MAY 27, 2016

Pathology Day 2016

ABSTRACT BOOK POSTERS & ORAL PRESENTATIONS

PATHOLOGY DAY 2016 SPEAKERS

JAMES HOGG LECTURE

ANDREW CHURG, MD, PHD

Professor of Pathology at the University of British Columbia and a Pathologist at Vancouver General Hospitala

GUEST SPEAKER

JACQUELINE QUANDT, PHD

Assistant Professor
Professor, Department of Pathology and Laboratory Medicine, UBC

GUEST SPEAKER

MARTIN WALE, BMBS, MBA

Acting Executive Vice President & Chief Medical Officer, Vancouver Island Health Authority

GUEST SPEAKER

KARAMA ASLEH-ABURAYA, PHD

Post Doctoral Fellow
Dr. Torsten Nielsen's lab

KEYNOTE SPEAKER

KENNETH ALDAPE, MD

Head of the MacFeeters-Hamilton Brain Tumour Centre at Princess Margaret Cancer Centre/Ontario Cancer Institute and Professor of the Department of Pathology, University Health Network

Research location: Toronto General Hospital/Research Institute (UHN)

Primary Research Area: Cancer
Secondary Research Area: Brain & Neuroscience

Research Statement: Dr. Aldape is a neuropathologist with a research interest in primary brain tumors.

He has experience with identification of biomarkers in gliomas, including microarray studies. He has identified a mesenchymal subgroup of glioma, which may be related to microenvironmental factors. His work characterizes glioma subtypes as well as identifies clinically relevant molecular alterations in these tumors.

Featuring oral and poster presentations from graduate students, residents and other trainees

UBC a place of mind

THE UNIVERSITY OF BRITISH COLUMBIA

PATHOLOGY laboratory medicine UNIVERSITY OF BRITISH COLUMBIA

Welcome

THANK YOU ALL FOR YOUR SUPPORTS

Pathology Day is a critically significant event in the departmental calendar as it serves as a time to showcase the broad spectrum of scholarly activities performed by our trainees and, by extension, our faculty. This gathering also provides a perfect venue to recognize outstanding contributions by members of the department. Pathology Day serves another very important function as well. It is an opportunity to get together to socialize and learn more about one another as well as gain an appreciation and understanding for the breadth of scholarly activities that take place in our geographically dispersed department. As for last year, a few members of our department will present a brief overview of their work as a way to facilitate this appreciation and understanding. We are very fortunate to have two outstanding individuals participate in the program this year, highlighting academic excellence and continuing in the tradition of having world leaders in their disciplines speak at Pathology Day. Dr. Andrew Churg will give the James Hogg Lecture, while Dr. Kenneth Aldape (Professor, Laboratory Medicine and Pathobiology, University of Toronto and Neuropathologist, Princess Margaret Cancer Centre, Toronto) is our Keynote Speaker.

I wish to extend my sincere thanks and gratitude to members of the committee responsible for organizing the event, including Dr. Avi Ostry, Dr. Corree Laule, Dr. Tony Ng, Cristina Low, and Adeline Chan, as well as all the other individuals whose efforts make the day a success.

MICHAEL ALLARD
PROFESSOR AND HEAD
DEPARTMENT OF PATHOLOGY & LABORATORY MEDICINE
FACULTY OF MEDICINE
UNIVERSITY OF BRITISH COLUMBIA

Acknowledgements

THE PATHOLOGY DAY COMMITTEE

Mike Allard

Corree Laule

Tony Ng

Avi Ostry

Pathology Day is a team effort and we would like to extend our thanks to everyone who contributed to the 2016 edition.

Cristina Low and Adeline Chan have been instrumental in handling the administrative and practical details of Pathology Day. Debbie Bertanjoli designed the website and managed the website tools in addition to preparing the abstract book.

We also wish to express our gratitude to the many department members who contributed their time and expertise to reviewing abstracts, moderating the oral sessions, and judging the oral and poster presentations.

This year these include:

- Lenka Allan
- MeiLin Bissonette
- Nevio Cimolai
- Katerina Dorovini-Zis
- Amal El Naggar
- Doug Filipenko
- Maria Issa
- Karuna Karunakaran
- Naimh Kelly
- Will Lockwood
- Honglin Luo
- Hamid Masoudi
- Bruce McManus
- Vicky Monsalve
- Jason Morin
- Muhammad Morshed
- Nick Myles
- John Priatel
- Steve Shen
- Tyler Smith
- Aleksandra Stefanovic
- Azi Taba
- Jeff Terry
- Peter van den Elzen
- Dragos Vasilescu
- Suzanne Vercauteren
- Bruce Verchere
- Cheryl Wellington
- Wei Xiong
- Decheng Yang

Finally, sincere thanks to the staff who kindly assisted with technical and administrative support throughout the day and our photographers: Heather Cheadle, Debbie Bertanjoli, Helen Dyck, Jenny Tai, Kay Chu, Dan Kim, Shelley Berkow and Jennifer Xenakis.

WE HOPE YOU ENJOY PATHOLOGY DAY 2016.

THE PATHOLOGY DAY COMMITTEE

MIKE ALLARD, CORREE LAULE, TONY NG AND AVI OSTRY

CONFERENCE OUTLINE

Cordula and Gunter Paetzold Health Education Centre, 1st floor, Jim Pattison Pavillion North, Vancouver General Hospital

7:45 am	Breakfast
7:55 am	Opening remarks – Dr. Michael Allard
8:00 am – 9:00 am	**James Hogg Lecture** in the Paetzold Lecture Theatre: Dr. Andrew Churg *"The separation of benign and malignant mesothelial proliferations"*
9:05 am – 9:25 am	**Guest speaker**: Dr. Jacqueline Quandt in the Paetzold Lecture Theatre *"Radical approaches to studying inflammatory neurodegeneration in models of multiple sclerosis"*

GRADUATE STUDENT ORAL SESSION (LECTURE THEATRE)	
9:30 am – 9:45 am	Samantha Burugu
9:45 am –10:00 am	Emily Button
10:00 am – 10:15 am	Deb Chen
10:15 am – 10:30 am	Jacky Leung

RESIDENT ORAL SESSION (MULTIPURPOSE ROOM)	
9:30 am – 9:45 am	Shazia Masud (MM)
9:45 am –10:00 am	Inna Sekirov (MM)
10:00 am – 10:15 am	Gang Wang (AP)
10:15 am – 10:30 am	Emilija Todorovic (AP)

10:30 am – 10:45 am	Break (Atrium)
10:45am – 11:05 am	**Guest speaker:** Dr. Martin Wale in the Paetzold Lecture Theatre *"BC Medical Quality Initiative & What It Might Mean For You"*

GRADUATE STUDENT ORAL SESSION (LECTURE THEATRE)	
11:10 am – 11:25 am	Stephanie Santacruz
11:25 am – 11:40 am	Nicholas Swyngedouw
11:40 am – 11:55 am	Thyrza May Toledo

RESIDENT ORAL SESSION (MULTIPURPOSE ROOM)	
11:10 am – 11:25 am	Audi Setiadi (HP)
11:25 am – 11:40 am	Yazeed Al Welaie (AP)
11:40 am – 11:55 am	Eric Bol (AP)
11:55 am – 12:10 pm	Maziar Riazy (AP)

Poster Session & Lunch [ICORD, Blusson Spinal Cord Centre, 818 West 10th Avenue, Vancouver, BC]

12:15 pm – 2:30 pm	Poster Session & Lunch at ICORD
2:45 pm – 3:05 pm	**Guest speaker:** Dr. Karama Asleh-Aburaya, Post Doctoral Fellow (Dr. Torsten Nielsen's Lab) in the Paetzold Lecture Theatre *"Basal biomarkers Nestin and INPP4b accurately identify intrinsic subtype in breast cancers that are weakly positive for estrogen receptor"*

GRADUATE STUDENT ORAL SESSION (LECTURE THEATRE)	
3:10 pm – 3:25 pm	Brennan Wadsworth
3:25 pm – 3:40 pm	Chris Wang
3:40 pm – 3:55 pm	Derek Wong
3:55 pm – 4:10 pm	Adam Ziada

RESIDENT ORAL SESSION (MULTIPURPOSE ROOM)	
3:10 pm – 3:25 pm	Basile Tessier-Cloutier (AP)
3:25 pm – 3:40 pm	Lawrence Lee (AP)
3:40 pm – 3:55 pm	Noorah Almadani (AP)
3:55 pm – 4:10 pm	Catalin Taraboanta (AP)

4:10 pm – 4:25 pm	Break (Atrium)
4:30 pm – 5:30 pm	**Keynote speaker** Dr. Ken Aldape in the Paetzold Lecture Theatre *"Molecular diagnosis of brain tumors"*
5:45 pm	Cocktails and Canapés (ICORD)
6:00 pm	Awards (ICORD) evening event closes at 8:30 pm

Thank you for making Pathology Day 2016 a success!

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YAZEED ALWELAIE	RESIDENT	ORAL SESSION
SUPERVISOR:	DOUGLAS WEBBER	

PATHOLOGIC FINDINGS IN GALLBLADDERS REMOVED FOR CLINICALLY BENIGN DISEASE: A PILOT STUDY OF 200 CASES AND LITERATURE REVIEW. -IS MICROSCOPY NECESSARY WHEN THE GALLBLADDER IS NOT MACROSCOPICALLY ABNORMAL?

BACKGROUND/OBJECTIVES: Neoplasms of gallbladder and biliary epithelium are relatively rare and their diagnosis can be challenging. The incidence of gallbladder carcinoma varies in different parts of the world and also differs among different ethnic groups within the same country. The practice of histopathological examination of gallbladder specimens removed for clinically benign conditions and its usefulness has been a subject of controversy. The aim of this study was to determine if sampling routine gallbladders removed for benign conditions is necessary and also to see whether it would be possible to limit those specimens to gross examination only preferably by pathology residents or pathologists.

METHODS: We retrospectively reviewed two hundred consecutive pathology reports and the corresponding histopathology slides of cholecystectomy specimens. We identified all relevant preoperative information and correlated those with the results of macroscopic and microscopic examination. The presence or absence of malignancy and dysplasia was noted and confirmed by a gastrointestinal pathologist.

RESULTS: A total of 200 gallbladder specimens were subjected to histopathological examination from October, 2014 to February 2015. We found three cases of primary gallbladder adenocarcinoma (1.5%). All three cases were suspected based upon clinical and radiological grounds and had an appropriate extended resection at the time of surgery. Only two cases of incidental low-grade dysplasia (1%) were identified and additional sections (six and seven sections, respectively) in both cases did not reveal high grade dysplasia, carcinoma in-situ nor invasive adenocarcinoma. 7.5% of cases were essentially normal gallbladders, some of which contained calculi. The remainder of cases (90.0%) showed only changes consistent with either acute or chronic cholecystitis with cholelithiasis. Gallbladder mucosal preservation was found to be suboptimal in 28% of cases due to a delay in formalin fixation.

CONCLUSIONS: A selective approach for submitting sections from cholecystectomy specimens may be appropriate in cases where the clinical and macroscopic suspicion for malignancy is low. Steps to ensure better fixation will be undertaken.

AUTHOR:

Yazeed Alwelaie[1], Douglas Webber[2]

AFFILIATIONS :

Department of Pathology and Laboratory Medicine, and the University of British Columbia

NOORAH ALMADANI	RESIDENT	ORAL SESSION
SUPERVISOR:	JEFFERSON TERRY	

AUTOPSY FINDINGS IN RMND1 -RELATED MITOCHONDRIAL CYTOPATHY

AUTHORS:

Noorah Almadani[1], Glenda Hendson[2], Hilary Valance[2], Jefferson Terry[2]

AFFILIATIONS:

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[2]Department of Pathology and Laboratory Medicine. Children's and Women's Health Centre of British Columbia, Vancouver, BC, Canada

RMND1 encodes a protein that localizes to the inner mitochondrial membrane where it plays a role in translation of the 13 mtDNA encoded polypeptides which are all structural subunits of the respiratory chain complexes (1). Mutations in RMND1 lead to development of a mitochondrial cytopathy characterized by lactic acidosis, deafness, renal dysfunction and myopathy (OMIM 614917) (2-4). Renal dysfunction is a relatively uncommon feature of mitochondrial disease suggesting RMND1 may preferentially affect kidney function. Presently there are no detailed published descriptions of the autopsy findings associated with RMND1 related mitochondrial cytopathy. Herein, we report the autopsy findings in a 4-year-old boy with mitochondrial cytopathy caused by pathogenic mutations in RMND1. Full clinical and biochemical features of the index case were recently published (3). Briefly, he was born at term following a pregnancy complicated by oligohydramnios. Mild sensorineural hearing loss was detected at birth through routine newborn screening. At 2 months of age, he presented with failure to thrive, diarrhea and epileptic encephalopathy. His unusual clinical renal presentation included lactic acidosis and renal dysfunction (declining glomerular filtration rate, hypertension, hyperkalemia, and hyponatremia).

Psychomotor development was delayed with severe hypotonia and myopathic features. Skeletal muscle complexes I and IV were significantly reduced. Magnetic resonance imaging (MRI) revealed increased T2 signals throughout the white matter and delayed myelination. His clinical condition deteriorated and he expired primarily due to progressive chronic renal failure at 4 years of age. At autopsy, renal abnormalities were noted including renal hypoplasia, diffuse glomerulosclerosis, tubular atrophy, calcification, interstitial fibrosis, and inflammation. The liver was enlarged secondary to steatosis. Histochemical analysis of skeletal muscle showed diffuse reduction in cytochrome C oxidase activity, abnormal NADH, SDH and PAS staining, increase in lipid and mild type-2 fiber atrophy. Non-specific chronic pancreatitis and adrenal cortical lipid depletion were also present. The spleen showed unusual subintimal deposits in the arterioles associated with the periarteriolar lymphoid tissue; this histological finding was not seen elsewhere. Ultrastructural analysis of kidney and skeletal muscle demonstrated rectilinear electron dense mitochondrial inclusions. This case report is the first detailed description of anatomical abnormalities associated with the RMND1 mitochondrial cytopathic phenotype, which is of value to recognizing RMND1 related mitochondrial cytopathy at autopsy and further characterizing the pathophysiology of this abnormality.

ERIC BOL	RESIDENT	ORAL SESSION
SUPERVISOR:	JASON MORIN	

STUDENT EXPOSURE TO POST-MORTEM EXAMINATION IN CANADIAN MEDICAL EDUCATION AND STUDENT VIEWS ON POST-MORTEM INVESTIGATIONS IN FUTURE CLINICAL PRACTICE

BACKGROUND/OBJECTIVES: A well-conducted autopsy can add important information regarding the direct cause of death, as well as important diagnoses that may have been missed clinically, and, in a significant proportion of cases, may offer up an entirely different cause of death which was not suspected clinically. As such, autopsies have the potential to improve clinical management, educate both seasoned clinicians as well as clinicians in training, provide important vital statistic information and provide closure to individuals when a loved one has passed.

Despite the recognized benefits of the post-mortem examination, the rate of hospital (non-medicolegal) autopsies has declined significantly over the past several decades. Numerous reasons have been proposed as to why the rate of the hospital post-mortem examination is declining, and many of them have been studied in detail. The lack of value placed on the autopsy, and personal distaste/misperceptions of the autopsy process has been attributed to declining exposure to autopsy exposure in training; however, few studies have addressed current exposure to autopsy practice in medical schools.

METHODS: We propose to survey medical students in their final year of training at several medical schools across Canada. The survey will be distributed to students via their respective undergraduate medical office. The survey has been designed to measure students' various types of autopsy exposure in medical training, personal experiences with the autopsy consent process in their medical training, their attitudes towards autopsies in their future practice and their level of knowledge with regards to death certification and referral to the coroner/medical examiner. Our analysis will focus on differences in autopsy exposure across training programs in Canada, and examine whether autopsy exposure in training correlates with attitudes towards autopsies in clinical practice.

AUTHORS:

Jason Morin, Eric Bol

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Department of Pathology and Laboratory Medicine, and the University of British Columbia

LAWRENCE LEE	RESIDENT	ORAL SESSION
SUPERVISOR:	MICHAEL NIMMO, DAVID SCHAEFFER	

RE-EVALUATING THE USE OF REFLEX HISTOCHEMICAL STAIN FOR DETECTING H. PYLORI IN GASTRIC BIOPSIES

AUTHORS:

Lawrence Lee[1], Michael Nimmo[1], David Schaeffer[1]

AFFILIATIONS:

[1]Department of Pathology and Laboratory Medicine, University of British Columbia

BACKGROUND/OBJECTIVES: *Helicobacter pylori* infection is the most common cause of chronic gastritis and peptic ulcers, in addition to being a risk factor for gastric adenocarcinoma and lymphoma. However, once diagnosed, *H. pylori* infection can be readily treated. The organisms can be difficult to identify using conventional H&E staining of endoscopic gastric biopsies, but its identification can be facilitated using special histochemical and immunohistochemical (IHC) stains. A modified toluidine blue stain has been done reflexively on all gastric biopsies evaluated at Vancouver General Hospital; however, the yield and cost-effectiveness of this strategy has not been evaluated since its introduction.

METHODS: All gastric biopsies evaluated at VGH during a six month period (April 20 to Oct 19, 2015) were identified through searching an electronic pathology archive (Sunset). All the pathology reports were reviewed, and the provided clinical history and pathologic findings were annotated.

RESULTS: A total 1030 cases were evaluated at VGH during the index period. 1351 modified toluidine blue stained histologic sections were created allowing for the identification of 106 cases of *H. Pylori* infection, and requiring an additional cost of $11,970 ($8.86/slide). H. pylori IHC was performed 76 times which identified an additional 20 cases, costing an additional $1,823.24 ($23.99/slide). All cases in which *H. pylori* was identified showed active chronic gastritis, a feature which was present in 276 cases.

CONCLUSIONS: The majority of gastric biopsies are normal or show only inactive chronic gastritis, and *H. pylori* infection highly unlikely in this setting. If reflex modified toluidine blue is discontinued and *H. pylori* IHC is performed on all cases of active chronic gastritis, a projected cost savings of $19,000 would be achieved without loss of sensitivity in identifying *H. pylori* infection.

SHAZIA MASUD	RESIDENT	ORAL SESSION
SUPERVISOR:	LINDA HOANG	

THE EPIDEMIOLOGICAL RISK FACTORS AND CLINICAL PRESENTATION OF RECTAL INFECTION WITH LYMPHOGRANULOMA VENEREUM VERSUS NON-LYMPHOGRANULOMA VENEREUM STRAINS OF CHLAMYDIA TRACHOMATIS, IN BRITISH COLUMBIA: A CASE CONTROL STUDY

BACKGROUND/OBJECTIVES: *Lymphogranuloma venereum* (LGV) is a re-emerging sexually transmitted infection (STI) among men who have sex with men (MSM) in North America. LGV infection rates in British Columbia (BC) have been increasing since 2010. A retrospective case control study of all identified LGV cases from 2011 to 2014 was undertaken to identify specific clinical features and risk factors associated with LGV infection.

