre

Introduction: Necroptosis has been shown to promote allograft injury following transplantation by promoting inflammation and ultimately contributing to graft rejection. Currently, there are no treatments targeting these cell death pathways. Previous studies describe necroptosis as directly initiated through RIPK1/3 and MLKL mediated cell lysis. However, recent studies have implicated MPTP, an inner membrane mitochondrial pore, as a significant player in this signaling pathway. Currently it is unclear how MLKL interacts with the mitochondria to induce necroptosis, and whether or not downstream mitochondrial dysfunction pathways are activated in necroptotic signaling. This study aims to investigate this knowledge gap by quantifying changes in necroptotic death following inhibition of downstream mitochondrial pathways.

Methods: Murine microvascular endothelial cells (MVECs) were cultured and treated with necroptosis inducing factors. Cells were treated with either caspase (-1,-2,-3,-5,-9), reactive oxygen species (ROS), or poly (ADP-ribose) polymerase (PARP) inhibitors. Apoptosis-inducing factor (AIF) was silenced in MVEC cells using siRNA and treated with necroptosis inducing factors. Level of necroptotic cell death was quantified using green fluorescent stain.

Results: No significant changes in necroptotic cell death were observed in cells treated with caspase or ROS inhibitors compared to control conditions. Experiments involving AIF are underway, however we expect to see a decrease in necroptotic cell death following AIF silencing.

Conclusion: These results suggest downstream caspase activation does not play a significant role in necroptotic cell death. This supports necroptosis as a caspase-independent form of cell-death. Future directions may include targeting additional downstream pathways to further clarify the effect of downstream mitochondrial signaling in necroptosis in the hopes of providing a therapeutic target to treat cell death and inflammation in organ transplantation.

POSTER ABSTRACT #59

Perceptions of pimping in a pathology residency program – a study using interviews and rich-picture drawings

*Emily A. Goebel*¹, Sayra M. Cristancho², Christopher J. Watling³, David K. Driman¹

¹ Department of Pathology and Laboratory Medicine, Western University

- ² Centre for Education Research & Innovation, Western University
- ³ Department of Clinical Neurological Sciences3, Western University

Introduction: Pimping is a distinct form of questioning in medical education that has proponents and detractors. It occurs in rounds and at one-on-one signout. Our study examines the use of and attitudes toward pimping in a pathology residency program to better understand its value and effectiveness.

Methods: Using grounded theory methodology, we conducted semi-structured interviews with 8 pathology trainees and 9 pathologists. As part of the interview process, we asked participants to draw a rich-picture of a pimping encounter. Consistent with grounded theory principles, we analyzed data iteratively using constant comparison.

Results: Negative emotions including anxiety and self-doubt occur during pimping (figure). For some, these resulted in motivation to study, while for others this was a futile, non-motivating experience. Most trainees felt they were being judged during pimping; however, they perceived that the intentions of pimping were not malicious, and in their best interests. This was supported by pathologists who stated that their motivation for pimping was to identify knowledge gaps, thus benefiting the trainee.

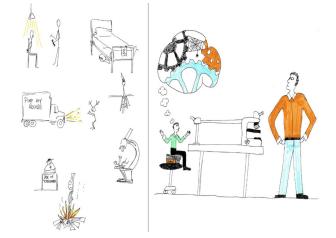


Figure: Two examples of rich-picture drawings illustrating perceptions of pimping encounters.

Conclusion: Recognizing when and how pimping may create negative emotions that may interfere with learning may enable educators to create more consistently meaningful interactions.

Transforming Growth Factor Beta 1 Signalling in Bone Marrow Precursor Cells

Sahana Nirmaiyan¹, Zia A. Khan^{1,2}

Pathology and Laboratory Medicine, Western University
Diabetes Research Program, Lawson Health Research Institute

Introduction: Transforming Growth Factor Beta (TGF β) signalling in stem cells has been demonstrated to be involved in the regulation of the stem cell phenotype. Previous studies on embryonic stem cells (ES cells) have reported TGF β 's role in promoting differentiation while others have reported its role in maintaining the stem cell state. In pursuit of gaining a better understanding of TGF β 's effect on differentiation, a study performed by our lab demonstrated that when bone marrow mesenchymal precursor cells (BM-MPCs) were exposed to TGF β 1, adipogenesis was inhibited. However, whether this inhibition is limited to adipogenesis and the signalling mechanism of TGF β in these cells are both unknown. In this study, we have attempted to examine if TGF β signalling leads to a specific differentiation outcome in BM-MPCs and have subsequently attempted to identify the signalling pathways that are altered due to TGF β signalling. We hypothesize that TGF β regulates bone marrow precursor cell differentiation through Smad-dependent and Smad-independent pathways.

Methods: To examine if TGF β signalling skews the differentiation of these cells, we performed RT-PCR to examine changes in the expression of cell lineage markers upon TGF β 1 exposure. To investigate the mechanism of signalling, BM-MPCs were exposed to either a control medium or TGF β 1 and the changes in intracellular signalling protein activation were examined using immunohistochemistry. We also investigated how the activation of these intracellular proteins changed when inducing the cells to differentiate into adipocytes.

Results: Our results show that TGF β 1 generally does not have an effect on the expression of pluripotency genes and reduces or completely shuts off the expression of most endoderm and ectoderm markers. Our results also show that although the expression of most mesoderm markers is decreased upon TGF β 1 exposure, the induction in expression of hematopoietic-related mesoderm markers CD34 and MIXL1 suggests that the hematopoietic lineage is promoted by TGF β . Immunohistochemistry data on TGF β signalling however was inconclusive.

Conclusion: The RT-PCR results demonstrate that TGF β 1 seems to inhibit most mesodermal differentiation while specifically promoting the hematopoietic mesodermal lineage. Also, further studies need to be done for better resolution on the TGF β signalling mechanism in BM-MPCs. These results in combination with future studies on other stem cell phenotype regulating pathways can be applied to more broad topics such as stem cell expansion and induced cell differentiation.

Characterization and the role of Ghrelin receptor in the vasculature of Duchenne Muscular Dystrophy

Maedeh Naghibosadat^{1, 5}, Leonard Luyt^{4, 6}, Lisa Hoffman^{2, 3, 5, 6}, and Savita Dhanvantari^{1, 2, 5, 6}

¹ Department of Pathology and Laboratory Medicine, Western University

- ² Department of Medical Biophysics, Western University
- ³ Department of Anatomy and Cell Biology, Western University
- ⁴ Department of Chemistry, Western University
- ⁵ Collabrative Program in MSK health, Western University
- ⁶ Lawson Research Institute

Introduction: Duchenne muscular dystrophy (DMD) is a severe neuromuscular disease caused by the absence of dystrophin. This deficit in dystrophin results in chronic skeletal and cardiac muscle degeneration. The absence of dystrophin in heart eventually results in dilated cardiomyopathy after 10 years of age. As the microvasculature of DMD is leaky and aberrant, for an effective therapy, there is a need to characterize and repair the microvasculature. The hormone ghrelin represents a potential therapeutic candidate to correct the cardiac vasculature. Ghrelin binds to the growth hormone secretagogue receptor (GHSR). Both ghrelin and GHSR are present throughout the cardiovascular system, particularly in vascular endothelial cells. My project will focus on characterizing changes in GHSR in the cardiac vasculature during the progression of DMD.

Methods: To test my hypothesis, I will use a murine model of DMD (mdx:utrn-/-) that phenocopies the human condition. We have previously shown that this cohort of mice has developed cardiomyopathy by 8-10 weeks of age. GHSR in the cardiac microvasculature was characterized using Cy5-ghrelin (1-18) at 15-17 weeks of age in both littermate control mice and mdx:utrn-/- mice. To determine whether GHSR levels correlate with an inflammatory vascular phenotype, the proposed inflammatory markers E-selectin, phosphorylated VE-cadherin and CD36 were quantified by immunofluorescence. Fibrosis was detected using Masson's trichrome stain.

Results: Our preliminary results indicate that there is an elevation in GHSR levels in the cardiac microvasculature in mdx:utrn-/- mice. In addition, there appeared to be dramatic increases in levels of the inflammatory markers, E-selectin, phosphorylated VE-cadherin and CD36. There appeared to be large amounts of collagen deposition as well as regions of necrosis throughout the cardiac tissue, indicating severe fibrosis.

Conclusion: Overall, it appears that increases in GHSR in the vasculature of mdx:utrn-/- mice is associated with increases in vascular inflammation. These changes parallel the onset of cardiomyopathy.

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

Milica Krstic^{1,2}, *Matthew J. Cecchini*¹, *Carl O. Postenka*^{2,3}, *Joseph Andrews*^{2,3}, Hon S. Leong ^{1,4}, *Muriel Brackstone*^{4,5}, *Ann F. Chambers*^{1,2,3}, *Alan B. Tuck*^{1,2,3}

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

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

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

Results: Overexpression of TBX3iso1 or TBX3iso2 in non-invasive 21NT cells resulted in increased survival, growth and invasiveness both in vitro and in vivo in the chick embryo. Genome-wide ChIP-array studies coupled to RNA-Seq revealed that both TBX3 isoforms promote invasiveness through altered expression of EMT-related genes, including direct up-regulation of SLUG. SLUG expression was required for TBX3-induced migration and invasion, likely through up-regulation of MMP14, which is particularly relevant in early stages of progression. Assessing TBX3 levels in early stage breast cancer by immunohistochemistry revealed high expression in low-grade lesions. Within our low-grade cohort, we saw an association between TBX3 expression in the DCIS and size of the invasive focus. All findings suggest the involvement of TBX3 in progression through the low-grade molecular pathway. We are currently assessing whether there is an association with TBX3 and downstream effectors identified by our genomic/transcriptomic studies, or with any clinical follow-up information. Interestingly, only TBX3iso1 overexpressing cells exhibited significant tumorigenic potential in nude mice. Data mining and supporting functional studies suggest this is likely due to promotion of angiogenesis and secretion of cancer-associated cytokines upon TBX3iso1 overexpression.

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

POSTER ABSTRACT #63

Acetylsalicylic acid reduces collagen contraction, remodelling and myofibroblast proliferation in subconjunctival tissue mimetic

James J. Armstrong, James Denstedt and Cindy M. L. Hutnik

¹ Department of Pathology and Laboratory Medicine, Western University

Purpose: Glaucoma surgery outcomes, traditional as well as MIGS, are hindered by inflammation-driven excessive wound healing responses. As such, perioperative corticosteroids are often used as a means to control pre-existing and/or surgically induced inflammation. NSAIDs are used to a lesser extent in glaucoma surgery, however are ubiquitous in cataract surgery. The more specific nature of COX1/2 inhibition by NSAIDs allows fewer unwanted side effects, such as steroid associated intra-ocular pressure (IOP) spikes. Long duration of action is beneficial for topically applied medications, as such, we assessed the efficacy of irreversible COX-1/2 inhibitor - acetylsalicylic acid (ASA) – to prevent collagen contraction, remodelling and myofibroblast proliferation *in vitro*.