METHODS: All individuals who tested positive for rectal LGV infection at the STI clinic and associated public health clinics of British Columbia Center for Disease Control (BCCDC) from January 1st 2011 to May 30th 2014 were included as cases. The controls were matched at 1:3 from individuals who tested positive with rectal *Chlamydia trichromatis* (CT) infection. The demographic, clinical and risk factor details were collected by retrospective review of clinical data and enhanced LGV surveillance forms. Descriptive statistical techniques were used to analyze clinical features, risk factors and co-infection variables. Odds ratios and multivariable logistic regression were used to explore the association of specific clinical features and risk factors with LGV infection between cases and controls.

RESULTS: A total of 259 cases with rectal CT infection were identified during the study period of which 63/259 (24%) cases tested positive for LGV serovars. LGV cases were associated with older age and were more symptomatic (OR= 4.15 [1.88-10.50], p< 0.001) at the time of presentation. LGV risk factors included HIV infection and history of other STI with the majority of LGV cases being co-infected with other STI at the time of diagnosis. HIV infected LGV cases were less symptomatic than HIV negative, LGV positive cases (p <0.0002).

CONCLUSIONS: MSM presenting with anorectal symptoms should be screened for rectal CT infection and if positive, the sample should be further tested for LGV serovar. In addition, asymptomatic HIV positive MSM should have regular rectal CT screen and LGV follow up if CT positive.

AUTHORS:

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MAZIAR RIAZY	RESIDENT	ORAL SESSION
SUPERVISOR:	DAVID SCHAEFFER	

THE PREDICTIVE EFFECT OF HUMAN EQUILIBRATIVE TRANSPORTER 1 STRATIFIED BY MISMATCH REPAIR DEFICIENCY IN RESECTED PANCREATIC DUCTAL ADENOCARCINOMA PATIENTS TREATED WITH ADJUVANT GEMCITABINE

AUTHORS:

Maziar Riazy[1], Steve Kalloger[1], Joanna Karasinska[2], Renata Peixoto[3], Setareh Samimi[3], Christine Chow[3], Brandon S Sheffield[1], Daniel Renouf[3], David F Schaeffer[1,2]

AFFILIATIONS:

[1]University of British Columbia, Vancouver, BC, Canada;
[2]Pancreas Centre British Columbia, Vancouver, BC, Canada;
[3]University of British Columbia, Genetic Pathology Evaluation Centre;
[4]British Columbia Cancer Agency, Vancouver, BC, Canada

BACKGROUND/OBJECTIVES: The predictive value of human equilibrative transporter 1 (hENT1) in pancreatic ductal adenocarcinoma (PDAC) patients treated with adjuvant gemcitabine remains ambiguous. We recently showed that mismatch repair (MMR) status is associated with adjuvant chemotherapy (AdjCTx) response in PDAC. Here, we report the predictive effect of hENT1 expression stratified by MMR status.

Design: A tissue microarray (TMA) from 246 resected PDAC patients acquired between 1987 - 2013 was stained using the SP120 antibody against hENT1 as well as four MMR proteins: MLH1, MSH2, MSH6, and PMS2. The predictive ability of hENT1 expression was quantified by modeling overall survival (OS) between the treated and untreated study arms in the four MMR/hENT1 factorials.

METHODS/ RESULTS: MMR deficiency (MMRd) was found in 39 (15.8%) cases and of these, hENT1 was expressed in 18 (46%) of cases. This was contrasted with 132/207 (63.8%) of the MMR proficient (MMRp) cases expressing hENT1 ($p = 0.048$). AdjCTx was given in 24% of cases and its rate of application did not differ across the four MMR / hENT1 factorials ($p = 0.94$). Age, sex, grade, lymphovascular invasion, perineural invasion, pT-Stage, pN-Stage did not significantly differ across the four groups ($p \geq 0.39$). Multivariable OS analysis revealed that AdjCtx was only effective in the 53% of cases that were MMRp / hENT1+ (RR = 0.36 [95%CI 0.21 - 0.58]). A secondary multivariable OS analysis without MMR status revealed that only cases, which expressed hENT1, responded to adjuvant gemcitabine (RR = 0.41 [95%CI 0.25 - 0.64]).

CONCLUSIONS: This finding is congruent with some of the previous investigations of the predictive value of hENT1 expression in resected PDAC treated with AdjCTx. However, the addition of MMR status suggests that there may be a synergistic effect with regard to treatment response and that its inclusion into the emerging mosaic of predicitve biomarkers in PDAC has served to potentially identify a small group (7%) of hENT1+ cases and a larger group of MMRp cases (30%) that do not benefit from AdjCTx. This result suggests that this population of non-responders should be triaged for trials using novel targeted molecular therapeutics.

INNA SEKIROV	RESIDENT	ORAL SESSION
SUPERVISOR:	LINDA HOANG	

USING WHOLE GENOME SEQUENCING TO TRACK THE SPREAD OF NDM-PRODUCING KLEBSIELLA PNEUMONIAE

BACKGROUND/OBJECTIVES: Carbapenemase producing organisms have been increasing in numbers world-wide. Carbapenemases are a class of enzymes able to break down carbapenems, one of the last resort antibiotics used in serious Gram negative infections. NDM is one of the carbapenemase types most prevalent in BC, and the number of NDM-producing organisms has been rising in our province, partly due to enhanced surveillance efforts. Infections with and colonization by these organisms pose increasing therapeutic and infection control challenges. Outbreaks due to these organisms are often complex and difficult to control. Our objective was to use whole genome sequencing data to track the transmission of a cluster of NDM-producing Klebsiella pneumoniae (NDM-Kpn) in one of BC health authorities.

METHODS: NDM-Kpn isolated in BC between 2008-2014 were sequenced at the National Microbiology Laboratory using Illumina sequencing platform. Sequences were assembled and analyzed at the BC Center for Disease Control. We obtained in silico multi locus sequence typing (MLST) of isolates, determined the assortment of carried plasmids and core single nucleotide variants (SNV) as compared to a reference strain. These data were used to track the spread of a cluster of NDM-Kpn in one of BC health authorities.

RESULTS: We identified a cluster of 32 genetically-related strains isolated from 27 patients from a single health authority belonging to sequence type (ST)340 isolated between 2011 and 2014. The strains exhibited a high degree of overall similarity, except for one strain that was more divergent from the rest (~10 fold more SNVs than the rest). This strain was isolated from a patient who was previously colonized with an unrelated ST15 NDM-Kpn. Four patients had more than one ST340 NDM-Kpn isolated in 2011-2014, with some of the isolates being 7 to 12 months apart, yet closely genetically related to each other. NDM-Kpn isolated from one patient in 2011 and 2012 were designated as putative “founder strains”, to which the rest of the isolates in the cluster were compared. NDM-Kpn strains genetically close to the “founder strains” were isolated up to 2 years later. Different plasmid profiles were carried by NDM-Kpn in the cluster. Different plasmid profiles were endemic to different hospitals and plasmid spread was demonstrated.

CONCLUSIONS: Whole genome sequencing provides insights into relatedness of NDM-Kpn strains and allows to track both clonal and plasmid-mediated transmission. This approach, in correlation with epidemiological data, has the potential to be used for outbreak investigations and control.

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AUDI SETIADI	RESIDENT	ORAL SESSION
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THE IMPACT OF GENETIC ABERRATIONS IDENTIFIED BY CHROMOSOMAL MICROARRAY AND NEXT GENERATION SEQUENCING ON PROGNOSTIC SCORING SYSTEM IN MYELODYSPLASTIC SYNDROMES

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BACKGROUND/OBJECTIVES: In myelodysplastic syndromes (MDS), current prognostic scoring system (IPSS-R) takes into account cytogenetics, along with clinical criteria to stratify patients into risk groups. This prognostic scoring system still relies on conventional methods such as standard karyotyping and fluorescence in situ hybridization. Recent advances in chromosomal microarray (CMA) technologies allow for high resolution analysis of unbalanced chromosomal lesions and detection of copy number-neutral loss of heterozygosity (CN-LOH), without relying on cell division. In addition to structural chromosomal abnormalities, somatic mutations in genes such as TP53, EZH2, DNMT3A, ASXL1, ETV6, RUNX1, SF3B1 and SRSF2 have been shown to have prognostic significance in MDS independently of other risk factors. In this study, we seek to determine whether cryptic chromosomal aberrations detected by CMA, in combination with gene mutation analysis by next generation sequencing (NGS) have any impact on IPSS-R prognostic scoring in MDS patients.

METHODS: Bone marrow or blood samples were obtained at diagnosis or prior to treatment from 29 MDS patients. We analyzed the samples using the high-resolution Affymetrix (n = 16) or Agilent (n = 13) microarray platforms, which combined comparative genomic hybridization and single nucleotide pleomorphism array technologies. DNA mutation analysis was performed using an in-house developed next-generation sequencing panel run on the Illumina MiSeq. Variants were annotated by comparing the sequences to the dBSNP and COSMIC databases to distinguish between germline and somatic variants.

RESULTS: In 19/29 (65.5%) of patients, all of the chromosomal aberrations detected by conventional cytogenetics were also identifiable by CMA. In 10/29 (34.5%) patients, CMA detected cryptic copy number aberrations or CN-LOH that were not seen by conventional cytogenetics. In 1/27 (3.7%) patients, IPSS-R scores would be re-classified as higher based on the additional findings by CMA. However, in the other 10/29 (34.5%) patients, CMA failed to detect some of the chromosomal abnormalities described by conventional cytogenetics, which resulted in lowering of IPSS-R scores in 4/27 (14.8%) patients due to the “missed” findings by array. In the latter group, 3/4 (75%) patients were also found to have poor prognostic mutations, namely ETV6, RUNX1 and K-RAS, which would be expected to re-stage them back into the higher risk category.

CONCLUSIONS: CMA in combination with gene mutation analysis could provide additional prognostic information than predicted by conventional cytogenetics alone in MDS patients. CMA technology allows for detection of cryptic chromosomal lesions that would not be otherwise detectable by karyotyping in MDS patients. However, CMA is inherently unable to detect balanced translocations and low level of mosaic clones, which led to downgrading of IPSS-R scores in a minority but not insignificant number of cases in our study. Mutational analysis of known prognostic genes in MDS may provide additional predictive value in the patients with discrepant array findings but larger studies are needed to determine whether these technologies can completely replace conventional cytogenetics in clinical settings.

CATALIN TARABOANTA	RESIDENT	ORAL SESSION
SUPERVISOR:	BLAKE GILKS	

FALSE NEGATIVE ENDOMETRIAL SAMPLING IN PATIENTS WITH ENDOMETRIAL CARCINOMA

BACKGROUND/OBJECTIVES: Endometrial cancer is the most common gynecological cancer, and the fourth most common cancer in women. Outpatient endometrial sampling has a high overall accuracy in diagnosing endometrial cancer, if an adequate sample is obtained. A negative result however is less reassuring. Clark et al. demonstrated a post-test probability of 0.9% (95% CI: 0.4% – 2.4%) for endometrial carcinoma after a negative endometrial biopsy. If symptoms persist, 11% of patients with a negative biopsy will have carcinoma or complex hyperplasia diagnosed in the ensuing two years. Our goal was to determine the false negative rate and sensitivity of endometrial sampling in patients with endometrial carcinoma from our region.

METHODS: In a retrospective cross-sectional design, we identified all hysterectomies with pathology reported between May 2011 and May 2015 in our regional anatomical pathology data base, by querying the terms “carcinoma”, “adenocarcinoma” or “hyperplasia” in the final diagnosis. Cases of primary ovarian, cervical, or fallopian tube carcinoma, metastatic carcinomas, outside consultations and cases with no previous endometrial sampling were excluded. The index cases identified were then cross-referenced for negative endometrial sampling results for that patient, reported up to five years prior to hysterectomy. We defined negative endometrial samples as either benign or insufficient/non-diagnostic, excluding cases of atypical hyperplasia and also those where follow-up sampling was recommended for any reason.

RESULTS: We reviewed 2,334 pathology reports and identified 1,677 cases of hysterectomy for endometrial carcinoma or atypical hyperplasia. In 179 cases there was a previous biopsy in which carcinoma or atypical hyperplasia was not identified: 122 benign and 57 insufficient/non-diagnostic. Out of these, 132 (74%) cases were diagnosed as endometrioid adenocarcinoma, 12 (7%) serous carcinoma, and 31 (17%) atypical hyperplasia on the hysterectomy specimen. There was a median of 230 days (IQR: 116 – 720) between the time of the negative biopsy and hysterectomy and FIGO stage was 1 in 67%, 2 in 19% and 3 in 14% of carcinoma cases. The total number of negative endometrial biopsies reported in our region between May 2011 and May 2015 was 24,380. The false negative rate (miss rate) was 10.6% for carcinoma or atypical hyperplasia. Calculated sensitivity was 89.3% with a post-test probability of endometrial cancer, given a negative endometrial sample, of 0.7%.

CONCLUSIONS: Our findings are comparable with those published more than a decade ago. There remains a significant lack of sensitivity with outpatient endometrial sampling in the diagnosis of endometrial malignancy and pre-malignancy; both pathologists and primary care physicians should be aware of this.

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BASILE TESSIER-CLOUTIER	RESIDENT	ORAL SESSION
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PROGRAMMED CELL DEATH LIGAND 1 (PD-L1) EXPRESSION IN PANCREATIC DUCTAL ADENOCARCINOMA

AUTHORS:

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BACKGROUND/OBJECTIVES: Immune checkpoint inhibitors have recently shown promising anti-cancer effects in a number of solid tumor types, typically through inhibition of the programmed cell death 1 (PD1) signaling axis. A predictive biomarker to this class of drugs has not been clearly identified; however, overexpression of the PD1 ligand (PD-L1) has shown particular promise in lung adenocarcinoma, and mismatch repair deficiency (dMMR) has been identified in colorectal adenocarcinoma. In this study, we explore the staining characteristics, prevalence, and clinicomolecular correlates of PD-L1 overexpression in pancreatic ductal adenocarcinoma (PDAC).

METHODS: A clinically annotated tissue microarray (TMA) was constructed using archival tissue from cases of resected PDAC. PD-L1 immunohistochemistry (IHC) was performed using the SP142 primary antibody, additional IHC stains for MMR proteins (MLH1, MSH2, MSH6, PMS2), as well as CD3 and CD8 were reviewed. PD-L1 status was assessed independently by two anatomical pathologists and consensus achieved on all cases. Cases were considered positive with membranous staining seen in >1% of tumor cells.

RESULTS: Two-hundred forty-one cases were included in the TMA and were evaluable by IHC. Thirty-one (13%) cases were positive for PD-L1. No statistically significant association was identified between PD-L1 status and MMR status or smoking history. CD3+ infiltrating lymphocytes were more prevalent in the epithelial component of PD-L1 positive cases (p=0.0015). Patients with PD-L1 positive tumors had shorter disease specific survival (p=0.0257).

CONCLUSIONS: PD-L1 positivity and MMR deficiency can be assessed by IHC in PDAC. Our data indicate that these properties are independent, but not necessarily mutually exclusive in the setting of PDAC.

EMILIJA TODOROVIC	RESIDENT	ORAL SESSION
SUPERVISOR:	NICKOLAS MYLES	

DIAGNOSTIC ACCURACY OF BETHESDA SYSTEM FOR FNA CYTOLOGY EVALUATION OF THYROID NODULES: PRACTICAL APPLICATION OF MULTI-LEVEL TEST LIKELIHOOD RATIOS

BACKGROUND/OBJECTIVES: **Objective:** Fine needle aspiration cytology (FNAC), based on The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC, NCI, USA) is an essential part of the clinical pathway guiding surgical decisions for patients with thyroid nodules. It encompasses six diagnostic categories, associated with increasing risk of thyroid malignancy. The diagnostic accuracy of Bethesda cannot be measured by binary test concordance, sensitivity and specificity, as it will lead to bias. TBSRTC represents a multi-level diagnostic test, which should be measured and monitored by applying likelihood ratios (LRs) for each level of the test result as they are expected to independent of prevalence, in contrast to the widely used "rates of malignancy". **Aim:** to measure the diagnostic accuracy of the TBSRTC system to detect malignancy in thyroid nodules for all six diagnostic levels of the test, using LRs for each level of the test in a large population-based patient cohort.

METHODS: Population: sequential patients (2010-2014) with thyroid nodules received thyroid FNAC, with or without follow-up surgery. Index test: FNAC categorized according to the TBSRTC. Reference test: final histopathology (surgical excision specimens). Outcome: diagnostic likelihood ratios (95% CI) for each test level; Bayesian probability revision based on prevalence of thyroid malignancies.

RESULTS: Of a total 3307 FNAC performed in patients with thyroid nodules 291 (8.8%) underwent surgical excision, followed by reference standard histopathology (including 3.7% of benign, 23 % of AUS/FLUS, and all 100 % of follicular neoplasms, suspicious for malignancy and malignant). There were 227 females and 64 males. There were no known thyroid malignancies diagnosed at 6-month follow-up in a subset of patients without surgery. TBSRTC categories "suspicious for malignancy", "malignant" and "benign" perform well and discriminate benign from malignant lesions accurately. For three other categories (unsatisfactory, atypia or unknown significance and follicular lesion) there was a significant diagnostic uncertainty, reflected by LRs.

CONCLUSIONS: Here we propose practical application of LRs as robust numeric determinants of TBSRTC accuracy, making test results comparable across different prevalence settings, published literature and actual observations in routine diagnostic practice. Due to TBSRTC limitations, surgeries continue to be performed for patients with a substantial number of benign lesions. Whether adjunct molecular methods in addition to FNAC improve the TBSRTC accuracy requires further research.

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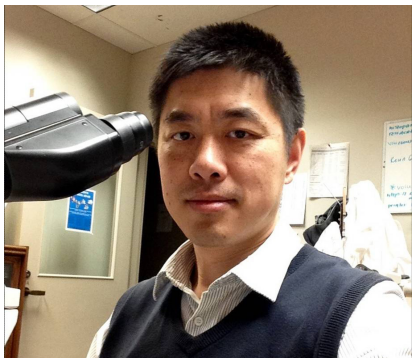

GANG WANG	RESIDENT	ORAL SESSION
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METASTATIC SPERMATOCYTIC SEMINOMA

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Spermatocytic seminoma is a rare germ cell tumor distinct from classical seminoma, both clinically and pathologically. It affects older men, with no association of cryptorchidism and no known counterpart in ovary or any other site. Pathologically, it is characterized by 3 distinct cell types and scant to absent lymphocytic infiltrate. Spermatocytic seminoma typically yields a good prognosis. Only two cases of metastatic spermatocytic seminoma have been reported in English literature, identified 10 months and 18 months after the diagnosis of the primary tumors, respectively. We report a case of a spermatocytic seminoma with metastases to retroperitoneal lymph nodes in a 51-year-old man, 12 years after orchiectomy for his primary tumor. Histological examination of the retroperitoneal biopsy fulfilled the microscopic criteria for spermatocytic seminoma.