Methods: Human Tenon's capsule fibroblasts (HTCFs) were cultured within both restrained and unrestrained collagen matrices that were augmented with HTCF secretory proteins. In the unrestrained, contraction was measured hourly for 12hr, then daily for 4 days. After which total RNA was extracted for gene expression analysis. The restrained matrices were allowed to mature under self-imposed tension for 7 days, then fixed and sectioned for histological analysis.

Results: At all time points, contraction was significantly reduced by ASA (0.02%) compared to control (p < 0.001). This effect was dramatically increased by gentamicin (0.01%), such that combination treatment resulted in no contraction (p < 0.001). Expression of Col3A1, Col2A1 and ACTA2 were all significantly decreased compared to control (p < 0.01). Restrained matrices treated with ASA displayed significantly smaller, less dense staining collagen fibers compared to control (p < 0.01). Immunostaining for alpha smooth muscle actin and Ki67, indicating proliferating myofibroblasts, was increased in control sections versus those treated with ASA. TUNEL staining revealed comparable rates of cell death across treatment groups (p > 0.05).

Conclusion: The results of this study demark acetylsalicylic acid as a candidate drug for perioperative inflammation control and wound healing modulation in glaucoma filtration surgery.

Partial depletion of regulatory T cells prevents secondary pseudomonas aeruginosa infection post sepsis by enhancing host inflammatory response

Guang-Ju Zhao^{1, 2, 3}, Zhi-Qiang Hu¹, Zhong-Qiu Lu¹, Tianqing Peng^{2, 3, 4}

¹ Emergency Department, the First Affiliated Hospital of Wenzhou Medical University

² Lawson Health Research Institute, London Health Sciences Centre

³ Department of Pathology and Laboratory Medicine, Western University

⁴ Department of Medicine, Western University

Introduction: Immune dysfunction contributes to secondary infection and worse outcomes in sepsis. It is well known that regulatory T cells (Tregs) induces immunosuppression. A recent study reported that complete deletion of Tregs failed to rescue septic mice. However, it remains to be determined whether inhibition of Tregs plays a protective role in secondary infection after sepsis.

Methods: Sepsis was induced in mice by Cecal Ligation and Puncture (CLP). After 3 and days of CLP, mice received pseudomonas aeruginosa via the trachea as a second hit, respectively. Anti-CD25 mAb was injected 24 hrs before the second hit. The protein levels of IL-17A, IL-1 β , IL-6 and IL-10 were measured in tissue lysates. Bacterial load was determined in bronchoalveolar lavage fluids. Histological changes were assessed in lung.

Results: Pathological changes and bacterial load in lung and mortality were increased by infection with pseudomonas aeruginosa in septic mice (3 days post CLP) compared with non-septic mice. These effects of existing sepsis on secondary pseudomonas aeruginosa infection were associated with an increased number of Tregs in both lung and spleen, leading to lower levels of IL-17A, IL-1 β and IL-6 production and higher levels of IL-10 production in lung. Furthermore, injection with PC61 (anti-CD25) mAb reduced the number of Tregs by 50% in spleen and 60% in lung of septic mice. This partial depletion of Tregs elevated IL-17A, IL-1 β and IL-6 production, and decreased IL-10 levels in septic mice with pseudomonas aeruginosa infection, leading to lower bacterial load, attenuation of lung injury and improvement of survival.

Conclusions: This study provided evidence demonstrating that enhanced secondary infection-induced tissue damage in septic mice correlates with an increased number of Tregs, and that partial depletion of Tregs rescues septic mice with secondary bacterial infection. Thus, Tregs may be a potential therapeutic target for limiting secondary infection in sepsis.

Comparing Bone Marrow Staging and Original Lymph Node Pathology in Non-Hodgkin's Lymphoma

Rachel Peet¹ and Ian Chin-Yee^{1, 2}

- ¹ Department of Pathology and Laboratory Medicine, Western University
- ² Department of Medicine, Division of Hematology,
- London Health Sciences Centre, Western University

Introduction: Non-Hodgkin's lymphoma (NHL) is a heterogeneous group of cancers that develops in the lymphatic system. Traditional staging involves lymph node (LN) or tissue biopsy for initial diagnosis, followed by bone marrow (BM) aspirate and biopsy to assess BM involvement (BMI), a common site for lymphomatous infiltration. Newer technologies such as imaging by 18-F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) are being increasingly used and have been reported in some studies as a more sensitive method for assessing BMI. Additionally, approximately 3-5% of the general population may harbour clonal B cells in their blood, a newly identified condition called monoclonal B cell lymphocytosis (MBL). The aim of this study is to determine if incidental MBL in the marrow may be a cause of upstaging with BMI in NHL patients.

Methods: We retrospectively reviewed charts of 489 patients who underwent BM biopsy for hematology/oncology studies, from August 2016-January 2018 at London Health Sciences Centers. We identified patients who underwent BM biopsy for initial staging for NHL (n = 66). For these patients, we collected data including patient demographic, NHL subtype and stage, initial LN histology, BM biopsy flow cytometry, and PET results (n = 58).