The tumor cells were negative for Oct3/4, glipican-3, alpha-fetoprotein, inhibin and SOX10, but showed scattered positive nuclear staining for SALL-4, which confirmed the diagnosis of metastatic spermatocytic seminoma. Thorough clinical and imaging work-up excluded presence of other primary or metastatic neoplasm.

SAMANTHA BURUGU	GRADUATE STUDENT	ORAL SESSION
SUPERVISOR:	TORSTEN NIELSEN	

PROGNOSTIC VALUE OF IMMUNE CHECKPOINT MARKERS IN BREAST CANCER PATIENTS

BACKGROUND/OBJECTIVES: The recent emergence of immune checkpoint inhibitors as effective cancer therapies has re-ignited interest in tumor immunology. These new immunotherapy drugs target regulatory pathways co-opted by cancers to evade the immune system, restoring anticancer immune activity, and providing a clinical benefit in immunogenic cancers such as melanoma. An increasing body of evidence shows that some breast cancers are also immunogenic, with the presence of tumor-infiltrating lymphocytes (TILs) associated with good prognosis in estrogen receptor negative cases and with response to certain conventional chemotherapy drugs. Our objective is to investigate the expression of targetable immune checkpoint markers (LAG-3, PD-1 and PD-L1) and to assess their prognostic value using breast cancer excision specimens linked to detailed patient follow-up.

METHODS: A tissue microarray was constructed from 330 breast carcinoma primary excision specimens from patients at UBC hospital. None of these patients had received neoadjuvant treatment. Serial 4µm sections were single stained with antibodies to LAG-3 and PD-1/PD-L1 (targetable T-cell checkpoint receptors and ligand) by immunohistochemistry using a Ventana Discovery Ultra automated slide stainer. LAG3+ and PD-1+ stromal and intra-epithelial immune cells were reported as absolute counts per TMA core. Stromal TILs (sTIL) were defined as lymphocytes present in the stroma not in direct contact with tumor nest whereas intra-epithelial TIL (iTIL) were lymphocytes in direct contact with carcinoma cells. PD-L1+ carcinoma cells were assessed by intensity. All descriptive and survival analyses were conducted using SPSS software (version 18).

RESULTS: We found LAG-3+ sTILs in 17% and PD-1+ sTILs in 40% of breast cancer cases; LAG-3+ iTILs were observed in 15%, and PD-1+ iTILs in 23% of cases. 8% of the cases had PD-L1+ carcinoma cells. All three biomarkers were significantly (P = < 0.001) associated with high grade, hormone receptor negativity, high Ki67 proliferation and with the HER2+ and basal-like subtypes. In survival analyses, the presence of LAG-3+TILs was significantly associated with better outcome among estrogen receptor negative breast cancer patients (PD-1 and PD-L1 showed no significant associations in this subgroup).

CONCLUSIONS: Although breast cancer has not generally been considered as an immunogenic cancer, recent data and our results show some cases are. Indeed, we observed the presence of immune checkpoints markers such as LAG-3+ and PD-1+ immune cells as well as PD-L1+ tumor cells and found them to have a significant association with major risk factors and aggressive breast cancer subtypes. These findings are being validated in an independent series powered for multivariable analysis, and may have treatment implications as anti-PD-1/PD-L1 drugs are already available and anti-LAG-3 drugs are currently in clinical trials.

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EMILY BUTTON | GRADUATE STUDENT | ORAL SESSION

SUPERVISOR: | CHERYL WELLINGTON

HIGH-DENSITY LIPOPROTEINS PREVENT INFLAMMATION IN HUMAN CEREBRAL MICROVASCULAR ENDOTHELIAL CELLS

AUTHORS:

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BACKGROUND/OBJECTIVES: Alzheimer's disease (AD) and related dementias affect one in 11 Canadians over the age of 65, however no cure is currently available. While AD is characterized by the accumulation of amyloid beta plaques and development of neurofibrillary tangles in the brain, it is becoming increasingly apparent that cerebral vascular dysfunction may also drive AD pathogenesis. High-density lipoprotein (HDL) is a target of interest for preventing cerebral vascular dysfunction in the context of AD. HDL is known to promote peripheral vessel health and epidemiological evidence suggests that high levels of serum HDL are protective of cognitive function. We hypothesize that the vasoprotective effects of HDL can extend to cerebral vessels and prevent AD pathologies.

METHODS: Established vasoprotective functions of HDL in peripheral vessels were tested in immortalized human cerebral microvascular endothelial cells (hCMEC/D3). hCMEC/D3 cells were incubated with 0.1 mg/mL HDL and 4,5-diaminofluorescein diacetate, a fluorescent reporter of nitric oxide, for 6 h then fluorescence was measured at 485 nm. To measure anti-inflammatory functions, HDL was added to confluent hCMEC/D3 for 2 h before cell stimulation with tumour necrosis factor alpha (TNFalpha), amyloid beta monomers, or amyloid beta fibrils for 3 hours, followed by the addition of fluorescently labelled peripheral blood mononuclear cells (PBMC) for 3 h. Adhered PBMC were counted after washing and fixing. Vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1) proteins were also measured by western blotting. Amyloid beta fibrillization with and without HDL was measured using thioflavin T, a reporter of beta sheet protein aggregates, and measuring fluorescence at 440 nm.

RESULTS: HDL from young, healthy donors prevented TNFalpha induced PBMC adherence to various endothelial cell types including primary and immortalized human umbilical vein endothelial cells (HUVEC and EA.hy296 respectively) as well as primary and immortalized human brain microvascular endothelial cells (HBMEC and HCMEC/D3 respectively). Other vascular functions of HDL, including the induction of nitric oxide, were conserved in brain endothelial cells (hCMEC/D3). HDL also prevented amyloid beta induced PBMC adherence and ICAM-1 expression in hCMEC/D3. The fibrillization of amyloid beta was delayed by HDL, however HDL prevented amyloid beta induced inflammation regardless of amyloid fibrillization status.

CONCLUSIONS: HDL maintains its anti-inflammatory functions in brain endothelial cells, reducing both TNFalpha and amyloid beta induced PBMC adherence and cell adhesion molecule expression. While HDL may delay the harmful fibrillization of amyloid beta from monomers into fibrils, it appears that another mechanism is responsible for the anti-inflammatory effects of HDL in hCMEC/D3. These data suggest that HDL may contribute to a reduced risk of dementia by decreasing vascular inflammation within the brain.

DEB CHEN	GRADUATE STUDENT	ORAL SESSION
SUPERVISOR:	DANA V. DEVINE	

PROCESS-INDUCED HEMOLYSIS: A COMPARATIVE ANALYSIS OF GAMMA-IRRADIATED AND PATHOGEN-INACTIVATED RED CELL CONCENTRATES

BACKGROUND/OBJECTIVES: Post-collection manipulations (PCMs) are aimed to further improve product safety, especially for vulnerable transfusion recipients. However, these PCMs of red cell concentrates (RCCs) often improve safety at a cost of product quality. One of the biggest challenges for blood banks lies in the balancing product safety and quality, warranting further investigation into the biochemical changes induced by PCMs. This comparative study aims to catalogue differences in various in vitro quality parameters and to identify changes in membrane protein profiles between standard-issue, gamma-irradiated, and pathogen-inactivated (PI) RCCs.

METHODS: Three ABO-matched whole blood (WB) units were pooled and split into three identical units. One WB unit was PI-treated with riboflavin and UV illumination prior to RCC production. The other two WB units were produced into RCCs, with one subjected to gamma-irradiation, while the other was left untreated as control. Samples were analyzed on days 5, 14, 21, 28 and 42 of storage. Hemolysis levels were obtained by the Harboe method. Microvesicles (MV) were enumerated using flow cytometry. Osmotic fragility was determined using a series of saline dilutions and the sodium chloride concentration that produced 50% hemolysis was reported as mean corpuscular fragility (MCF). Membrane protein profiles between standard-issue, gamma-irradiated, and PI RCCs were assessed using a quantitative proteomics approach based on iTRAQ. Group means were compared using two-way ANOVA with repeated measures.

RESULTS: PI-treated RCC exhibited significantly higher hemolysis than gamma-irradiated and standard-issue RCCs at each time point measured ($p<0.0001$). MV release displayed a similar trend as a function of both storage and PCMs ($p<0.0001$). MCF increased with gamma-irradiation and was significantly more pronounced with PI-treatment ($p<0.0001$).

CONCLUSIONS: Overall, the in vitro quality parameters indicated that PI-treatment impacts RBC quality more severely compared to gamma-irradiation. iTRAQ proteomic analysis will identify membrane protein changes induced by PCMs, which will be placed in the context of more conventional quality parameters. These protein profiles can be used to generate mechanistic models of process-induced RBC damage, which may inform future blood banking practices in order to improve RCC quality.

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JACKY LEUNG | GRADUATE STUDENT | ORAL SESSION

SUPERVISOR: | MARIANNE SADAR

PROLINE ISOMERASE PIN1 MODULATES THE N-TERMINAL DOMAIN OF THE ANDROGEN RECEPTOR

AUTHORS:

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BACKGROUND/OBJECTIVES: The progression to lethal, castration-resistant prostate cancer (CRPC) is related to a resurgence of transcriptionally active androgen receptor (AR), despite aggressive therapy aimed at depriving the AR of its natural ligand. Mechanisms presumed to allow the AR to bypass androgen blockade include activation of AR by signal transduction pathways initiated by growth factors, and/or the expression of constitutively active AR splice variants that lack the C-terminal ligand-binding domain. This is because the N-terminal domain (NTD) of AR is a powerful transactivation domain that mediates most if not all AR's transcriptional activity, and can also be activated independently of androgen. Curiously, the AR NTD lacks intrinsic structure, but harbors a number of putative binding sites for Pin1, a proline isomerase whose primary function is to regulate the structure of proteins. Elevated levels of Pin1 in patient specimens are associated with increased risk for developing CRPC, but whether Pin1 may interact and regulate the AR is unclear. We hypothesized that Pin1 serves as a coactivator of AR by regulating the AR NTD.

METHODS: Luciferase reporter assays for AR transcriptional activity were carried out in the presence of siRNAs targeting Pin1 or the Pin1 inhibitor juglone (5-Hydroxy-1,4-naphthoquinone). Androgen-dependent AR activity was measured in the presence of synthetic androgen, R1881; whereas androgen-independent mechanisms were assayed by transactivation of the AR NTD or transcription mediated by truncated AR splice variants (ARv567es or V7). In addition, BrdU incorporation assays were performed on prostate cancer cells expressing full-length AR only (LNCaP) and cells which express both full-length AR and AR splice variants (LNCaP95).

RESULTS: Our results demonstrated that inactivation of Pin1 interrupted the activity of the AR NTD. Juglone effectively and specifically inhibits transcription driven by full-length AR, ARv567es and V7, and attenuated AR-dependent growth of LNCaP (androgen-dependent) and LNCaP95 cells (androgen-independent). Furthermore, juglone selectively blocked transactivation of the AR NTD in the presence of interleukin-6 by preventing interactions between endogenous AR and STAT3. Through mapping experiments, we determined the region of the AR NTD most vulnerable to Pin1 antagonism.

CONCLUSIONS: These results provide valuable insight into the role of Pin1 in prostate cancer, and suggest that Pin1 is an important coactivator of AR NTD that can mediate ligand-independent activation. Currently there is no cure for lethal CRPC, and perhaps targeting specific Pin1 motifs on the AR may serve as a valuable therapeutic target.

STEPHANIE SANTACRUZ	GRADUATE STUDENT	ORAL SESSION
SUPERVISOR:	DAVID GRANVILLE	

GRANZYME B DISRUPTS EPITHELIAL BARRIER FUNCTION

BACKGROUND/OBJECTIVES: The epithelium functions as a barrier to the external environment and this barrier function is regulated by cell junctions. This barrier can be found in the skin, lung, gastrointestinal tract and blood-cerebrospinal fluid barrier. Age-related and chronic inflammatory conditions result in a loss of epithelial barrier integrity. Loss of epithelial barrier disruption can result in increased infection, allergen exposure, moisture/temperature loss, inflammation, and other events. Granzyme B (GzmB) is a serine protease originally studied in immune cell-mediated apoptosis. However, recent work has found that GzmB is expressed by other cell types, can accumulate in the extracellular milieu, and is associated with aging, chronic inflammation and impaired tissue repair due to extracellular matrix (ECM) cleavage. As such, GzmB may contribute to the progressive loss of epithelial barrier function. We hypothesized that GzmB disrupts epithelial barrier function through the proteolytic cleavage of cell junction proteins.

METHODS: Human keratinocytes were treated with exogenous GzmB, in the presence or absence of a GzmB inhibitor. Subsequently, epithelial barrier function was assessed by Electric Cell-substrate Impedance Sensing (ECIS) and immunofluorescence (confocal) microscopy was used to visualize E-cadherin. Afterward, cell culture supernatants were assessed for presence of cadherin fragmentation by western blot analysis.

RESULTS: GzmB treatment resulted in a loss of E-cadherin staining on the cell membrane which was further supported by western blot analysis of the cell supernatants and biochemical cleavage assays, where we observed a dose-dependent increase in E-cadherin fragmentation. Moreover, E-cadherin cleavage was markedly decreased when treated with a GzmB inhibitor. Confluent monolayers of keratinocytes measured by ECIS show a marked decrease in resistance (barrier function) when treated with GzmB while cells treated with GzmB and inhibitor remained unaffected.

CONCLUSIONS: In summary, GzmB contributes to a decline in epithelial barrier function in part through the proteolytic cleavage of E-cadherin. Further work will look at permeability experiments of differentiated keratinocytes as well as obtaining primary cells from human and mouse skin for further GzmB treatments. Clinical relevance of this project could lead to topical inhibitors that will aid in treatment of chronic skin wounds commonly seen in diabetic foot ulcers, pressure ulcers, and autoimmune disorders.

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NICHOLAS SWYNGEDOUW | GRADUATE STUDENT | ORAL SESSION

SUPERVISOR: | CHUN SEOW & PETER PARÉ

THE INFLUENCE OF INTERLEUKIN-13 ON FORCE GENERATION IN AIRWAY SMOOTH MUSCLE TISSUE

AUTHORS:

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BACKGROUND/OBJECTIVES: Asthma is a chronic inflammatory disease of the lungs that currently affects an estimated 300 million people globally. It is characterized by airway hyperresponsiveness (AHR) and remodeling which lead to excessive airway narrowing and obstruction. Airway smooth muscle (ASM) has been implicated in the pathophysiology of asthma by contributing to this excessive airway narrowing and AHR. Furthermore, inflammation has also been suggested as a mechanism contributing to AHR in asthmatics. Levels of Interleukin-13 (IL-13), an inflammatory mediator, are increased in asthmatic sera and can alter the expression of specific contractile genes and proteins in cultured ASM cells. In cultured cells, IL-13 can cause increased ASM contractility and force generation in response to different contractile agonists such as acetylcholine (ACh), KCl, or histamine. However, there remains a lack of consensus regarding whether IL-13 can induce changes in mechanical properties of ASM tissue in response to all, or only some, contractile agonists. Our objective was to investigate the influence of IL-13 on the mechanical properties of isolated ASM tissue in response to a variety of agonists.

METHODS: Sheep tracheal smooth muscle was isolated, bathed in Krebs saline solution, and then equilibrated using electrical field stimulation. In order to obtain baseline mechanical measurements, tissues were either contracted with a range of ACh concentrations (10-8 to 10-3 M), pre-stimulated with 10-5 M ACh then relaxed with progressive doses of isoproterenol (ISO; 10-9 to 10-4 M), or contracted with single doses of KCl (80 mM) or histamine (10-4 M); n=5 per condition. Paired samples from each tissue were then pinned at constant (in situ) length and incubated for 24h or 72h with or without IL-13 (50 ng/mL) in serum-free DMEM. Tissue responses were compared to their baseline (t=0) measurements after incubation to determine the influence of IL-13 (two-tailed paired t-test or 2-way ANOVA).

RESULTS: Compared to non-exposed tissues, IL-13 did not increase maximal force or sensitivity to a range of ACh concentrations after either 24 or 72h (n=5 each), nor did it impede the relaxation of ASM induced by ISO after 24h (n=5). Likewise, response to KCl was not changed by IL-13 after 72h (n=5). Response to histamine was 2.2-fold higher compared to control (t=72h) after treatment (% of baseline maximal force, n=5, p=0.03).

CONCLUSIONS: These findings contrast previous work done in ASM cell culture experiments. In tissue strips, IL-13 did not induce significant changes to ASM mechanics in response to ACh, ISO, or KCl treatment. However, IL-13 did influence histamine-induced contractile response suggesting a potential avenue that airway inflammation influences ASM contraction. Elucidation of the complex relationship between airway inflammation and ASM will be necessary for identifying novel targets for therapeutic intervention to alleviate severe asthma exacerbations.

THYRZA MAY TOLEDO	GRADUATE STUDENT	ORAL SESSION
SUPERVISOR:	SUZANNE VERCAUTEREN	

COMPARISON OF THE QUANTITY AND QUALITY OF MONONUCLEAR CELLS ISOLATED BY THE CONVENTIONAL DENSITY GRADIENT CENTRIFUGATION METHOD VERSUS USING SEPMATE ISOLATION TUBES FOR BIOBANKING PURPOSES

BACKGROUND/OBJECTIVES: Providing high quality biospecimens to researchers while saving cost is important in Biobanking practices. Isolation of mononuclear cells is an expensive and time consuming practice. The objective of this study was to compare the quantity and quality of mononuclear cells isolated using the traditional density gradient centrifugation method versus a recently developed method using SepMate™ isolation tubes from STEMCELL Technologies.

METHODS: We isolated mononuclear cells from blood using the traditional density gradient centrifugation method or SepMate™ isolation tubes. The mononuclear cell yields were determined manually using a hemocytometer using a Crystal Violet dye and an automated cell counter. Cells were frozen and stored at -80°C. Cells were then thawed and the cell yield, recovery, purity and viability were evaluated. Flow cytometric analysis was performed to determine mononuclear composition and viability.