Results: 10 patients had positive BM biopsy results. We observed concordant immunophenotypes for LN and BM biopsy results in 5 of these patients, the remaining required additional interpretation. 45 of 58 patients exhibited concordance between BM biopsy and PET results. For discordant results, we examined the interpretation of clinical staging to determine whether the patient was upstaged based on BMI due to MBL as opposed to the original lymphoma.

Conclusion: Our findings suggest that incidental monoclonal B lymphocytosis is not of concern for clinical staging of NHL patients. Understanding the implications of incidental monoclonal populations in the BM is essential for appropriate staging and treatment of lymphoma patients.

Nrf2 regulation in the antioxidant response in cancer

Vy Ngo, Anne Brickenden, Nadun Karunatilleke, Zheng Song, James Wing-Yiu Choy and Martin Duennwald

¹ Department of Pathology and Laboratory Medicine, Western University

Introduction: Nrf2 is the master transcription factor that regulates antioxidant response pathways crucial for detoxifying reactive oxygen species (ROS) in cells. However, hyperactive Nrf2 is also linked to in poor prognosis in numerous cancers, therefore, elucidating the mechanisms of Nrf2 regulation in cancer is of utmost importance. Specific protein-protein interactions of Nrf2 determine its protein folding status, stability, subcellular localization, and activity and thus, selectively alter the oxidative stress response in cellular homeostasis and cancer.

Methods: To study Nrf2 regulation, we have established and characterized yeast models expressing human Nrf2, its cancer-associated mutants, and its interacting proteins. We furthermore use cancer cell lines to determine aberrant Nrf2 activity.

Results: Our studies show that Nrf2 activity is tightly controlled by its interaction with select proteins. Surprisingly, we show that cancer-associated mutants of Nrf2 and its key repressor, Keap1, are prone to misfold and aggregate upon exposure to high levels of oxidative stress. Finally, we discovered that both Nrf2 and Keap1 interact with the molecular chaperone Hsp90, a major heat shock protein, indicating an intriguing link between the antioxidant and the heat shock response. We will build on these interesting results and elucidate the functional significance of Nrf2 protein-protein interactions in the antioxidant response in cancer.

Discussion: Our research provides novel insight into previously unexplored aspects of Nrf2 regulation by aggregation and interaction with Hsp90 and may thus indicate previously unexplored therapeutic strategies for the treatment of cancer.

A Case of Hirschsprung Disease with Total Colonic Aganglionosis and Small Bowel Involvement

Alyshia Phillips¹, Matthew J. Cecchini¹, Joanna C. Walsh¹

¹ Department of Pathology and Laboratory Medicine, Western University

Introduction: Hirschsprung disease (HD) or congenital aganglionic megacolon is characterized by the absence of enteric ganglia along a variable length of intestine. Affected patients typically present during the neonatal period with obstructive symptoms. The pathogenesis underlying HD involves a failure of neural crest cell migration from cecum to rectum, producing a portion of intestine that lacks both the Meissner submucosal plexus and Auerbach myenteric plexus. Peristalsis is thus absent, resulting in intestinal obstruction.

Case: A three-day old male initially presented with blood-tinged emesis and failure to pass meconium. Pregnancy was unremarkable apart from maternal drug use, spontaneous vaginal delivery was uncomplicated and no other congenital abnormalities were identified. He underwent an exploratory laparotomy and resection for ischemic bowel. No ganglion cells were identified in the resected bowel and he continued to have feeding intolerance and signs of obstruction on imaging. Rectum and ileostomy were biopsied and found to be negative for ganglion cells. He underwent resection of the aganglionic segment (which showed prominent enterocolitis) with ganglion cells identified in the jejunum on frozen section. This left only 65 cm of functional bowel remaining distal to the ligament of Treitz.

Discussion: Worldwide, HD is estimated to occur once in every 5000 live births with a male predominance. Most affected individuals (80%) present with aganglionosis in the rectosigmoid colon. Less commonly, HD extends to total colonic aganglionosis (8-10%) and even more rarely (1%) the disease involves the proximal small bowel. This manifestation of long segment HD has increased morbidity and mortality as it puts the patient at greater risk for short bowel syndrome (SBS), long-term TPN-dependence and enterocolitis. SBS predisposes individuals to hepatic failure due to prolonged TPN, nutrient malabsorption, infections and other health complications. The presented case highlights a rare pathological variant of HD involving proximal small bowel.

Models and Peer Teaching as Pedagogical Methods in the Surgical Pathology Laboratory

Emily G. M. Kyle¹, Jose A. Gomez^{1, 2}, Nancy G. Chan^{1, 2}

- ¹ Department of Pathology and Laboratory Medicine, Western University ² Department of Pathology and Laboratory Medicine,
- London Health Sciences Centre

Introduction: Pathologists' Assistants (PAs) are highly trained health care professionals who examine ("gross") patients' surgical specimens for diagnosis and treatment planning. During their training, graduate students learn using real patient material.

Aim: To help prepare students for approaching patient samples, hands on activities using clay models and cheese strings were used to imitate prostatectomy and salpingectomy specimens respectively. Peer teaching was also assessed as a pedagogical method.