RESULTS: There was no significant difference in the mean mononuclear cell yield obtained with the Ficoll and SepMate™ method (n=13, p=0.7369). After freeze-thaw, the mononuclear cell recovery and purity between the Ficoll and SepMate™ method were not significantly different (recovery, n=11, p=0.1651; purity, n=8, p=0.5789). Flow cytometry showed that the two methods were comparable in terms of total lymphocyte yield (Ficoll: 44.6±14.3% vs SepMate: 43.6±13.6%), total CD3+ T-cell yield (Ficoll: 29.0±15.5% vs SepMate: 30.3±14.3%), and within the CD3+ population, CD4+ T-cell yield (Ficoll: 46.1±18.3% vs SepMate: 45.1±19.2%) and CD8+ T-cell yield (Ficoll: 43.4±17.8% vs SepMate: 44.2±18.7%). Also, the lymphocyte viability from the Ficoll method of (90.9±1.7%) was comparable with the SepMate™ method of (91.2±1.4%). Lastly, we found that using the SepMate™ tubes was more time efficient and cost effective for isolating mononuclear cells from blood.

CONCLUSIONS: We conclude that using SepMate™ tubes can be a time and cost effective method for the isolation of mononuclear cells for Biobanking purposes.

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BRENNAN WADSWORTH | GRADUATE STUDENT | ORAL SESSION

SUPERVISOR: KEVIN BENNEWITH

[18F]-FLUORO-ETHANOL IS A NOVEL POSITRON EMISSION TOMOGRAPHY (PET) REPORTER OF TUMOUR PERFUSION

AUTHORS:

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AFFILIATIONS:

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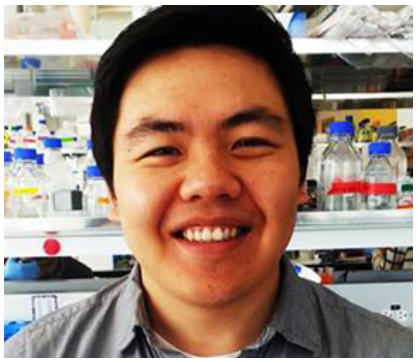
BACKGROUND/OBJECTIVES: Positron emission tomography (PET) is a non-invasive imaging technique that is frequently used in oncology. In brief, a biologically active molecule is conjugated to a gamma radiation-emitting radioisotope and introduced into the bloodstream. At this point a PET machine can detect the gamma radiation and produce a 3D image representing the radiotracer biodistribution. For the purpose of identifying aggressive tumours, we are interested in developing a PET radiotracer that will report the regional perfusion status of tumours. Tumour perfusion is a variable of interest because aggressive tumours tend to develop dysfunctional intra-tumoural blood vessels. When these vessels fail, large tumour regions can lose perfusion and become necrotic. This presence of necrosis is associated with therapy resistance and poor patient survival. We hypothesize that a reporter of tumour perfusion will identify intra-tumoural necrosis. Our research group previously published 2-[18F]-Fluoroethanol (FEtOH) as a candidate perfusion reporter in non-tumour bearing mice. This study aimed to validate that FEtOH reports regional tumour perfusion via PET.

METHODS: Balb/C mice were each orthotopically implanted with two tumours derived from murine mammary carcinoma cell lines; the 4T1 and 67NR cell lines. Established characterization of these cell lines shows that 4T1 cells develop metastatic tumours with central necrosis, while 67NR cells develop non-metastatic, well-perfused tumours. We administered the FEtOH to these mice in order to assess if the reporter could differentiate the two tumour types fitting with their distinct perfusion phenotypes. In addition, we administered mice with one of three drugs previously validated as modifiers of tumour perfusion, Hydralzine, Nicotinamide, or Pentoxifylline, to observe if the FEtOH reporter would respond as expected to these agents.

RESULTS: Current results indicate that FEtOH reliably reports central necrosis in 4T1 tumours and not 67NR tumours. Necrosis was confirmed using fluorescent imaging of Hoechst 33342 on tumour sections. Nicotinamide- and Pentoxifylline-induced increases in tumour perfusion were reflected by an increase in detected FEtOH tumour activity. Similarly, Hydralazine-induced decreases in tumour perfusion were reflected a decrease in FEtOH activity.

CONCLUSIONS: FEtOH is a robust reporter of regional tumour perfusion status. Future study will assess the applicability of the reporter in the context of monitoring tumour perfusion status over time during treatment with anti-angiogenic therapies.

CHRIS WANG	GRADUATE STUDENT	ORAL SESSION
SUPERVISOR:	MADS DAUGAARD	



ONCOFETAL CHONDROITIN SULFATE MODIFICATIONS IN NUCLEAR FACTOR κB PATHWAY ACTIVATION

BACKGROUND/OBJECTIVES: The Nuclear Factor κB (NF-κB) pathway plays a key role in innate immunity, inflammation, as well as cancer development and progression. It is a rapid response signaling pathway where the transcription factor, p65, is sequestered in the cytoplasm by IκB. Activators of the NF-κB pathway will produce a signaling cascade involving multiple different factors that ultimately leads to the proteasome-mediated degradation of IκB. This is a key event in the NF-κB signaling cascade that frees p65 to localize to the nucleus and alter gene transcription. Signaling pathways are complicated and are often controlled through many different mechanisms. One of which is glycosylation of surface proteins that affects protein structure and stability. Recently our team discovered that a recombinant malarial protein, rVAR2, specifically binds to unique oncofetal chondroitin sulfate A (CSA) modifications expressed on placental trophoblasts and cancer cells. In an unbiased functional genomics gain-of-binding screen for rVAR2-interacting chondroitin sulfate proteoglycans (CSPGs), a small repertoire of proteoglycans highly upregulated in different cancers including CC Chemokine Receptors known to be involved in NF-κB signalling. We hypothesize that there is an oncofetal CSA component in the regulation of the NF-κB signaling pathway.

METHODS: MG-63 osteosarcoma cell lines were used as an in vitro model system. Affymetrix whole transcriptome microarray was used to determine gene expression changes following rVAR2 treatment. Gene expression profiling from transcriptome analysis was validated by qPCR. Degradation of IκB was determined by immunoblotting following rVAR2 treatment in the presence and absence of a proteasome inhibitor (MG-132). Nuclearization of the NF-κ B transcription factor p65 was determined by confocal microscopy. ELISA was performed on media following rVAR2 treatment to investigate changes in cytokine secretion.

RESULTS: Transcriptome microarray analysis on MG-63 osteosarcoma cells revealed a strong induction of NF-κB target genes such as IL-8, IL-6 and IL-1B which peaked 2-3h after rVAR2 treatment. Validation by qPCR, immunoblotting and ELISA showed that this activation step was dependent on specific interaction between rVAR2 and oncofetal CSA chains. Furthermore, the increase in NF-κB target mRNA levels occurs in the absence of serum in the media indicating that this activation is a result of specific interaction between rVAR2 and oncofetal CSA on cancer cells. LPS mediated activation of NF-κB remains unaffected by the presence of oncofetal CSA indicating a unique pathway through which rVAR2 acts to initiate NF-κB signaling.

CONCLUSIONS: Here we report the discovery that the NF-kB pathway can be regulated by rVAR2 in a strictly CSA-dependent manner. Our data provides evidence for the presence of a CSA glycosaminoglycan component in the NF-κB pathway. With this project we would ultimately determine how oncofetal CSA is involved in modulating immune and inflammatory responses in tumors. This will have a significant impact on our understanding of glycosylation, NF-κB signaling, and their combined role in cancer biology.

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DEREK WONG | GRADUATE STUDENT | ORAL SESSION

SUPERVISOR: | STEPHEN YIP

FUNCTIONAL INVESTIGATIONS OF CAPICUA PROTEIN IN OLIGODENDROGLIOMA

AUTHORS:

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BACKGROUND/OBJECTIVES: Oligodendroglioma (ODG) is a type of primary brain cancer marked by unique clinical, histological, and genetic characteristics. These tumours have slow and predictable progression and are more sensitive to treatment leading to favorable prognosis for patients. Recently, mutations in the Capicua (CIC) gene, have been found in up to 70% of ODGs. The high frequency of CIC mutations indicates that loss or altered function of the CIC protein may be crucially associated with the unique biology of ODG. Although much is not known about mammalian CIC, it has been shown to be a transcriptional repressor whose activity is inhibited upon receptor tyrosine kinase (RTK) activation in Drosophila. The mechanism by which CIC represses the expression of its targets in mammals remains poorly understood. In previous studies, ataxin-1-like protein (ATXN1L), another transcriptional repressor, and CIC are closely involved in the pathology of spinocerebellar ataxia, a degenerative brain disease; however, their relationship and role in brain tumour pathology has yet to be elucidated. We hypothesize that CIC and ATXN1L interact with each other and are involved with the repression of growth pathways downstream of RTK activation.

METHODS: CIC knockout (KO) HEK293 cell lines (CIC-KO) were previously established using CRISPR genetic modification technique. FLAG- tagged CIC (CIC-flag) was stably reintroduced into the CIC-KO lines. Using these cell lines and isogenic unmodified control; CIC and ATXN1L were immunoprecipitated (IP) and samples were run on SDS-PAGE. Protein detection was done by Western Blot. ATXN1L was knocked down using siRNA and changes in transcript and protein levels were detected using RT-qPCR and WB.

RESULTS: IP of both CIC-flag and endogenous CIC also resulted in co-immunoprecipitation (co-IP) of ATXN1L. This was validated and confirmed with ATXN1L IP which resulted in co-IP of CIC-flag. Knockdown of ATXN1L using siRNA in HEK293 cells resulted in an increase of ETV1/4/5 transcripts which was similar to changes seen in CIC KO HEK293 cells using RT-qPCR. Increases in pERK, and DUSP6 in ATXN1L siRNA HEK293 cells were seen using Western blot, also consistent with CIC KO HEK293 cells. All transcripts and proteins analyzed are targets downstream of RTK activation.

CONCLUSIONS: CIC and ATXN1L interact physically with each other and are both known transcriptional repressors. Our data suggests that knockdown of ATXN1L may produce a phenotype similar to CIC-KO. Thus, disruption of the CIC-ATXN1L repressive complex through mutation may be selected for during ODG tumourigenesis and progression.

ADAM ZIADA | SUMMER STUDENT | ORAL SESSION

SUPERVISOR: HÉLÈNE CÔTÉ

ELEVATED BLOOD MITOCHONDRIAL DNA MUTATIONS RATES ARE ASSOCIATED WITH INCREASED AGE AND, PEAK VIRAL LOAD, BUT NOT HIV STATUS

BACKGROUND/OBJECTIVES: According to the oxidative stress theory of aging, somatic mtDNA mutation accumulation leads to tissue aging and dysfunction. Both antiretrovirals, and HIV, can negatively impact mtDNA, increase oxidative stress, and contribute to accelerated aging such as that observed in persons living with HIV. We investigated leukocyte somatic mtDNA point mutations in HIV+ and HIV- participants enrolled in the CARMA cohort study.

METHODS: Participants in this cross-sectional study were HIV+ (n=92, including n=12<19y) and HIV- (n=72, n=13<19y) females, not infected with HCV or HBV, and either current or never smokers. Whole blood DNA was extracted and somatic mtDNA substitution mutation rates per 100,000bp were quantified via next generation sequencing. Factors associated with mtDNA mutations were investigated through logistic regressions Spearman's correlations, Mann-Whitney tests, and ANCOVA of log10 values as appropriate.

RESULTS: For 78 HIV+ and 64 HIV- individuals, aged 1-75 years, blood somatic mtDNA mutation rates met quality control (median [IQR] (range) of 3.6 [2.3-6.1] (0.0-25.0)). Ages were similar between the two groups (p=0.15) and all HIV+ children had undetectable HIV plasma viral load (pVL) while this was true for 59% of adults. In each group, current smokers made up approximately 40% of adult population. A significant correlation was seen between mtDNA somatic mutation rates and age (rho=0.29, p<0.001) but no association was observed with smoking status (current vs. never, p=0.84) or HIV+ status (p=0.96). Among HIV+ adults, higher mtDNA somatic mutation rates were associated with peak HIV pVL recorded (> vs. ≤100,000 copies/mL, p=0.015) but not current HIV pVL (rho=0.003, p=0.67), CD4+ count (rho=0.015, p=0.34), or CD4 nadir (rho=0.021, p=0.25). In a multivariable model of HIV+ adult participants that included age and peak pVL, only peak pVL (p<0.01) remained independently associated with mtDNA mutations rates. Among age, HIV status, and smoking, only age remained significantly associated (p<0.01) with a higher mtDNA somatic mutation rate in a multivariable model among all participants.

CONCLUSIONS: Our preliminary results suggest that somatic mtDNA mutations can be measured in blood and do increases with age, consistent with current theories of aging. In addition, mtDNA somatic point mutations are associated with an elevated peak viremia.

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KARAMA ASLEH-ABURAYA	POST-DOCTORAL FELLOW	ORAL SESSION
SUPERVISOR:	TORSTEN NIELSEN	

BASAL BIOMARKERS NESTIN AND INPP4B ACCURATELY IDENTIFY INTRINSIC SUBTYPE IN BREAST CANCERS THAT ARE WEAKLY POSITIVE FOR ESTROGEN RECEPTOR

AUTHORS:

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BACKGROUND/OBJECTIVES: Recent evidence indicates that weakly positive immuno histochemical staining of estrogen receptor (ER) is not reliably associated with a luminal subtype, with the majority re-classified as basal-like by gene expression profile. In this study we assessed the capacity of recently-identified immunohistochemical markers of basal-like subtype not dependent on ER status– positive expression of nestin or loss of inositol polyphosphate-4-phosphatase (INPP4b)– to discriminate intrinsic subtypes, focusing on clinically-problematic cases with weak ER positivity.

METHODS: Formalin-fixed paraffin embedded blocks, enriched for large proportions of ER negative and ER weakly positive breast cancers, were selected from two previous studies conducted in the period 2008-2013 and used for (1) RNA extraction for PAM50 intrinsic subtyping and (2) tissue microarray construction for immunohistochemical assessment of nestin and INPP4b.

RESULTS: Fifty-eight cases were weakly-positive for ER (Allred 3-5), among which 28 (48%) were assigned as basal-like by PAM50 gene expression. In these 58 cases, nestin/INPP4b panel identified 23 basal-like cases with a positive predictive value of 87% (95%CI; 78%-95%) and excluded luminal subtype with a negative predictive value of 95% (95%CI; 88%-100%). Weakly positive ER patients assigned as basal-like by nestin/INPP4b definition demonstrated a median survival time of 45.8 months, significantly lower than 65 months among other ER weakly positive cases (p=0.012).

CONCLUSIONS: Immunohistochemical assessment of nestin and INPP4b provides an accurate, accessible and inexpensive tool to identify basal-like breast cancer subtype in the clinically problematic setting of weak ER positivity. This panel identifies poor prognosis patients who might need strong considerations for non-endocrine-based therapies.

USAMA ABBASI | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: | JAYACHANDRAN KIZHAKKEDATHU

MACROMOLECULAR ENGINEERING OF A LIVER SPECIFIC IRON CHELATING SYSTEM FOR THE TREATMENT OF SYSTEMIC IRON OVERLOAD

BACKGROUND/OBJECTIVES: Iron is vital for life. It has the capacity to undergo cyclic redox reactions, which makes it an especially useful component in many enzymes and proteins. However, excess iron has an enormous damaging potential; free cellular iron catalyzes the generation of highly reactive oxygen species, which can lead to organ dysfunction and death. The ability to offload excess iron remains challenging since there is no known physiological pathway to actively excrete iron. This poses a particular challenge in transfusion-dependent hemoglobinopathies, including sickle cell disease and thalassemia. Approximately, 5% of the world's population carries trait genes for such disorders. Transfusion therapy is the current standard of care for patients. Since humans lack an iron excretion pathway, chronically transfused patients accumulate excess iron, which overwhelms the primary storage sites, particularly the liver and spleen, and disrupts iron homeostasis. Consequently, organ toxicity is a major complication associated with iron overload, particularly with iron-induced cardiac and hepatic failure accounting for many deaths despite intensive chelation therapy. To date, no methods are available to target iron chelators to susceptible organs. The ability to selectively excrete iron will be a paradigm shift in the treatment of transfusion iron overload. *We hypothesize that a macromolecular iron chelating system conjugated with liver targeting groups that achieves optimized blood circulation time would enhance the iron removal from liver, thereby preventing hepatic complications in patients.*

METHODS: Parenchymal liver cells express the unique asialoglycoprotein receptor (ASGP-R), a lectin that binds strongly to liver targeting ligands (LTGs), which has been viewed as a promising target in multiple drug delivery studies. A polymeric scaffold was used to conjugate LTGs and this system was optimized for ideal multivalent receptor interaction. A time based uptake profile and a concentration based uptake profile was conducted on fluorescently labelled polymer with 60 LTGs; 250 nM polymeric solutions were incubated for several time points over 24 hours, and several concentrations, ranging from picomolar to micromolar concentrations, at 1 hour incubations. Cellular uptake was investigated through flow cytometry using HepG2 cells, a human hepatic cell line, as our in vitro model.

RESULTS: With respect to the time based uptake profile, polymer-LTGs showed significant uptake as early as 30 minutes with 3.5 fold increase in median fluorescence intensity ($p<0.05$) compared to the control polymer without LTGs. Over the course of 24 hour incubation period, the uptake continued to increase linearly, exceeding 50 fold increase after 24 hours. On the other hand, with respect to the concentration based uptake profile, polymer-LTGs showed significant uptake at as little as 50 nM with 2.7 fold increase in median fluorescent intensity ($p<0.05$). The uptake profile increases exponentially and reaches a plateau at 2uM with 6.2 fold increase in median fluorescent intensity. Median fluorescent intensity was normalized against untreated HepG2 cells. ANOVA was performed on all median fluorescent intensity at $p=0.05$.

CONCLUSIONS: Polymer with 60 LTGs show promising results with regards to receptor specific uptake. Future studies will optimize polymer molecular weight as well as number of LTGs to provide maximum multivalent interaction with our receptor, ASGP-R. These studies provide novel insight for the development of non-toxic polymeric systems and have potential application for iron overload diseases. Future experiments will address chelating efficiencies and characterize mechanisms of uptake and iron sequestration, as well as investigate changes in the iron regulatory elements.

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ABHINAV AJAYKUMAR | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: | HÉLÈNE CÔTÉ

HOW DOES THE NUMBER OF ANTIRETROVIRAL DRUGS HIV+ PREGNANT WOMEN RECEIVE INFLUENCE THEIR INFANT'S MITOCHONDRIAL DNA?

AUTHORS:

Abhinav Ajaykumar[1,2], Hélène Côté[1,2] and the CIHR Team on Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

AFFILIATIONS:

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BACKGROUND/OBJECTIVES: The introduction of Zidovudine (AZT) treatment in pregnancy during the mid-1990's saw a rapid decline in the risk of mother-to-child transmission, from 40% to as low as 8%. This was followed by the advent of more effective dual, then triple-drug antiretroviral therapy (ART) that further reduced perinatal HIV transmission to ~1%. However, many ART drugs can cross the placenta and could affect cellular processes in the developing fetus. ART drugs that target the HIV reverse transcriptase enzyme are known to inhibit the mitochondrial polymerase κ enzyme responsible for mitochondrial DNA (mtDNA) replication. This may result in mitochondrial alterations, including mitochondrial dysfunction which can be reflected by changes in mtDNA content. Indeed, we and others have observed differences in HIV-exposed uninfected (HEU) infant mtDNA content at birth compared to controls. However, little is known on how this may be affected by the duration, type and number of ART drugs the infant was exposed to during pregnancy. Our objective was to determine the impact of increased maternal cART drug burden during pregnancy on infant mtDNA content in a unique cohort that dates back to the early 1990's and included untreated and monotherapy-treated HIV+ mothers.

METHODS: mtDNA content was measured via qPCR in dried blood spots collected between birth and one month of age from 121 HEU infants enrolled in the Centre Maternel et Infantile sur le SIDA (CMIS) Mother–Child Cohort. Univariate analyses were performed to investigate associations between mtDNA content and infant characteristics such as sex, gestational age (GA) at birth, birth weight, and duration of in utero exposure to maternal ART. Maternal characteristics examined were age, ethnicity, smoking during pregnancy, illicit drug use during pregnancy, Hep B or C virus history, number of ART drugs during pregnancy, as well as viral load and CD4 count closest to delivery. Important factors ($p<0.10$) were included in multivariable linear regression models.

RESULTS: Among the 121 infants, 51 were exposed to maternal AZT monotherapy in utero, 23 to dual therapy (mostly AZT+ lamivudine (3TC)), 17 to triple therapy (mostly AZT+3TC+ nelfinavir (NFV)) and the remaining 30 were born to ART naïve mothers. Univariately, infants born to mothers who took ≥2 ART drugs during pregnancy had significantly higher mtDNA content compared to infants born to untreated ($p=0.016$) or mono-treated ($p=0.001$) mothers. However, we found no association between infant mtDNA content and the duration of in utero ART exposure. In a multivariable model that included GA at birth and number of ART drugs during pregnancy, GA at birth was significantly associated with higher mtDNA content ($p=0.012$), while the association between number of ART drugs and mtDNA content was no longer statistically significant ($p=0.09$).

CONCLUSIONS: Results from this small cohort suggest that infant mtDNA content may be related to maternal ART drug burden during pregnancy. More specifically, the addition of 3TC appears to drive higher mtDNA content in infants. This is somewhat unexpected since 3TC is widely believed to exert little mitochondrial toxicity.

AHMAD ARBAEEN	GRADUATE STUDENT	POSTER PRESENTATION
SUPERVISOR:	DANA DEVINE	

HEMOSTATIC FUNCTION OF STORED BUFFY COAT PLATELET CONCENTRATE IN PLASMA TREATED WITH PATHOGEN INACTIVATION SYSTEM

BACKGROUND/OBJECTIVES: Pathogen inactivation (PI) systems have been introduced to blood banking to decrease transfusion-transmitted infections (TTI). It has been shown that PI-treatment is leading to an accelerated development of platelet storage lesion and might affect the hemostatic functionality of platelet concentrate (BCPC). This study is to determine the effect of PI by riboflavin/UV light (Mirasol) on the hemostatic potential of BCPC produced in plasma using the technology of Thromboelastometry (ROTEM), in parallel, other in vitro tests were investigated.

METHODS: Paired BCPCs that produced in plasma, were pooled and split to illumination bags on the day of production. Six BCPCs were treated with riboflavin and UV Light and six were kept un-treated as a paired control. Samples were drawn on days 2, 5, 7, and 9 of storage, and reconstituted with fresh frozen plasma to a platelet count of 100 x109/L. To assess the dynamics of the clot development in the ROTEM system, Kaolin was used as an activator for the intrinsic coagulation pathway, and tissue plasminogen activator was applied to investigate fibrinolysis resistance. In parallel, P-selectin expression and pH level of these samples were determined.

RESULTS: The illumination of BCPC did not result in changing the clotting time or the clot forming time between the two groups. After day 7 of storage, there was significant decrease in the rate of the fibrin –platelet interaction (alpha value) in illuminated BCPC which is primary influenced by the functionality of the platelets and the contribution of the fibrinogen. Maximum clot formation (MCF) was significantly reduced in the illuminated BCPC compared to the control BCPC during the whole storage time (MCF, P value: < 0.0001), but was steady consistent in both groups. The fibrinolysis resistance was slightly decreased in the illuminated BCPC with significant decrease after 7 days of storage. The activation level of platelets during storage time increased significantly in day 5, 7, and 9 compared to day 2 of storage ($p < 0.001$), and was significantly higher in the illuminated BCPC ($p < 0.001$, compared to the control). The pH value change significantly during storage in any of the groups, and there was significant dropped in the illuminated BCPC compared to control BCPC ($p < 0.001$). The pH value dropped under 6.8 in illuminated BCPC at day 9. Significantly, there was no correlation between ROTEM profile of the Mirasol treated PC and the parallel in vitro tests.

CONCLUSIONS: This study show that the illumination of BCPC has an impact on the platelet functionality, and while the other in vitro tests shown more deterioration of the platelet, ROTEM profile indicate that illuminated PC by Mirasol is probably save to be transfused up to day 7.

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ABIGAIL BATICADOS | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: | CHRISTIAN STEIDL

FUNCTIONAL SIGNIFICANCE OF TRANSMEMBRANE PROTEIN 30A (TMEM30A) MUTATIONAL PATTERNS IN DIFFUSE LARGE B-CELL LYMPHOMA

AUTHORS:

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BACKGROUND/OBJECTIVES: TMEM30A is a transmembrane protein that functions as a flippase and actively holds phosphotidlyserine (PS) in the inner cytosolic leaflet of the cell. During apoptosis, the flippase action of TMEM30A is inhibited and this causes PS to be externalized to the surface of the cell. Externalization of PS represents an “eat-me” signal, which promotes phagocytosis from macrophages, and thus damaged cells are engulfed and removed from the organism. Current comprehensive genomic data generated in our laboratory revealed novel and recurrent loss of function mutations and structural alterations in TMEM30A (29%; 96 of 336 cases). Importantly, 84% of all mutations co-occurred with a deletion in the other allele postulating a role of TMEM30A as a novel tumour suppressor gene in B-cell lymphoma. Moreover, clinical correlates in cases homogeneously treated with RCHOP (Rituximab, Cyclophosphamide, Hydroxydaunomycin, Oncovin and Prednisone) showed that TMEM30A inactivating genetic alterations are significantly associated with favourable outcomes. Therefore, we hypothesize that PS externalization occurs as a consequence of TMEM30A inactivation and this contributes to increased sensitivity to externally induced apoptosis in DLBCL.

METHODS: DLBCL-derived DOHH2 wild type (WT), nonsilence control (NS) and shRNA TMEM30A stable knock down (KD) clones were utilized as in-vitro model systems. Quantitative reverse transcription PCR (qRT-PCR) was used to validate the success of the RNAi experiment on the DOHH2 transduced cell line. To characterize the biology of TMEM30A inactivation, WST-1 cell proliferation assay and trypan-blue exclusion cell viability assay was performed. In addition, Annexin V (apoptotic marker) and 7-AAD (necrotic marker) flow cytometric analysis was used to examine differences in PS externalization of mutant KD clone 3 (CL3) versus NS control after hypothermic stress. CRISPR genome editing was done to generate a TMEM30A knockout (KO) DOHH2 cell line. Furthermore, a TMEM30A expression vector was also constructed to rescue the TMEM30A phenotype in the CRISPR KO DOHH2 cell line.

RESULTS: qRT-PCR shows that the KD mutant clones had decreased levels of messenger RNA expression with 85% KD compared to the WT and NS control. Functional characterization using flow cytometry with APC-Annexin V (apoptotic marker) and 7-AAD (necrotic marker) demonstrated CL3 had consistent shifts toward higher Annexin V binding (apoptosis) up to the end (Day 5) of the time course hypothermic stress experiment. In addition, cell viability assays on serum-starved cells demonstrated that CL3 was more sensitive to serum starvation-induced stress than NS. On the other hand, cell proliferation assay did not show differences in growth rates between KD and NS. For the KO experiments, PCR colony screen from a TMEM30A expression vector transfection revealed clones with the expected insert size of ~1.4 kilobases and these were verified by sequencing and are therefore ready for use in further rescue experiments.

CONCLUSIONS: Based on the results of the cell viability assay and flow analysis, CL3 had higher sensitivity to serum starvation and hypothermia induced apoptosis compared to NS. In the future, I will be using drugs as external stressors in my cell lines in my two in vitro model systems i.e shRNA DOHH2 KD clones and CRISPR KO DOHH2 clones to recapitulate the findings in diagnostic patient cases that showed better outcomes with RCHOP treatment. **Significance:** These results indicate that exposure of PS through TMEM30A inactivation may promote the anti-tumour activity of external stressors against DLBCL. Overall, these findings are suggestive of a novel therapeutic option for a specific subset of DLBCL patients with this genotype.

NICHA BOONPATTRAWONG | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: | ANGELA DEVLIN

MATERNAL OBESITY AND EXERCISE PROGRAMS CARDIOVASCULAR HEALTH IN OFFSPRING

BACKGROUND/OBJECTIVES: In Canada, approximately 50% of women of childbearing age are overweight or obese. This raises a concern as obesity has been found to be strongly associated with development of type 2 diabetes and cardiovascular disease (CVD). In addition, there are evidence from both population-based and rodent studies supporting the theory of developmental programming that fetal and early postnatal environment can influence one's risk for chronic diseases, such as CVD. Several rodent studies investigating maternal obesity demonstrated that offspring from obese dams have higher adiposity, more insulin resistant, and exhibit vascular endothelial dysfunction – an early indicator of CVD. Current theories suggest endothelial dysfunction is characterized by reduced nitric oxide-mediated vasodilatation and is a result of endothelial nitric oxide synthase uncoupling. As such, factors that have been shown to increase availability of nitric oxide, such as exercise, should improve vascular endothelial function. We hypothesize that changes in epigenetic regulation and expression of genes required for endothelial function and promoting oxidative stress and inflammation will contribute to the programming of vascular dysfunction in offspring from dams with obesity and exercise intervention will help mitigate the adverse effects.

METHODS: Female (C57BL/6) mice are fed from weaning control (10% kcal fat) or western diet (45% kcal fat) to induce excess adiposity. At 13 weeks, female mice were put into cages with or without access to running wheel for voluntary exercise throughout breeding, pregnancy and lactation. Exercise in dams will be confirmed by quantification of citrate synthase activity. The male offspring are then weaned onto either control or western diet. Glucose homeostasis will be assessed by intraperitoneal glucose and insulin tolerance test in male offspring after 13 weeks of feeding. Vascular endothelial function (ex vivo) will be assessed in the aorta by isometric force measurement for vasoconstriction in response to phenylephrine and vasodilatation in response to acetylcholine (endothelial-dependent) and sodium nitroprusside (endothelial-independent). Endothelial cells will be isolated from whole aortas to determine cell-specific whole genome differential gene expression pattern via RNA-seq. DNA methylation and histone methylation/acetylation of differentially expressed genes will be quantified by bisulfite Pyrosequencing and ChIP, respectively.

RESULTS: We are in the process of breeding, diet and exercise intervention. Our preliminary result show that offspring weights at weaning were significantly greater in offspring from dams fed western diet compared to those from control diet-fed dams ($p<0.05$). We also saw that maternal obesity is associated with glucose intolerance and insulin insensitivity. We predict that offspring from dams fed the western diet will have impaired endothelial-dependent vascular function and glucose homeostasis compared to offspring from lean dams (control diet); offspring fed western diet will augment this effect and exhibit epigenetic changes and a gene expression profile that promotes vascular dysfunction, oxidative stress, and inflammation. Maternal exercise in obese dams will mitigate the adverse effect of maternal obesity.

CONCLUSIONS: This research will improve our understanding of the effects of maternal exercise on cardiovascular health in offspring and developmental programming of CVD via epigenetic mechanisms. The findings will infer as to the beneficial effects of exercise during pregnancy in women with obesity and can be useful in setting guidelines for exercise during pregnancy.

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GUILAINE BOYCE | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: CHERYL WELLINGTON

THE ANTI-INFLAMMATORY EFFECT OF HIGH DENSITY LIPOPROTEINS ON CEREBRAL LEUKOCYTE INFILTRATION IN ALZHEIMER'S DISEASE PATHOGENESIS

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BACKGROUND/OBJECTIVES: Alzheimer's Disease (AD) is a devastating neurodegenerative disorder that represents the most common form of dementia worldwide. While the aetiology of AD has not been fully elucidated it is accepted that cerebral accumulation of the aggregates of the peptide amyloid beta (Ab) is key to the molecular pathology of AD. Deposits of Ab aggregates in the brain can indirectly cause neurotoxicity by promoting damaging chronic neuroinflammation via the local immune response in the brain. Cerebral Ab deposition may further propagate this inflammatory response by inducing selective recruitment of immune cells (leukocytes) from the periphery into the brain across the blood brain barrier (BBB) in a multi-step process known as leukocyte extravasation. Epidemiological evidence points to high density lipoproteins (HDL) or "good cholesterol" as a potential resiliency factor for AD. HDL is known to possess anti-inflammatory properties in peripheral vessels, however, whether these effects extend to the cerebrovasculature has yet to be determined. The objective of this study is to utilise an in vitro system to determine whether HDL can counteract the inflammatory effect of Ab on the brain vasculature to inhibit leukocyte extravasation.

METHODS: Vascular inflammation was assessed by using Western blot to measure the protein levels of the key leukocyte adhesion proteins, vascular cell adhesion protein 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1), expressed on immortalized brain microvascular endothelial cell line (HCMEC/D3) in response to inflammatory stimulus of Ab peptides (Ab 1-40 and Ab1-42). A static in vitro model of the human BBB was constructed by co-culturing primary human astrocytes with HCMEC/D3 on transwell inserts, with astrocytes and HCMEC/D3 forming the abluminal (brain) and luminal (blood) sides of the BBB system respectively. To assess the effect of peripheral HDL on leukocyte extravasation, either freshly isolated human peripheral blood mononuclear cells (PBMCs) or immortalized human monocytic cells (THP1) were fluorescently labelled and +/- freshly isolated HDL, were allowed to migrate from the luminal side of the in vitro BBB system to the abluminal side, in response to Ab peptide stimulus.

RESULTS: Treatment of HCMEC/D3 cells with HDL reduced protein levels of ICAM but not VCAM in response to Ab stimulus. Preliminary data shows that in the *in vitro* BBB system, there was a trend for Ab1-42 to increase transendothelial migration of the monocyte and lymphocyte population. The concurrent incubation of HDL with PBMCs on the luminal side of the BBB system decreased leukocyte migration. These patterns in transendothelial migration were similarly observed with THP1 cells.

CONCLUSIONS: Data from this study so far suggests that HDL may exert an anti-inflammatory effect by preventing leukocyte migration into the brain in response to Ab stimulus. A possible mechanism for this may be HDL's ability to limit expression of the inflammation-induced adhesion molecule ICAM on the luminal side of the BBB.

JACK CALDER	GRADUATE STUDENT	POSTER PRESENTATION
SUPERVISOR:	WILL LOCKWOOD	

TRANSFORMATION INDUCED DEPENDENCY OF THE NORMALLY NON-VITAL RNA HELICASE DDX3X PROVIDES A NOVEL THERAPEUTIC STRATEGY IN LUNG CANCER

BACKGROUND/OBJECTIVES: Lung cancer is the leading cause of cancer death worldwide, mainly due to the lack of effective drugs available. Difficulty in target-based drug discovery has led to a renewed interest in phenotypic/function based screening for the identification of novel small-molecule inhibitors. Through a screen of 189, 290 small molecules, we have identified a compound known as Lung Cancer Screen 3 (LCS3) that is structurally different from most known drugs. Using two different quantitative affinity approaches – SILAC and PLATO – we identified the protein DDX3X as the most likely molecular target of LCS3. DDX3X, located on the X chromosome, is a known member of the DEAD-box family of ATP dependant RNA helicases and has previously been implicated in cancers other than lung. Interestingly, expression and mutational status of DDX3X remain relatively constant across non-malignant and cancerous lung cell lines, indicating that cancer cells may be more dependent on DDX3X function than non-malignant cells. Recently, a study suggested that cancer cells can become dependent on genes that do not typically perform vital functions in normal cells in order to compensate for transformation induced stresses and that targeting these genes in the context of a transformed genotype leads to synthetic lethality. We therefore hypothesize that transformed LC cells have become dependent on a ubiquitous, non-vital normal gene, DDX3X, offering a novel therapeutic target and strategy.

METHODS: To confirm LC dependency on DDX3X I will be utilizing cDNA expression vectors to "rescue" sensitive LC cells through DDX3X overexpression. If confirmed, I look to establish whether non-malignant lung cells become dependent on DDX3X following transformation. Normal bronchial epithelial cells will be transformed by conditional expression of LC mutant oncogenes EGFR or KRAS or through exposure to the tobacco carcinogen NNK. Transformation will be assessed through soft-agar colony formation and positive transformants will be assessed on their dependency for DDX3X through treatment with LCS3.

RESULTS: To establish that DDX3X inhibition kills lung cancer cells we used an RNAi mediated knockdown approach in sensitive lung cancer cell lines. We found that knockdown of DDX3X selectively kills cancer cell lines but not normal or resistant cell lines, providing additional evidence for DDX3X as the target of LCS3-mediated killing. Additionally, a cell line found to have homozygous deletion of DDX3X was shown to be more resistant to LCS3 treatment as compared to other lung cancer cell lines. Through "rescue" experiments we look to confirm that LC cells are dependent DDX3X, as overexpression of DDX3X should make sensitive LC cells more resistant to LCS3 treatment by compensating for the inhibition of endogenous DDX3X.

CONCLUSIONS: The inhibitory characteristics of LCS3 on LC cells suggest that DDX3X may be vital to the cells survival. As DDX3X is expressed in non-malignant lung cells this may open a new avenue for lung cancer treatment not focused on targeting mutated oncogenes or tumor suppressor genes. Not only will this work provide insight into other "druggable" vulnerabilities in LC, but it will also further develop characterization of LCS3-like compounds and DDX3X inhibitors for therapeutic purposes, which may ultimately lead to better outcomes in LC patients.