Methods: This study's first author was taught to section an imitation prostate using a clay model and use the SEE-FIM protocol for salpingectomies using cheese strings. The clay model mimicked the anatomy and orientation of radical prostatectomy tissue encountered in the hospital laboratory. The cheese string was "stripped" on one end to imitate fimbriae. The student "teacher" then taught her classmates as well as first year pathology residents how to "gross" these cases.

Results: Students and residents generally felt more confident about their ability to handle prostatectomy and salpingectomy cases in the gross room after this hands on, peer led activity. Some trainees noted they felt no significant difference between asking questions of a peer teacher compared to a pathologist.

Discussion: Hands on activities using models provide a safe environment for teaching the approach to patients' surgical specimens. Peer teaching is an effective pedagogical method.

The Role of Cyclooxygenase in Colitis-Associated Colon Cancer

*Hayley J. Good*¹, *Elena N. Fazio*¹, *Alice E. Shin*¹, *Liyue Zhang*¹, and Samuel Asfaha¹

¹ Department of Medicine, Department of Pathology and Laboratory Medicine, Western University, London Regional Cancer Program, Lawson Health Research Institute

Introduction: Inflammatory bowel disease (IBD), a chronic state of colonic inflammation, is a major risk factor for the development of colorectal cancer (CRC). Despite the clear link between inflammation and cancer, the mechanism by which colitis leads to cancer is unknown. Doublecortin-like kinase-1 (Dclk1) is a marker of tuft cells, a rare and ill-defined cell type of the colon. We previously showed that Dclk1+ cells are quiescent and long-lived, and remain resistant to proliferation even upon mutation of the tumour suppressor APC, but become cancer-initiating cells upon exposure to inflammation. Interestingly, Dclk1+ tuft cells express high levels of cyclooxygenase (COX)-1 and -2, the direct enzyme target of non-steroidal anti-inflammatory drugs (NSAIDs) which are known chemopreventative drugs in CRC. Thus, we aimed to determine the effects of COX inhibition by NSAIDs on colitis-associated cancer.

Methods: Dclk1^{CreERT2}/APC^{flox/flox} mice were administered tamoxifen to induce an APC mutation in Dclk1-expressing cells. Mice were then exposed to the colitis-inducing agent dextran sodium sulfate (DSS), followed by daily treatment with Aspirin (non-selective COX inhibitor), celecoxib, rofecoxib (COX-2selective inhibitors), or vehicle for the remainder of the experiment. 16 weeks post-tamoxifen, colonic tumour number and size were analyzed to determine the effect of NSAIDs on tumour initiation and growth, respectively. Extent of inflammation was assessed by myeloperoxidase activity and histology, and colonic tissue was analyzed for measurement of inflammatory mediators influencing tumourigenesis by qRT-PCR and protein array.

Results: Treatment with Aspirin, but not celecoxib or rofecoxib, significantly reduced the number of Dclk1+ cell-derived colonic tumours. There was no significant difference in tumour size or degree of colitis between vehicle and NSAID-treated groups.

Conclusions: These findings suggest a role for COX-mediated inflammation in colonic tumourigenesis arising from Dclk1+ cells. Our results suggest that Aspirin, rather than COX-2-selective NSAIDs, may be useful for chemoprevention of CRC in patients with IBD.

Characterization of Effects of Growth Differentiation Factor 15 on T Cells

Vitali Veramkovich¹, James Koropatnick², Lisa Cameron¹, and Xiufen Zheng¹

¹ Department of Pathology and Laboratory Medicine, Western University ² London Regional Cancer Program, Lawson Heath Research Institute

Introduction: Elevated plasma levels of growth differentiation factor 15 (GDF15) have been observed in patients suffering from a wide variety of ailments. Particular attention has been devoted to studies focusing on patients affected with heart disease and cancer. In relation to cardiomyocytes, GDF15 has been previously shown to have a protective role. In cancer patients, GDF15 has been associated with both pro-tumoral and anti-tumoral activities. 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.

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, Western blot, 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: Preliminary results show that GDF15 reduces the rate of proliferation and increases T cell viability. Furthermore, the data suggests that GDF15 modulates cytokine secretion profile of T cells. Treatment of GDF15 deficient cells with exogenous GDF15 at concentrations below 25 ng/ml is only able to partially rescue the phenotype.

Results: Students and residents generally felt more confident about their ability to handle prostatectomy and salpingectomy cases in the gross room after this hands on, peer led activity. Some trainees noted they felt no significant difference between asking questions of a peer teacher compared to a pathologist.

Conclusion: Preliminary findings suggest that GDF15 is involved in differentiation, viability and activity of T cells. The results implicate GDF15 as an important factor in T cell immunoregulation.

Understanding the Post-surgical Wound Healing Response of Tenon's Capsule Fibroblasts in Glaucoma Patients

Josh Dube¹, James J. Armstrong^{1, 2}, Cindy Hutnik^{1, 2, 3}

¹ Department of Pathology and Laboratory Medicine, Western University

- ² Department of Ophthalmology, Western University
- ³ Ivey Eye Institute, St. Joseph's Health Care

Introduction: Excessive scarring following glaucoma filtration surgery is a common cause of surgical failure. Glaucoma patients often display exuberant post-surgical scarring which may be due to excessive inflammation caused by poor inflammation resolution. In addition, glaucoma patients' ocular tissue is routinely exposed to prostaglandin analogue medication for extended periods of time to reduce intraocular pressure. However, it is unclear whether chronic exposure to prostaglandin analogue medication affects specialized pro-resolving mediator (SPM) receptor expression. SPM's are a growing class of signaling molecules important in mediating inflammation resolution and controlled wound healing. The present study is aimed at investigating the expression of a particular SPM RvD1 receptor known as GPR32, in Tenon's Capsule of glaucoma patients. In addition, the effect of PGF2 α on fibroblast scarring propensity will also be assessed.