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WAI HANG CHENG | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: CHERYL WELLINGTON

CHIMERA (CLOSED-HEAD IMPACT MODEL OF ENGINEERED ROTATIONAL ACCELERATION) TRAUMATIC BRAIN INJURY EXACERBATES NEUROPATHOLOGY IN APP/PS1 MICE

AUTHORS:

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BACKGROUND/OBJECTIVES: Traumatic brain injury (TBI) may raise Alzheimer Disease (AD) risk up to 10 fold. Mild TBI, which comprises over 75% of all TBI cases, can lead to long-term neurodegeneration with AD-like neuropathologies upon repetitive exposure. This study aims to study how mild repetitive TBI (mrTBI) exacerbates neuropathology in an APP/PS1 amyloidosis model by age.

METHODS: The experimental rodent TBI model recently developed by our laboratory - CHIMERA - is used to induce impact-acceleration closed-head TBI. Male APP/PS1 mice at 5-mo or 13-mo were subjected to 2 mild TBI spaced 24hr apart, and sacrificed at 2-14 days after. Behavioral tests assessed neurological, motor, and cognitive functions. Histological and biochemical assays assessed amyloid-beta, axonal, and microglial changes.

RESULTS: Compared to sham-operated APP/PS1 mice, mrTBI prolonged loss of righting reflex, increased neurological deficits (neurological severity score), and exacerbated motor discoordination (Rotarod) in APP/PS1 mice. Preliminary data suggest that mrTBI resulted in poorer Barnes Maze performance in APP/PS1 but not WT littermates. By 2-day post-TBI, 5-mo APP/PS1 showed increased 6E10+ve amyloid-beta deposits with no change in amyloid-beta deposit in 13-mo APP/PS1. MrTBI also increased Iba-1 microglial activation and argyrophilic fibres in white matter areas, including optic tract, with similar effects in WT and APP/PS1. Blood brain barrier integrity is being assessed by serum protein extravasation.

CONCLUSIONS: Clinically around 30% of post-TBI brains have amyloid deposits, and the mechanisms by which TBI is involved not yet clear. Our results suggest that age may contribute to amyloid-beta neuropathology after mrTBI.

JENNA COLLIER	GRADUATE STUDENT	POSTER PRESENTATION
SUPERVISOR:	KEVIN BENNEWITH	

CYTOKINE AND IMMUNE CELL PROFILES OF EARLY LUNG CANCER DEVELOPMENT

BACKGROUND/OBJECTIVES: Low dose computed tomography (LDCT) screening of smokers at high-risk of developing lung cancer identifies cancerous and pre-cancerous lesions early, and substantially improves patient survival. However, physicians require biomarkers to accurately predict which lung nodules detected by LDCT are malignant and how fast they will grow or spread. The development of these biomarkers has been problematic since most proposed biomarkers lack sensitivity and specificity to identify patients with early lung cancer with minimal tumour burden. With the recent success of immune modulatory agents in the treatment of advanced lung cancer, aberrant regulation of the immune response to tumour cells has emerged as a critical contributor to lung cancer progression. In particular, the frequency of exhausted T lymphocytes in patients—T cells with reduced functional and proliferative capabilities—may predict response to checkpoint inhibitor therapy.

We postulate that the immune system is capable of detecting neoplastic transformation in lung cells, resulting in quantifiable changes in cytokines, chemokines and immune cells in lung tissue and peripheral blood (PB). We will use in vitro and novel mouse models of oncogene-driven lung tumourigenesis to identify and functionally validate cytokines and immune cell profiles in the lungs and blood during lung tumour development with subsequent validation in unique lung cancer patient samples. **Objective:** To optimize the use of Luminex and mass cytometry for the quantification of temporal changes in cytokines and immune cell populations in murine lung tissue and in human PB.

METHODS: Temporal changes in cytokine production by oncogenic-driven transformation of 3T3 cell lines expressing the oncogenes EGFRL858R, wtEGFR, or KRASG12V under the control of a doxycycline-inducible promoter (TetO) were analyzed in vitro by Luminex over 5 days of induction. To optimize the panel of 27 murine antibodies to be used for mass cytometry, metastatic lungs of mice bearing orthotopic 4T1 or 4T07 mammary tumours were analyzed. Peripheral blood mononuclear cells (PBMCs) were isolated from the PB of lung cancer patients and analyzed by mass cytometry using a 29 antibody panel including various markers for the identification of exhausted T cells.

RESULTS: The secretion of the cytokine RANTES (CCL5) increased 100-fold during oncogenic transformation driven by TetO-EGFR^{L858R} compared to a 20 to 30-fold increase by 3T3 TetO-KRASG12V or 3T3 TetO-wtEGFR. Mass cytometry was used to identify various immune cell populations—including potentially exhausted T lymphocytes—in metastatic murine lungs and in the PB of lung cancer patients, though further optimization of cell processing for mass cytometry analysis is necessary.

CONCLUSIONS: We found that cells transformed with mutant EGFR express different cytokines than cells transformed with a mutant form of KRAS, suggesting that specific immune cell subsets may infiltrate lung tumours driven by each oncogene.

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TANYA DE SILVA | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: WILLIAM LOCKWOOD

CHARACTERIZATION OF THE NOVEL CANDIDATE TUMOR SUPPRESSOR GENE SNF2 HISTONE LINKER PHD RING HELICASE E3 UBIQUITIN PROTEIN LIGASE (SHPRH) IN LUNG CANCER SUSCEPTIBILITY AND DEVELOPMENT

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BACKGROUND/OBJECTIVES: Lung cancer is the leading cause of cancer related death worldwide, mainly due to late stage diagnosis. Early detection of lung cancer is essential in order to improve patient outcomes. Using comprehensive genomic characterization of a panel of lung adenocarcinomas (LAC), our group has identified a candidate tumor suppressor gene (TSG), SHPRH, based on its high frequency of disruption. SHPRH is located on chromosome 6q, a genetic locus associated with familial lung cancer susceptibility, and is an evolutionarily conserved E3 ligase that mediates crucial processes related to DNA repair. We hypothesize that SHPRH has critical roles in regulating lung cancer susceptibility and development.

METHODS: I will utilize LAC cell lines with biallelic inactivation (e.g. homozygous deletion) of SHPRH and engineer these cells to reactivate SHPRH using tetracycline-inducible lentiviral vectors. The effects of SHPRH expression will be assessed at multiple levels in vitro using proliferation, migration and invasion assays and also determined in vivo using tumour xenografts in immune compromised mice. To assess the impact of SHPRH on human lung cancer initiation, I will use normal human peripheral (small) airway epithelial cells for transformation assays using shRNAs targeting SHPRH in conjunction with exposure to tobacco carcinogens. Given the immense genetic instability in subsets of LACs, I will also determine the effects of SHPRH reconstitution on genomic instability using DNA damage assays to quantify and analyze DNA defects and repair in individual cells.

RESULTS: Our genomic data identified frequent, biallelic inactivation of SHPRH in LACs, highlighting it as a candidate TSG. SHPRH plays fundamental roles in DNA repair and lies within the lung cancer susceptibility locus on chromosome 6q, it is possible that its inactivation could lead to increased lung cancer risk by promoting an impaired response to DNA damaging agents such as cigarette smoke. Our results indicate that SHPRH may promotes growth arrest in LAC cell lines using cell viability assays. SHPRH knockdown promotes human lung cancer initiation in normal human peripheral (small) airway epithelial cells as measured by their ability to transform by soft agar colony formation after treatment with tobacco carcinogens. These results could be explained as DNA repair deficiencies due to loss of SHPRH may increase their transformability in response to carcinogens due to the increased accumulation of chromosome aberrations.

CONCLUSIONS: The ubiquitous expression of SHPRH as well as its genetic alterations in SHPRH may contribute to carcinogenesis as cells are exposed to DNA damage agents, by incremental accumulation of vulnerable defects in DNA repair, which may be exploited for LC therapy. The DNA repair properties of SHPRH and its location within the lung cancer sensitivity region suggest that this gene, once verified in our model systems, may be useful as a risk marker for lung cancer. Future studies on the disruption of SHPRH in lung cancer may help us predict who may be at risk, and ultimately, new avenues for therapies designed.

LISA DECOTRET	GRADUATE STUDENT	POSTER PRESENTATION
SUPERVISOR:	CATHERINE J. PALLEN	

PROTEIN TYROSINE PHOSPHATASE ALPHA (PTP ALPHA) REGULATES INVADOPODIA-MEDIATED MATRIX DEGRADATION

BACKGROUND/OBJECTIVES: Normal cell migration is a highly conserved process that plays a critical role in many physiological processes including wound healing and immune cell response. Aberrant cell migration is implicated in cancer metastasis, which is the leading cause of cancer related mortalities. In normal cells, cell migration is a dynamic process in which the cell adheres to and then releases from the extracellular matrix (ECM), which involves specialized structures called focal adhesions (FAs). In highly invasive tumour cells, invasive ability is mediated by similar integrin-mediated structures called invadopodia. These are Src-regulated dynamic actin-based protrusions of the plasma membrane that mediate extracellular matrix (ECM) degradation. Protein tyrosine phosphatase alpha (PTP alpha), a widely expressed transmembrane protein, positively regulates integrin signaling and promotes cell migration. Recent findings have demonstrated that PTP alpha is a critical Src activator and that subsequent Src-mediated phosphorylation of PTP alpha at Tyr789 is required for normal cell migration. However, little is known about the role of PTP alpha in cancer cell motility. Our goal is to determine the roles of PTP alpha in Src-signaling mechanisms that regulate invadopodia structure and function to promote the invasive motility of malignant cells.

METHODS: We are currently investigating the structural and functional consequences of the loss of PTP alpha on invadopodia-mediated cancer metastasis. I first used siRNA to knockdown PTP alpha in the invasive MDA-MB-231 breast cancer cell line. Then, I performed an invadopodia-mediated ECM-degradation assay using Oregon Green-488 conjugated gelatin coated coverslips to determine the ECM-degradation ability of wild-type vs. PTP alpha-depleted MDA-MB-231 cells. Finally, I confirmed that ECM-degradation occurs where invadopodia form (marked by co-localized actin/cortactin) using immunofluorescence microscopy.

RESULTS: Small interfering-RNA mediated depletion of PTP alpha reveals an impaired ability of invadopodia to degrade ECM, indicating the importance of PTP alpha in invadopodia function. However, through the use of immunofluorescence microscopy, it appears that PTP alpha may not regulate the formation of invadopodia.

CONCLUSIONS: Our preliminary data suggest that PTP alpha positively regulates the ECM-degradation ability of invadopodia. Thus, PTP alpha may play a role in invadopodia-mediated cancer invasive motility, which may lead to new mechanistic targets for therapeutic interventions to prevent cancer metastasis and limit mortality.

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NATALIE FIRMINO	GRADUATE STUDENT	POSTER PRESENTATION
SUPERVISOR:	KEVIN BENNEWITH	

HYPOXIA IS INDUCED IN THE TUMOR-DRAINING LYMPH NODE

AUTHORS:

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BACKGROUND/OBJECTIVES: Lymph node metastasis has historically been considered a passive process, with cancer cells draining through the lymphatic system and becoming trapped in downstream lymph nodes; however, recent evidence suggests that the development of metastases in the tumour-draining lymph nodes (TDLN) requires pre-metastatic changes in the lymph node microenvironment. Such changes include increased lymphangiogenesis and the dilation of the subcapsular sinus (SCS), a region of the lymph node that lymph-borne tumour cells first encounter. Since the (SCS) is devoid of blood vessels, we hypothesized that tissue near the (SCS) of (TDLNs) and therefore local immune cell populations are hypoxic, resulting in impaired anti-tumour immune responses and a permissive environment for metastatic colonization of the lymph node.

METHODS: Murine mammary carcinoma cells (4T1 and 4TO7) were injected into the fat pad of immune-competent BALB/c mice. After the establishment of mammary tumours (3 weeks post-implant), the hypoxia-specific marker pimonidazole was administered to label all hypoxic cells in tumour-bearing and control mice. Tissue sections of (TDLNs) and healthy inguinal lymph nodes were stained with antibodies against pimonidazole to assess local hypoxia.

RESULTS: Our results show pimonidazole staining within the cortex and (SCS) of (TDLNs). Pimonidazole staining was not present in the inguinal lymph nodes of non-tumour bearing mice. These preliminary results indicate that these regions of hypoxia develop prior to the establishment of lymph node metastases.

CONCLUSIONS: Initial results suggest that (TDLNs) are hypoxic relative to control lymph nodes. Future studies will determine which immune cells are exposed to (TDLN) hypoxia and how this environment affects their phenotype.

MARYAM GHAEDI	GRADUATE STUDENT	POSTER PRESENTATION
SUPERVISOR:	FUMIO TAKEI	

NEW INSIGHTS INTO THE PATHWAYS OF INNATE AND T LYMPHOCYTE DEVELOPMENT

BACKGROUND/OBJECTIVES: All lymphocytes, including T, B and innate lymphocytes, are thought to develop from common lymphoid progenitors (CLPs). However, the upstream lymphoid progenitors to CLPs, lymphoid- primed multipotent progenitors (LMPPs) are more efficient than CLPs in differentiating into T cells and group 2 innate lymphoid cells (ILC2s) in transplantation assays.

METHODS: Here, we have divided LMPPs, based on the expression of the lymphoid marker CD127, into CD127- (LMPP-s) and CD127+ (LMPP+s) subsets and compared them with Ly6D- and Ly6D+ CLPs. It has been previously shown that Ly6D- CLPs have potent T and NK cell potentials and Ly6D+ CLPs mainly behave as B cell progenitors. Ly6D- CLPs, Ly6D+ CLPs, LMPP-s, or LMPP+s were purified by cell sorting from C57BL/6 mice (CD45.2+), and 2,000 cells of each population were intravenously injected with helper BM cells from NSG mice (CD45.1+) into lethally irradiated congenic B6.Ly5SJL recipients (CD45.1+). The tissues of the recipient mice were analyzed by flow cytometry at 7, 10, 17, and 27 days post-transplantation. We performed global gene expression analysis of these progenitor populations by Affymetrix microarray. RNA was extracted from purified LMPP-s, LMPP+s, Ly6D- CLPs, and Ly6D+ CLPs and analyzed for gene expression. We also performed comprehensive analysis on the neonatal lymphoid progenitors and assessed their lymphoid potential in transplantation assays.

RESULTS: Adult LMPP+s differentiated into T cells and ILCs more rapidly and efficiently than other progenitors in transplantation assays. The development of T cells and ILC2s is highly active in the neonatal period. Neonatal CLPs are rare and, un-like prominent neonatal LMPP+s, do not efficiently differentiate into T cells and ILC2s. ILC2s generated in the neonatal period are long lived and persist in adult tissues.

CONCLUSIONS: Our data suggest that, some long-lived T cells and ILCs develop from LMPP+s bypassing the CLP stage at the neonatal stage and persist into the adulthood, whereas short-lived B cells are continuously produced from the prevalent BM Ly6D+ CLPs in adults. Ly6D- CLPs may also contribute to the T and ILC lineages in adult mice. However, the development of T cells and ILCs from lymphoid progenitors in adult mice appears limited.

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AMANDA HENDERSON | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: | ANGELA DEVLIN

THE ROLE OF INSULIN-LIKE GROWTH FACTOR-1 IN PROGRAMMING OF OFFSPRING ADIPOSITY BY MATERNAL FOLATE/ VITAMIN B12 IMBALANCE

AUTHORS:

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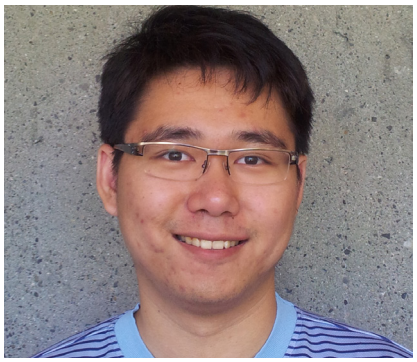
BACKGROUND/OBJECTIVES: Developmental programming suggest that prenatal and early postnatal environment, such as maternal nutrition, can impact risk for chronic disease later in life. Recent population studies have reported greater insulin resistance and adiposity in children from mothers with adequate folate but low vitamin B12 (B12) status during pregnancy. Grain products in North America are fortified with folic acid to reduce the incidence of neural tube defects. Folate is required for methyl metabolism and is metabolically linked to vitamin B12. Low B12 status, even when folate is adequate, can trap folate in a metabolically inactive form. Folate deficiency is rare in Canada, yet approximately 1 in 20 Canadians are deficient in B12. The mechanisms underlying the relationship between maternal imbalance of folate/ B12 status and offspring adiposity and insulin resistance is not understood. A study recently reported that maternal B12 deficiency during pregnancy disrupts growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis, resulting in growth retardation and bone malformation in the offspring. The objective of this study is to determine if programming of offspring adiposity by maternal folate/ B12 imbalance involves disturbances in the GH/IGF-1 axis.

METHODS: Female mice (C57BL/6J) were fed one of 3 diets: control (2mg folic acid/kg diet, M-CON), supplemental FA (10mg/kg diet) with adequate B12 (SFA+B12), or supplemental FA without B12 (SFA-B12). Dams were fed the diets 6 weeks prior to conception, through breeding, pregnancy, and lactation. One male and one female pup from each dam were weaned onto a control diet or a high-fat (45% energy) diet. Tissue was harvested from offspring mice at 20 weeks post-weaning. Serum IGF-1 concentrations were quantified by ELISA. Hepatic Igf1 mRNA, Igfbp2 mRNA, and Cpt1 mRNA expression was quantified by Real-Time PCR using the ΔΔCt method of relative quantification. The effect of maternal diet was determined by 1-way ANOVA, separately in offspring fed the post weaning control diet and western diet.

RESULTS: Female SFA-B12 offspring fed the post weaning control diet had lower ($p<0.05$) serum IGF-1 concentrations than M-CON offspring and SFA+B12 offspring. Female SFA-B12 offspring fed the post weaning western diet had lower ($p=0.08$) serum IGF-1 concentrations than SFA+B12 offspring. No effect of maternal diet on serum IGF-1 concentrations was observed in male offspring. Female SFA+B12 offspring fed the post weaning western diet had higher ($p=0.008$) hepatic Igf1 mRNA than M-CON offspring. No effect of maternal diet on hepatic Igf1 mRNA was observed in male offspring. Male SFA-B12 offspring fed the post weaning control diet had higher ($p=0.028$) hepatic Igfbp2 mRNA than M-CON offspring. No effect of maternal diet on hepatic Igfbp2 mRNA was observed in female offspring.