Methods: To assess GPR32 levels, a primary culture of human Tenon's capsule Fibroblasts (HTCF's) as well as Tenon's capsule whole tissue from glaucoma and non-glaucoma patients were stained with antibodies against the GPR32 receptor using immunocytochemistry and Western blot. To determine the effect of PGF2 α on fibroblast scarring propensity, HTCF's were treated with TGF β for 24h to induce differentiation into pro-scarring myofibroblasts. The cells were then treated with a single dose of PGF2 α (0.5ng/mL, 5ng/mL, 50ng/mL) for 3 days followed by measurement of the change in α -SMA protein levels using Western blot.

Results: The RvD1 receptor is expressed in the Tenon's capsule of patients with glaucoma. In addition, PGF2 α treatment decreases the level of α -SMA protein expression in HTCF's.

Conclusions: These findings indicate that a deficit in RvD1 receptor expression is not a likely mechanism of increased scarring following glaucoma filtration surgery. In addition, these results show that prostaglandin treatment does not appear to promote, and may even reduce, fibroblast scarring propensity in Tenon's capsule of glaucoma patients.

c-Kit and IR interplay on β -cell proliferation and intracellular signalling in INS-1 cells

Amanda Oakie^{1, 2}, Jinming Li^{1, 3}, and Rennian Wang^{1, 3}

- ¹ Children's Health Research Institute
- ² Department of Pathology and Laboratory Medicine
- ³ Department of Physiology and Pharmacology, University of Western Ontario

Introduction: Receptor tyrosine kinases (RTK) are present on β -cells and activate intracellular signalling pathways involved in their proliferation, survival, and function. The RTKs c-Kit and insulin receptor (IR) are required for maintaining β -cell mass, and the loss of function in either receptor promotes the development of glucose intolerance in mice. Previous work from our lab demonstrated that overexpression of c-Kit in β -cells could enhance insulin secretion with increased IR and insulin receptor substrate (IRS) expression. This study examines the interplay between c-Kit and IR receptor signalling in β -cell function and proliferation.

Methods: INS-1 832/13 cells were used to determine RTK interplay with the following treatments: 1) siRNA knockdown of c-Kit or IR, with or without SCF stimulation; and 2) co-stimulation of c-Kit and IR with ligands stem cell factor (SCF; 50 ng/ml) and insulin (0.2 nM), respectively.

Results: Under 24-hour SCF treatment alone, INS-1 cells displayed increased IR and IRS mRNA and protein levels. Knockdown of c-Kit using siRNA reduced IR and IRS levels, phosphorylation of Akt (S473), and cell proliferation. Preliminary findings from IR siRNA disruption studies showed that SCF increased phosphorylated Akt and cyclin D1 levels in control siRNA cells, yet both were inhibited in IR siRNA + SCF cells. Treating INS-1 cells with a combination of SCF and insulin increased phosphorylated Akt and GSK3 β when compared to untreated cells. However, there was no compounded increase in co-stimulated cells when compared to SCF alone or insulin alone.

Discussion: The reduction of c-Kit signalling adversely affects IR and IRS levels and compromises proliferation. The disruption of IR signalling may interfere with SCF –stimulated β -cell function. Co-stimulation of both receptors did not lead to synergistic intracellular signalling. Further experiments will focus on how c-Kit and IR stimulation and disruption affect factors downstream of PI3K/Akt signalling and proteins of insulin exocytosis.

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

Madison Wallace^{1, 2}, Rennian Wang^{1, 3}

- ¹ Children's Health Research Institute, Lawson Health Research Institute
- ² Department of Pathology and Laboratory Medicine
- ³ Department of Physiology and Pharmacology, Western University

Introduction: Quiescent pancreatic stellate cells (PaSC) can become activated (α -SMA+) due to stress or injury. Activated PaSCs produce extracellular matrices (ECM), growth factors, and cytokines that, if over produced, result in increased fibrotic and inflammatory responses within pancreatic islets. We hypothesize that (1) activated PaSCs increase β -cell proliferation through cell-cell, cell-matrix, and/or growth factor interactions; however, (2) chronic activation of PaSCs will lead to β -cell dysfunction via increased immune and fibrotic changes.

Methods: Male C57BL/6J mice are fed a 60% kcal high fat diet (HFD) starting at 6-weeks of age to stimulate a progressive T2DM model. Control mice are maintained on a normal diet (ND). Mice will be examined at 4-, 8-, and 22-weeks for the activation of PaSCs and subsequent effects on β -cell proliferation and function. The effects of inhibiting PaSC activation will be assessed by (1) treating HFD mice with NADPH oxidase inhibitor diphenylene iodonium (DPI) one week prior and throughout a 4-week HFD feeding period; and (2) administering DPI after 10-15 weeks on HFD to examine whether reducing the number of chronically active PaSCs can decrease negative impact on β -cell function.

Results: Preliminary data revealed that HFD mice began to develop increased IPGTT AUC levels after 4-weeks on their diet, and this was significantly increased after 10- and 20-weeks on the HFD when compared to ND mice. Preliminary HFD studies at 20-weeks have shown significantly enlarged β -cell mass, impaired glucose tolerance and intra-islet inflammation. Significantly increased intra-islet α -SMA+ signalling was observed.

Summary: Future studies will focus on determining the point of PaSC activation in vivo during a HFD, and their subsequent effects on the progression towards T2DM. Increased expression of α -SMA, along with other PaSC markers, will be used to assess activation. This study will help enhance our understanding of factors that effect β -cell viability and function.

Prevalence of Thrombophilia in Suspected Stroke Patients.

*Christopher Tran*¹, Omar Raslan², Luciano Sposato^{3, 4}, Alejandro Lazo-Langner^{2, 4}

¹ Department of Pathology and Laboratory Medicine, Western University

- ² Department of Medicine, Division of Hematology, Western University
- ³ Department of Clinical Neurological Sciences, Western University
- ⁴ Department of Epidemiology and Biostatistics, Western University

Introduction: Ischemic strokes are very common and can be classified based on etiology. If no etiology is found after a standard stroke evaluation, the stroke is deemed cryptogenic and requires additional evaluations. One of these advanced evaluations is the genetic screening for inherited thrombophilia. Current evidence suggests that thrombophilia screening is over utilized in stroke patients and may provide more risks than benefits.

Methodology: 500 suspected stroke/TIA patient who had a thrombophilia screen test will be included in this retrospective study. We will determine the proportions of positive thrombophilia screen in our population using Wilson's score. Finally, if appropriate, we will identify the relationship between inherited thrombophilia and suspected stroke/TIA events.

Expected Results: We expect a low prevalence of inherited thrombophilia in our patient population. We also believe that there will be no relationship between inherited thrombophilia and suspected stroke/TIA events.

Significance: The results of this study can help clarify whether thrombophilia screening is beneficial for ischemic stroke patients. Our assessment may lead to developing more defined criteria when ordering a thrombophilia screen for stroke patients.

Effects of Acute Sepsis in the Liver of an Adult Intrauterine Growth-Restricted Offspring

Anastasiya Vinokurtseva¹ and Edith Arany^{1, 2, 3}

¹ Department of Pathology and Laboratory Medicine, Western University

² Diabetes Research Program, Lawson Health Research Institute

³ Children's Health Research Institute

Introduction: Intrauterine Growth Restriction (IUGR) increases the predisposition to metabolic diseases in adulthood. One risk factor is maternal protein restriction (LPD) during gestation and lactation. Interestingly, an acute insult such as sepsis has not been studied in offspring of mothers exposed to protein deficiency. Recently, a family of nuclear receptors that regulate the expression of genes that modulate transcriptional activities and epigenetic changes were identified. One of them, peroxisome proliferator-activated receptor α (PPAR α) is a key regulator of nutrient/energy homeostasis and a modulator against chemical toxicants in the liver. Downstream of PPAR α , a novel hepatokine FGF21 may have an important role in preventing oxidative stress in sepsis. In this study we aim to understand the role of these nuclear receptors and their downstream targets in our LPD model in the presence of an acute sepsis.

Methods: Offspring of mothers treated with control (20% protein) or low protein (8% protein) diets were studied. At 4 months of age, they received a single intraperitoneal injection of fecal slurry and were sacrificed 6 hours later. Liver was dissected and frozen in liquid nitrogen or fixed in 10% formalin. Tissues were embedded in paraffin and sectioned for histological analysis. Ten fields of view were imaged per animal and fat inclusions were quantified by image analysis. RNA was extracted using Trizol. Expression profiles of PPAR α , FGF21, TNF α , TGF- β , IL-1 α , IL-1 β , IL-6 were analyzed by qPCR.

Results: By Hematoxylin and Eosin (H&E) and Oil Red O (ORO) staining, we found fat droplets within hepatocytes surrounding the periportal areas in male LPD offspring. Masson Trichrome (MT) staining showed a thickening of the Glisson capsule in both female and male LPD septic offspring.

Conclusions: Understanding the underlying molecular mechanisms in the first stages of sepsis in IUGR individuals may help improve treatment strategies in these patients.

MALAT1 and HOTAIR: Key Epigenetic Regulators in Diabetic Retinopathy

Saumik Biswas¹, Anu Thomas¹, Biao Feng¹, Shali Chen¹, Erfan Aref-Eshghi¹, John Gonder^{1, 2}, Bekim Sadikovic¹, Subrata Chakrabarti¹

¹ Department of Pathology and Laboratory Medicine, Western University ² Department of Ophthalmology, Western University

Introduction: With the increasing incidence of diabetic retinopathy (DR), the need for novel and specific therapy is essential. Genomic studies have identified several long non-coding RNAs (IncRNAs). However, majority of IncRNAs have not been characterized in DR. We examined the roles of IncRNAs MALAT1 and HOTAIR in DR.

Methods: Human retinal endothelial cells (HRECs) were cultured in 5 mM/L (NG) or in 25 mM/L (HG) glucose. RNA expressions of MALAT1, HOTAIR, and inflammatory and angiogenic cytokines were tested. HRECs were similarly examined following siRNA-mediated MALAT1 or HOTAIR knockdown, or treatment with histone (DZNep) and DNA methylation (5'-aza-dC) blockers. DNA methylation patterns and RNA-protein interactions were analyzed. Retinal tissues from Malat1 knockout (KO) and wild-type (WT) mice with or without diabetes were examined. We further assessed MALAT1 and HOTAIR in human diabetic and non-diabetic vitreous.