CONCLUSIONS: These findings suggest a role for IGF-1 in programming of offspring adiposity by maternal folate/ B12 imbalance. It is vital to understand the implications of folate/B12 imbalances, particularly during pregnancy, to optimize the health of Canadians.

ANTHONY HSIEH	GRADUATE STUDENT	POSTER PRESENTATION
SUPERVISOR:	HÉLÈNE CÔTÉ	



NEUROCOGNITIVE IMPAIRMENT IN PEOPLE LIVING WITH HIV MAY BE RELATED TO BLOOD MITOCHONDRIAL DNA

BACKGROUND/OBJECTIVES: Over the past 30 years, robust data from multiple studies have revealed a connection between HIV and neurocognitive impairment, and HIV is now associated with a spectrum of neurological disorders. Although treatment with combination AntiRetroviral Therapy (cART) has been successful in extending lifespan and preventing AIDS, it does not reduce the severity or prevalence of HIV-related neurocognitive deficits, which can be as high as 50%.

Studies have implicated mitochondrial dysfunction in HIV-related neurocognitive impairment. HIV has been linked to elevated mitochondrial oxidative stress resulting in the accumulation of endogenous reactive oxygen species (ROS), which can directly damage the mitochondria and it DNA (mtDNA). MtDNA content is a biomarker used to investigate mitochondrial health. Recently, large studies have linked HIV-associated neurocognitive disorders to higher mtDNA content in blood as well as increased cell-free mtDNA in cerebrospinal fluid. In 2014, we showed neurocognitive impairments associated with HIV infection in women compared to an HIV- control group with similar demographic backgrounds and comorbidities. Here, our objective is to assess mtDNA content as a secondary use of data in the same cohort, to determine whether results from neurocognitive assessments, including the computerized Cambridge Neuropsychological Test Automated Battery (CANTAB), are associated with blood mtDNA content. We hypothesize that higher blood mtDNA content is associated with measures of neurocognitive impairment in HIV+, but not in HIV- women.

METHODS: Blood mtDNA content was measured by qPCR in 81 HIV+ and 45 HIV- women enrolled in the 5.1 substudy of the Children and Women: AntiRetrovirals and Markers of Aging (CARMA) cohort. Data from CANTAB, along with conventional tests for fine motor speed and dexterity, learning and memory, speed of information processing, and working memory were collected (Giesbrecht et al. PlosONE, 2014). Group comparisons by HIV serostatus were performed using Mann-Whitney U tests. Univariate associations were analyzed using Spearman's correlation. Multivariable analyses were by linear regression modelling.

RESULTS: Among all participants (n=125), lower blood mtDNA content was associated with better scores on the Cambridge Gambling Task (CGT)-Risk Adjustment test (p=0.024), a CANTAB assessment for executive functioning, after adjusting for age and years of education. Repeating the same model in each group reveals that this association is driven by the HIV+ group (HIV+ n=80, p=0.018 vs. HIV- n=45, p=0.264). Univariately, mtDNA was not associated with any individual cognitive measure previously found to differ by HIV status and was not significantly different between the HIV+ and HIV- groups.

CONCLUSIONS: These results suggest that blood mtDNA content may be related to executive functioning in the context of HIV. Although our study did not consider cART effects and is likely insufficiently powered to detect the influence of HIV on mtDNA associations, it lends support to the notion that blood mtDNA content may be related neurocognitive impairments.

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HANI BAGHERI | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: EVICA RAJCAN-SEPAROVIC

DECIPHERING THE IMPORTANCE OF NON-CODING ELEMENTS DELETED IN PATIENTS WITH 2P15P16.1 MICRODELETION SYNDROME

AUTHORS:

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BACKGROUND/OBJECTIVES: The 2p15p16.1 microdeletion syndrome (OMIM 612513) is a rare genetic disorder caused by de novo deletion of a small segment of chromosome 2 that we first described in two phenotypically similar individuals with intellectual disability (ID). So far, more than 30 subjects with this microdeletion have been reported in literature who share common clinical features including delayed neurocognitive development and consistent craniofacial defects e.g. microcephaly and unique facial dysmorphism. Microdeletions in the subjects vary in size and we have previously shown using a multifaceted approach that any of the 3 commonly deleted genes, XPO1, USP34, and BCL11A could be a strong candidate for the syndrome. However, the existence of 2 patients with deletions in the 2p15p16.1 region which do not contain any of the 3 genes suggests that non-coding elements regulating the 3 genes could contribute to common syndromic features.

METHODS: We reviewed the characteristics of the two deletions without the 3 candidate genes using UCSC and VISTA enhancer browsers. The VISTA Enhancer Browser was also used to find and compare the distribution of all positive enhancer elements and neurospecific enhancer elements in the human genome. To identify whether 2p15p16.1 enhancer elements are expression quantitative trait loci (eQTLs), we extracted SNPs involved within the enhancer sequences and investigated if they were significantly associated with expression levels of 2p15p16.1 genes and other genome-wide genes in the brain using several webtools e.g. braineac.org. The effect of enhancer SNPs on transcription factor (TF) binding was also assessed using regulomedb.org.

RESULTS: Our VISTA enhancer browser analysis suggested that chromosome 2 contains the highest number of enhancers in the genome (104 of 897 VISTA-positive and 68 of 617 brain/neuronal-expressed enhancers, of which approximately one-third are present in the 2p15p16.1 region). The non-coding elements in the two patient deletions without the 3 genes included 11 enhancers. Our preliminary data also suggests that some of the 11 2p15p16.1 enhancers are regulating the 3 genes and are important TF binding sites.

CONCLUSIONS: Using webtools, we described a bioinformatics-assisted approach to decipher the effects of non-coding enhancer elements on the 2p15p16.1 syndrome. Our findings will set the foundation for improved understanding of the role of enhancers in regulation of genes from 2p15p16.1 region.

CHRISTA KLEIN-BOSGOED | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: DANA V. DEVINE

THE IMPACT OF PATHOGEN INACTIVATION TREATMENT ON SELECTED PLATELET MRNA TRANSCRIPTS

BACKGROUND/OBJECTIVES: Pathogen inactivation treatments (PIs) use UV-light with or without a photosensitive agent to modify viral and bacterial RNA and DNA. Even though platelets are anucleate, it has been demonstrated that they can synthesize proteins using RNA and the ribosomal machinery derived from megakaryocytes. Pathogen-inactivated platelet concentrates show signs of accelerated storage lesion, but the effect of PI on platelet mRNA and subsequent protein synthesis remains unclear. In this study we analyzed the effect of the Mirasol Pathogen Reduction Technology on total platelet RNA and on specific platelet mRNA transcripts. We hypothesize that the application of this Pathogen Inactivation Treatment, which is designed to target viral and bacterial RNA and DNA, has a negative impact on platelet mRNA.

METHODS: Apheresis PCs were collected from healthy volunteer donors. In a pool and split design ($N \geq 3$), one unit was PI treated while the other remained untreated. Total RNA was extracted using TRIzol (Invitrogen, Burlington ON), normalized by platelet count, and quantitated by measuring the absorbance at 260 nM. Total RNA was then treated with DNase, followed by cDNA synthesis. The transcripts selected for assessment were the glycoproteins (GP)IIIa, GPIIb and GPIb, the alpha-granule proteins platelet factor 4 (PF4), osteonectin, thrombospondin (TSP) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Quantitative polymerase chain reaction with primers specific for the selected transcripts was used to determine the relative mRNA amounts.

RESULTS: After treatment all analyzed mRNAs were significantly reduced ($P < 0.05$), but to different degrees. For GAPDH and PF4, transcripts appeared less susceptible to the treatment, with 70% remaining one hour after UV illumination. For GPIIIa and TSP, less than 15% remained after treatment. There was a correlation ($R2 = 0.85$) between transcript length and amount of mRNA remaining one hour after treatment. All transcripts, except PF4 mRNA, demonstrated an increase in mRNA half-life post-treatment. Total RNA demonstrated a life span equal to the platelet life span of 10 – 11 days.

CONCLUSIONS: Treatment of platelets with riboflavin and UV illumination has detrimental effects on platelet mRNA. Remarkably, the impact of the treatment was different among the tested transcripts. Here we demonstrate that there is a strong correlation between the impact of the treatment on platelet mRNA and the length of the transcript. In treated platelets, the selected mRNA targets showed a different degradation pattern during storage compared to untreated control platelets, resulting in a longer mRNA half-life post-treatment for most of the platelet transcripts similar to what can be seen in nucleated cells. Our findings suggest mechanisms might be in place in platelets, protecting platelet mRNA from rapid degradation.

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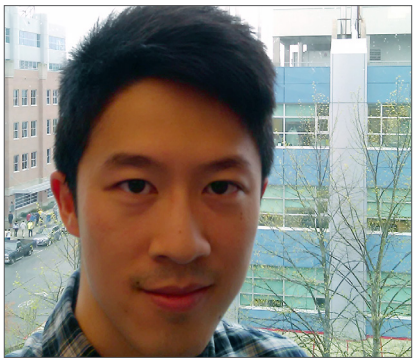

BRYAN LIN | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: ED PRYZDIAL

VIRUS ASSIMILATION OF HOST COAGULATION FACTORS

AUTHORS:

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BACKGROUND/OBJECTIVES: Viruses pose a pervasive risk in our blood and blood systems. Many of which have an outer envelope structure containing host-derived membrane lipids and proteins, as well as virus-encoded proteins. We have shown that the herpes simplex virus type 1 (HSV1) envelope acquires tissue factor (TF) and anionic phospholipids (aPL) from the host, both vital in physiological blood coagulation. Viral TF and aPL accelerates the enzyme factor (F) VIIa in activating FX to FXa, leading to clot formation. Without TF, virus infectivity is compromised in vitro and in vivo, as evidenced by our lab. As well, the multi-functional HSV1-encoded glycoprotein C (gC) can enhance FVIIa activity and bind to FX on the virus surface and in solution, suggesting that gC may have evolved to affect TF function. Objectives: 1) To determine if other important blood-related enveloped viruses (e.g. dengue virus) obtain TF and/or aPL. 2) To define the role of viral TF as well as gC in promoting coagulation.

METHODS: Immunogold electron microscopy was used to simultaneously visualize virus markers gC or E protein (for HSV1 and dengue virus, respectively), TF and Annexin V binding as a probe for aPL on the virus surface. FX activation by virus or a soluble form of recombinant gC was followed through the use of a FXa-selective chromogenic substrate. Plasma clotting induced by HSV1 was characterized in human normal pooled plasma and factor VIII deficient plasma (to prevent non-TF dependent FX activation).

RESULTS: My results have shown that HSV1 and dengue virus can incorporate TF into their envelope. HSV1 displayed functional TF as followed by FX activation. Furthermore, TF presence was essential for a two-fold enhancement of FX activation by virus-bound gC. Similarly, a soluble form of gC also enhanced TF activity in a dose-dependent manner. In plasma, gC enhanced TF-dependent clotting times induced by HSV1, although factor VIII compensated for the absence of gC.

CONCLUSIONS: Enveloped viruses steal host coagulation factors, expressing them on their surface thereby predisposing an individual to hypercoagulability leading to vascular disease. Targeting TF on viruses as a broad-spectrum anti-viral may decrease infectivity, prevent vascular complications and improve the safety of blood transfusion products. HSV1 has a virus-encoded cofactor for FX activation; further studies can allow the design of new therapeutics that improve clotting in patients with bleeding disorders.

YULIA MERKULOVA	GRADUATE STUDENT	POSTER PRESENTATION
SUPERVISOR:	DAVID GRANVILLE	

GRANZYME B INHIBITS KERATINOCYTE MIGRATION BY DISRUPTING EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)-MEDIATED SIGNALLING

BACKGROUND/OBJECTIVES: Chronic non-healing wounds including diabetic, venous, and decubitus skin ulcers are a persistent problem due to the lack of effective therapies and have an annual treatment cost in the U.S. in excess of $25 billion. These wounds can become infected and result in amputation. In fact, globally every 30 seconds a lower limb is lost to diabetic chronic wounds. Growth factor therapies have been attempted, however, their effects are modest, and these treatments lead to increased risk of skin malignancies. Thus there is a great need for development of therapeutic strategies to treat non-healing skin wounds. Granzyme B (GzmB) is a serine protease that was, until recently, believed to function exclusively in cytotoxic lymphocyte-mediated apoptosis. However, during excessive or chronic inflammation, GzmB can accumulate in the extracellular milieu, retain its activity, and cleave a number of important extracellular proteins. GzmB is abundant in diseases associated with aging and chronic inflammation such as non-healing skin wounds, rheumatoid arthritis, and cancer. Epidermal growth factor receptor (EGFR) is a transmembrane receptor involved in cell proliferation and migration, and its signalling is essential during wound healing. The present study investigated the effect of GzmB on keratinocyte migration and EGFR-mediated signaling. We hypothesize that GzmB impairs EGFR-mediated cell migration.

METHODS: Scratch assay and Electric Cell-Substrate Impedance Sensing (ECIS) were used to simulate wound healing in vitro and assess epithelial cell migration. Immunofluorescent staining of GzmB-treated epithelial cells followed by confocal microscopy was used to show changes in migratory cell morphology. Cells were treated with GzmB (0-200 nM for 6 h) and EGF-induced EGFR phosphorylation was monitored by immunoblotting for phosphorylated EGFR (tyrosine 1068). Biochemical assays were used to investigate direct proteolytic effects of GzmB on extracellular EGFR domain and EGF. Lastly, cell viability was assessed by flow cytometry. Data analyzed by one-way ANOVA with Tukey's multiple comparisons test or Mann-Whitney test.

RESULTS: GzmB inhibited the migration of both unstimulated and EGF-stimulated keratinocytes in vitro. While no migration was observed in GzmB-treated/EGF-unstimulated cells over 18 hours, GzmB-treated/EGF-stimulated cells showed a significant delay in migration compared to GzmB-untreated/EGF-stimulated cells, indicating that GzmB impairs keratinocyte migration through an EGFR-dependent mechanism. Additionally GzmB reduced the phosphorylation levels of EGFR in a dose-dependent manner. GzmB did not cleave the extracellular domain of EGFR or EGF and did not affect cell viability.

CONCLUSIONS: GzmB may contribute to pathogenesis of chronic wounds by disrupting the EGFR signalling pathway, to result in impaired keratinocyte migration. Further work is required to delineate the mechanism behind GzmB-induced EGFR pathway impairment. Additional cytoskeletal staining and immunofluorescence will be performed to support current immunofluorescence data. This work may lead to improved wound healing therapies for chronic non-healing wounds.

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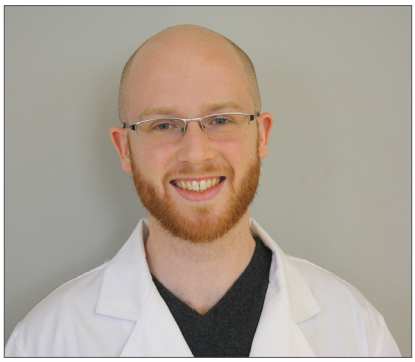

FRASER MUIR | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: G.R. WAYNE MOORE, JACQUELINE QUANDT

EVIDENCE FOR LIPID ANTIGEN PRESENTATION IN DISORDERS OF THE CENTRAL NERVOUS SYSTEM

AUTHORS:

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BACKGROUND/OBJECTIVES: The inflammatory immune response has long been held to be a key component to the disease process of Multiple Sclerosis (MS), which is believed to be autoimmune in nature. Despite the myelin sheath being composed of 70 to 85% lipids the literature has primarily focused on the immune response against the peptide components of the myelin sheath, with researchers only recently turning their attention to the possibility of lipid antigens playing a role in the pathogenesis of MS. Research conducted in our lab looking at the diffusely abnormal white matter in archival MS brain tissue has shown that there is a lipid-specific depletion, with preservation of myelin proteins within the diffusely abnormal white matter; suggesting the possibility of an immune response against myelin lipids in MS. The lipid antigen presenting molecules of the immune system are the five members of the CD1, Class I MHC-like, protein family. Of these five the one of most interest to researchers has been CD1d; it the only one of the five present in mice, and has been shown to present sulfatide (a component of myelin) to immune cells such as natural killer T cells. Human studies have found changes in the regulation and number of circulating monocytes expressing CD1 in MS and suggest a role for them in the disease, yet to date no studies have investigated their presence in tissues of the CNS.

METHODS: Archival formalin-fixed, paraffin-embedded MS, non-MS focal, destructive lesions, and normal control brain tissues were sectioned and then stained with H&E, luxol fast blue (LFB for myelin), and HLA-DR (class II MHC). Tissue blocks were next selected which contained lesions, and - in the case of MS tissues - were categorized as either active, chronic active (CA), or chronic silent (CS) based upon the HLA-DR staining characteristics. Sections were triple stained for CD1d, Iba-1 for microglia, Glial Fibrillary Acidic Protein (GFAP) for astrocytes, and 4',6-diamidino-2-phenylindole (DAPI) for nuclei. Slides were examined using an epifluorescent microscope, and CD1d-positive cells were quantified per mm2 and the numbers of cells double labeling for CD1d with Iba-1 or GFAP were noted.

RESULTS: CD1d-positive cells were present in both MS, and non-MS lesions and absent in healthy tissue. In both cases CD1d colocalized with either microglia or reactive astrocytes. Overall, active lesions/acute pathologies tended to present with primarily CD1d-positive astrocytes, whereas the chronic lesions/older pathologies showed more microglial CD1d expression.

CONCLUSIONS: The localization of CD1d to distinct glia during disease is novel, and suggests the type of antigen presenting cell may vary with the age of the lesion. These findings support the idea that there is an anti-lipid autoimmune response in the pathogenesis of MS, as well as other CNS pathologies.

JON OBST	GRADUATE STUDENT	POSTER PRESENTATION
SUPERVISOR:	MARIANNE SADAR	

RESISTANCE TO A NOVEL, ANDROGEN RECEPTOR (AR) N-TERMINAL DOMAIN INHIBITOR OCCURS VIA A SELECTIVE METABOLISM PATHWAY

BACKGROUND/OBJECTIVES: Prostate cancer (PCa) represents the second leading cause of all cancer related mortality in North American men. The androgen receptor (AR) has long been recognized as playing a crucial role in tumour maintenance and progression; therefore its inhibition has been the cornerstone of modern therapy for men who fail primary treatment. Current treatment options are initially effective, however resistance ultimately develops and the disease progresses to a lethal form termed castration-resistant prostate cancer (CRPC). Our lab has discovered a new class of molecules (EPI) which inhibit the AR by binding to the N-terminal domain (NTD). The NTD interacts with transcriptional machinery, and its presence is absolutely necessary for a transcriptionally functional receptor. We have previously shown that EPI-002 specifically inhibits both full length AR, and constitutively active AR splice variants. The efficacy of our lead compound in the context of drug resistant CRPC is currently being tested in a Phase I/II clinical trial. Here we attempt to identify novel resistance mechanisms arising from sustained AR-NTD inhibition.