Results: HG caused upregulations of MALAT1, HOTAIR, IL-6, TNF- α , and VEGF-A transcripts in HRECs. HG increased binding of EZH2 (a PRC2 component) with MALAT1 and HOTAIR and evoked unique DNA methylation patterns in the MALAT1 and HOTAIR CpG regions. DZNep, 5-aza-dC and knockdown of MALAT1 or HOTAIR reduced mRNA expressions of IL-6, TNF- α , and VEGF-A. Similarly, diabetes-induced elevations of these inflammatory and angiogenic cytokines in the retina were prevented in the Malat1 KO mice. Furthermore, MALAT1 and HOTAIR levels were elevated in the diabetic vitreous.

Conclusions: Our findings allude to the importance of IncRNAs in influencing DR through epigenetic mediators. Understanding the role of IncRNAs may allow the development of better-targeted therapies in DR.

Characterizing the Mechanisms of LETM1 EF-hand Function

Rachel Lee¹ and Peter B. Stathopulos²

¹ Department of Pathology and Laboratory Medicine, Western University ² Department of Physiology and Pharmacology, Western University

Introduction: Monoallelic deletion of leucine-zipper EF-hand-containing transmembrane protein 1 (LETM1), a Ca²⁺/proton (H⁺) antiporter on the inner mitochondrial membrane, is responsible for development of seizures in Wolf-Hirschhorn Syndrome (WHS). Previous work from our lab demonstrated that the presence of Ca²⁺ and low pH increase the α -helicity and thermal stability of the Ca²⁺-binding EF-hand motif. Since LETM1 is postulated to have insufficient transmembrane domains for channel formation and that EF-hands tend to function in pairs, we hypothesize that EF-hand oligomerization may play an important role in LETM1 function and regulation.

Methods: We first purified LETM1 643-699 – a construct containing its EFhand. Oligomerization in Ca²⁺-loaded and Ca²⁺-free conditions, as well as at pH 6, 7, and 8 was studied using size-exclusion chromatography with in-line multiangle light scattering (SEC-MALS). Intrinsic protein fluorescence is sensitive to conformational changes; thus, we inserted a tyrosine at position 667 (LETM1 643-699 Y-insert) to 1) determine if Ca²⁺ binding and pH affects conformation of the EF-hand, and 2) to measure macroscopic Ca²⁺-binding affinity of the EF-hand.

Results: Our results show an increase in molar mass in the Ca²⁺-loaded state across all pH conditions. This increase corresponds to a shift from the theoretical monomeric weight to a dimeric weight, suggesting that Ca²⁺ induces LETM1 EF-hand oligomerization. Ca²⁺ titrations showed that the EF-hand undergoes the canonical closed-to-open conformational transition when binding Ca²⁺, indicating an increased polar environment near the inserted Tyr. Further, Ca²⁺-binding affinity is relatively weak, suggesting a regulatory role when Ca²⁺ levels near the EF-hand are high.

Discussion: We show that Ca²⁺ induces dimerization of the LETM1 EFhand and that this important regulatory motif is conformationally sensitive to relatively high levels of Ca²⁺. These findings indicate that the EF-hand structure is sensitive to Ca²⁺ and pH, suggesting a conformation-based regulatory mechanism, dependent on local Ca²⁺ and H⁺ concentrations.

Characterization of Gingival Fibromas

Nicole Smith¹, Linda Jackson-Boeters¹, Georgia Nikoloudaki², Zia Khan¹, Douglas Hamilton², Mark Darling^{1,3}

¹ Department of Pathology and Laboratory Medicine, Western University

- ² Department of Anatomy and Cell Biology, Western University
- ³ Department of Pathology and Laboratory Medicine, London Health Sciences Centre

Introduction: Gingival fibromas (GFs) are proliferations of fibrous tissue which present as pink-red, sessile or pedunculated, often non-ulcerated gingival masses. Peripheral ossifying fibromas (POFs) are gingival growths that present similarly to GFs but are more commonly ulcerated. Histologically, both lesions demonstrate a cellular fibrous proliferation; however, POFs also contain areas of ossification. GFs have not been well described in the literature; therefore, our aim was to characterize and compare these lesions to POFs using histochemistry and immunohistochemistry.

Methods: GFs presented at a later average age than POFs (49 vs. 29 years). Both lesions were more common in females. Histologically, both lesions demonstrated dense fibrous connective tissue covered by stratified squamous epithelium. Foci of ossification were present in all POFs. Mild surface erosion was present in 20% of GFs and 60% of POFs were partially ulcerated. Chronic inflammation was present in 70% of GFs and 80% of POFs. All lesions stained with aSMA, SATB2, and periostin with the following immunoreactive scores:

GF:	aSMA 3.7±1.33	SATB2 6.4±0.97	periostin 5.4±1.13
POF:	aSMA 5.4±1.52	SATB2 7.4±0.55,	periostin 6.4±0.55

with no significant differences (aSMA p=0.0553, SATB2 p=0.0849, periostin p=0.124).

Conclusions: Our research demonstrated that although GFs and POFs appear to be distinct lesions, their immunohistochemical staining profiles are similar.

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