METHODS: The androgen sensitive human PCa cell line LNCaP was used in all experiments. A potentially resistant cell line (LNCaP-EPIR) was generated by serially passaging parental LNCaP cells once weekly in media supplemented with EPI-002 beginning in September 2012. Growth curves were generated for both lines following treatment with EPI-002 and anti-androgen enzalutamide (ENZ) with and without androgen stimulation. LNCaP-EPIR cells were used as a xenograft model confirming biological resistance also occurs in vivo. A human transcriptome microarray (Affymetrix) was used to identify possible resistance mechanisms and validated using qRT-PCR.

RESULTS: LNCaP-EPIR cells treated with 25 μM EPI-002 displayed similar growth rates to vehicle treatment, both in vitro and in vivo. Conversely, parental LNCaP cells showed significant growth inhibition in response to drug treatment. Intriguingly, growth of LNCaP-EPIR cells was significantly inhibited by anti-androgen ENZ, and sensitivity was readily seen in the xenograft study as well. This phenomenon implies functional AR-mediated transcription remains an integral factor in driving proliferation, despite chronic EPI-002 treatment. qRT-PCR data demonstrated that EPI-002 failed to prevent AR mediated transcription for number of AR regulated genes. Interrogation of microarray data revealed candidate genes (UGT2B family) which were specifically upregulated in the resistant line, and may function to metabolize EPI-002. Supporting this hypothesis, an EPI-045 analog resistant to UGT2B metabolism was able significantly reduce LNCaP-EPIR proliferation and AR transciption.

CONCLUSIONS: Taken together these data point to an EPI-specific mechanism of resistance, whereby EPI-002 is preferentially metabolized and removed from the cell. LNCaP-EPIR cells remain dependent upon AR signalling, and are sensitive to anti-androgens used clinically. This work highlights the potential for combination or sequential therapy in the context of drug-resistant CRPC. and ILCs from lymphoid progenitors in adult mice appears limited.

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SARA SABERI | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: HELENE COTE

CONCORDANCE BETWEEN PLASMA COTININE CONCENTRATION AND SMOKING SELF-REPORTING BY PREGNANT WOMEN IN THE CARMA (CELLULAR AGING AND HIV COMORBIDITIES IN WOMEN AND CHILDREN) COHORT STUDY

AUTHORS:

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BACKGROUND/OBJECTIVES: Although it is a well-established risk factor for adverse pregnancy outcome, smoking during pregnancy is still common, with smoking rates varying from 10% in Japan to 24% in Scotland, and 30-35% in Spain. In Canada, the 2001 smoking rate among pregnant women was estimated at 17% in. Most cohort studies collect information on smoking, and the vast majority use self-report data. Due to stigma associated with smoking during pregnancy, there is a risk that the behavior may be underreported, leading to study biases. However, the concentration of cotinine, a metabolite of nicotine with a ~16h half-life in serum, plasma, urine, or saliva is a more accurate measure of smoking. The objective of this study was to examine the reliability of self-reported smoking during pregnancy in the CARMA cohort by measuring plasma cotinine concentration.

METHODS : CARMA is a prospective cohort study in Cellular Aging and HIV Comorbidities in Women and Children. For the purpose of this study, we used plasma samples collected during the third trimester (31-37 weeks of gestation) of pregnancy from 47 women. Substance use information including smoking was collected on the same day as blood collection. Cotinine was measured by ELISA (enzyme-linked immunosorbent assay). We conducted a descriptive analysis by comparing the expected proportion of cotinine-negative among self-reported non-users (expected 100%) and of cotinine-positive among self-reported users and reported the percentage of agreement.

RESULTS: The mean maternal age at delivery was 31±6 (range 17-42). There was no significant difference in maternal age between the self-reported smokers and nonsmokers, but there were fewer aboriginal women in the nonsmoker group (p=0.013). Pregnant women with income <$15000/year were more likely to smoke (p=0.003). Daily smoking was reported by 30% of pregnant women, weekly smoking by 6%, and nonsmoking by 49%. Smoking with unknown frequency was also reported by 15%. Using a cotinine cut-off of 5 ng/ml, we observed a 90% concordance between self-reported smoking and plasma cotinine. For the self-reported non-smokers, concordance was 88%. Two pregnant women classified as smoker according to their self-report data (yes vs. no) showed plasma cotinine level <5 ng/ml but they reported smoking on average fewer than 2 cigarettes per week during pregnancy. Given the half-life of cotinine, the timing of maternal sampling could influence its detection. If women reporting less than 2 cigarettes per week are re-categorized as non-smokers, the concordance between the self-reported smoking and self-reported non-smoking rises to 100% and 96%, respectively.

CONCLUSIONS: These findings demonstrate that study participants are highly accurate in their self-report of smoking during pregnancy. This confirms that within the CARMA cohort study, self-reported smoking is a valid measure for tobacco exposure during pregnancy and does not support the existence of bias due to underreporting.

SARA SABERI	GRADUATE STUDENT	POSTER PRESENTATION
SUPERVISOR:	HÉLÈNE CÔTÉ	

PHARMACOLOGICAL CONCENTRATIONS OF ANTIRETROVIRAL THERAPY DRUGS AFFECT MITOCHONDRIAL DNA (MTDNA) QUANTITY AND QUALITY IN CELL CULTURE MODELS

BACKGROUND/OBJECTIVES: Despite the great success of combination antiretroviral therapy (cART), HIV+ individuals are at increased risk of premature aging and age-related diseases. CART regimens usually contains two nucleoside reverse transcriptase inhibitors (NRTIs) combined with either a non-NRTI (NNRTI) or a boosted protease inhibitor (PI). NRTIs inhibit mitochondrial polymerase gamma, which can deplete mtDNA. However, mitochondrial dysfunction is not always associated with mtDNA depletion. PIs and NNRTIs can induce oxidative stress and mtDNA damage that can stimulate compensatory mitochondrial biogenesis. MtDNA damage leads to mitochondrial dysfunction and forms the basis of the oxidative stress theory of aging. We evaluated the effects of individual antiretrovirals (ARV) and five cART regimens on mtDNA quantity and quality in cultured cells.

METHODS: Immortalized human placental (JEG-3) and T lymphoblast (CEM) cells were cultured in the presence of NRTIs: ABC (Abacavir), AZT (Zidovudine), FTC (Emtricitabine), TDF (Tenofovir), 3TC (Lamivudine), d4T (Stavudine) (+control), NNRTIs: EFV (Efavirenz), NVP (Nevirapine), and PIs: LPV (Lopinavir), NFV (Nelfinavir), all at 1x, 10x and 20× Cmax for 3 days, then harvested. Growth rate, viability, mtDNA content and mtDNA AOD (apparent oxidative damage) were measured in 3 technical replicates. Somatic mtDNA mutation burden was also quantified in a subset using an ultra-deep sequencing strategy. The JEG-3 cells were also exposed to clinical (1× Cmax) concentrations of five cART regimens used in HIV pregnancy: ABC/3TC with either EFV, LPVr (ritonavir boosted LPV) or RAL (raltegravir), AZT/3TC/ LPVr and TDF/3TC/EFV. After 21 days, the cells were returned to ARV-free medium for 10 days, to allow recovery. Cells were counted and collected at baseline and every three days during the experiment. These longitudinal experiments were performed in three independent experiments.

RESULTS: Both cell lines showed similar responses to ARVs. MtDNA content increased at 1x but depleted at 10x and 20x Cmax d4T while mtDNA content and AOD both increased in cells exposed to ABC, LPV and NFV. These effects were concurrent with substantially reduced growth rates. In cells exposed to EFV, a mixed effect was seen whereby mtDNA increased at 1× and 20x Cmax but decreased at 10× Cmax. Among all ARVs tested, EFV exerted the largest effect on growth rate, mtDNA content and AOD. Somatic mtDNA mutation burden (n=1, 20x Cmax ARV) was d4t=EFV>NFV≈control. In JEG-3 cells longitudinally exposed to all non-LPV/r regimens, mtDNA content increased during treatment phase and then decreased after removal of drug pressure, during the recovery phase. In JEG-3 cells treated with both LPV/r-containing regimes, mtDNA content increased after day 24 in recovery phase.

CONCLUSIONS: The opposite effects of some ARVs on mtDNA content illustrate the need to evaluate these drugs alone and in combinations, using multiple mtDNA measures, as various mtDNA alterations could affect mitochondrial function, cellular metabolism and aging. The mtDNA effects seen here with EFV, LPV and ABC warrant further research given their increasing use in pregnancyand ILCs from lymphoid progenitors in adult mice appears limited.

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MARTA SALVADOR | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: | HELENE COTE

PLACENTAL MITOCHONDRIAL DYSFUNCTION IN RELATION TO PRETERM BIRTH IN HIV PREGNANCY

AUTHORS:

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BACKGROUND/OBJECTIVES: *In utero* exposure to combination antiretroviral therapy (cART) greatly reduces mother-to-child transmission of HIV. However, there is an increased risk of preterm delivery (<37 weeks of gestation) and small for gestational age newborns among HIV+ women compared to HIV- women. This has been linked to maternal cART exposure, and possibly protease inhibitors (PIs). Some cART drugs cross the placenta, and several can induce oxidative stress and/or mitochondrial dysfunction. This could consequently impair placental function and affect fetal growth. Progesterone, a steroid hormone synthesized by the cytochrome p450 in the placental mitochondria, is essential to maintain a uterine environment suitable for the developing embryo: it therefore plays a pivotal role in the physiological progression of pregnancy. Reduced plasma progesterone levels have been reported in HIV+ pregnant women treated with cART. In addition, mouse studies have suggested an association between decreased levels of plasma progesterone, negative pregnancy outcome, and treatment with PIs. However, the effect of HIV/cART exposure on human placental progesterone levels remains unknown. Our lab recently observed an association between altered mitochondrial DNA (mtDNA) content and exposure to PI-based cART in placenta from HIV+ women, as well as in cultured cells. Because HIV and cART could both affect placental mitochondrial function and thereby progesterone production, our aim is to investigate the relationship between PI exposure, placenta mitochondrial alterations and progesterone levels, in both human placenta and a cell culture model. **HYPOTHESES:** 1) HIV+ women exposed to cART will have significantly lower placental progesterone levels and/or altered mtDNA content compared to HIV- controls. 2) Among HIV+ women, those treated with PI-based cART will show lower placental progesterone levels and/or altered mtDNA content than those treated with PI-sparing cART. 3) PIs will affect progesterone production *in vitro*.

RESEARCH PLAN: Placenta and blood specimens, as well as clinical and sociodemographic data have already been collected for pregnant women enrolled in the Canadian prospective cohort study, the Children and Women: AntiRetrovirals and Markers of Aging (CARMA) cohort, which started in 2008. Our study sample will include 103 HIV+ women and 60 HIV- controls (likely more) from the CARMA cohort who share similar sociodemographic characteristics. Placental and plasma progesterone levels as well as mtDNA content will be measured using ELISA and qPCR respectively. We will extend these investigations to in vitro models using two human placental choriocarcinoma cell lines, JEG3 and BeWo. Cells will be treated in a dose-dependent manner with PI-based cART, and induced to produce progesterone. Progesterone synthesis and mtDNA measures will be done as above. Additionally we will use immunofluorescence to visualise changes in mitochondrial membrane potential and morphology using laser scanning confocal microscopy. If we establish a link between PI-induced mitochondrial dysfunction and progesterone synthesis, gene expression studies will be undertaken to identify the pathway affected.studies will be undertaken to identify the pathway affected.

ZHOUCHUNYANG XIA | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: DAVID HUNTSMAN

BEYOND CODING MUTATIONS: USING LINE 1 RETROTRANSPOSON AS BIOMARKERS TO TRACK CANCER DEVELOPMENT AND PROGRESSION

BACKGROUND/OBJECTIVES: Women with endometriosis, a painful condition caused by displaced endometrial tissue, have a 3-fold increased risk of developing endometriod ovarian cancer (ENOCa) and ovarian clear cell cancer (OCCC). How two distinct cancers arise from the same precursor lesion is unknown. Sensitive biomarkers are needed to identify women with high risk endometriosis. As protein coding mutations implicated in this malignant transformation only occur in subsets of the patients, its utility as biomarker is limited. Our whole genome sequencing of 29 ENOCa and 36 OCCC cases revealed a highly frequent insertion event originating from an active LINE-1 (L1) element in the TTC28 gene. L1 is a mobile genetic element that can take DNA pieces and insert them into random genomic locations. L1s have been known to become activated in cancer. Previous L1 insertion and methylation analysis in different cancer types showed the potential of L1 as a diagnostic and prognostic marker. We hypothesize that TTC28 L1 retrotransposon is an early event in the transformation of endometriosis into ENOCa and OCCC and such events could be used as biomarkers for endometriosis with high cancer risk.

METHODS: We will compare the presence of TTC28 L1 insertions to six SNVs and frame shifts mutations in normal, endometriosis, and tumor tissues from different anatomical sites in four ENOCa and four OCCC cases. PCR followed by Sanger sequencing will be used to detect TTC28 L1 insertions, and micro-fluidic PCR assay followed by MiSeq sequencing will be used to detect SNV/frameshift mutations. We will use a target capture sequencing method to track novel TTC28 L1 insertions in tissues. In brief, probes tiling a portion of TTC28 L1 3' end plus 1kb downstream will be used to capture DNA fragments containing the TTC28 L1 insertion, and the fragments will be sequenced on the MiSeq. We will assess the difference in TTC28 L1 methylation status between normal, endometriosis, and tumor tissues via the sequencing of bi-sulfite treated DNA in the same eight cases.

RESULTS: Comparison of TTC28 L1 in normal versus tumor tissues show that TTC28 L1 insertion is an early event preceding all SNV and/or frameshift mutations, and occurs in tumor tissues across all cases. Unexpectedly, TTC 18 insertions were found in normal tissues in one ENOCa and one OCCC cases, and such presence is currently being validated to eliminate PCR errors. We are currently designing probes for the target capture sequencing method. Preliminary results show that TTC28 L1 promoters are unmethylated in tumors with L1 insertions. Future experiments involving additional cases plus endometriosis tissues will be performed. We expect to see L1 insertions and promoter hypomethylation in endometriosis tissues.

CONCLUSIONS: Our preliminary results show that TTC28 L1 promoter hypomethylation and TTC28 L1 insertions occur early and can be explored as a method to trace tumor development and progression. The development of a target capture assay to detect novel L1 insertions will be crucial for looking at cases that do not have WGS data. Ultimately, we hope to be able to detect L1 insertions in plasma samples, and use L1 insertions as a biomarker to identify high-risk endometriosis cases, especially in cases where common coding mutations are not found.

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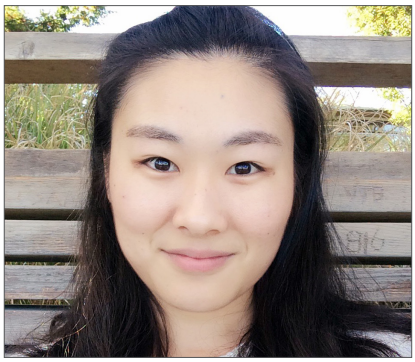

LINDA YANG | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: MARK SCOTT

ENHANCING THE PROINFLAMMATORY IMMUNE RESPONSE USING MIRNA-BASED THERAPEUTICS

AUTHORS:

Xining (Linda) Yang[1,3] and Mark Scott[1,2,3]

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BACKGROUND/OBJECTIVES: Effective proinflammatory response protects the human body from a number of challenges including cancer. Therapeutics are needed when the immunity fails to recognize and eliminate cancer cells. While many immune components are well known clinically (e.g., IVIG), other emerging plasma constituents may prove to be of equal or greater therapeutic potential. Recent research by our laboratory has discovered a novel therapeutic which is capable of modulating the body's own immune system to enhance an inflammatory response. This therapeutic consists of a complex mixture of microRNAs (miRNAs). First recognized as biologically functional in 2000, miRNAs are small, non-coding RNAs that regulate expression of genes at the post-transcriptional level and are involved in a wide range of biological responses. However, miRNA regulation is complex and a single miRNA can affect hundreds of genes and individual genes can be regulated by multiple miRNAs. From a bioregulatory approach, 'pattern of miRNA expression' (encompassing increased, decreased and static levels) must be mimicked to achieve pharmacologically effectiveness. Using bioreactor systems, our laboratory has generated a pharmacologically effective miRNA-based therapeutic (denoted as Inflammatory Agent 1, IA1) capable of producing in vitro and in vivo inflammatory responses. Unprocessed IA1 (uIA1) was produced using human and murine in vitro bioreactor systems. We hypothesized that human- and murine-source uIA1 would enhance a proinflammatory response characterized by proliferation of effector T cells (both CD8+ and CD4+ Th17 cells) while decreasing T regulatory (CD4+) cells.

METHODS: Mixed Lymphocyte Reaction (MLR) based bioreactor systems were used to produce uIA1 using either allogenic human peripheral blood mononuclear cells (PBMC) or H-2 disparate murine splenocytes. Each MLR reaction well contained a total of 2 x 106 cells (single donor for resting or equal numbers for disparate donors for MLR). Cell free MLR conditioned media were collected at Day 5 as optimal human- or murine-source uIA1 preparations. Proliferation of human resting T cells (CD3+, CD8+ and CD4+) and murine lymphocytes in a secondary MLR was assessed using CFSE Cell Proliferation Kit at Day 10. CD4+ Th17 (proinflammatory) and Foxp3+ Treg (tolerogenic) subsets were also determined at Day 10.

RESULTS: Both human- and murine-source uIA1 preparations promoted a proinflammatory response in lymphocytes. A significant (10-fold) increase of resting CD3+ T cell proliferation was induced by human-source uIA1 treatment. Murine-source uIA1 also doubled the proliferation of lymphocytes in a secondary MLR treated with uIA1. Most significantly, both human- and murine-source uIA1 resulted in an expansion of CD8+ subpopulation (mainly cytotoxic T cells). Within the CD4+ population, uIA1 resulted in a skewed proliferation toward Th17 cells with a concomitant decrease in Treg cells resulting in an increase in the Th17:Treg ratio.

CONCLUSIONS: uIA1 demonstrated significant proinflammatory effects on T cell proliferation and subset differentiation. Enhancing the proinflammatory state may be useful in treating diseases such as cancer.

ADA YOUNG	GRADUATE STUDENT	POSTER PRESENTATION
SUPERVISOR:	KEVIN BENNEWITH	

CLINICALLY RELEVANT, LOW-DOSE IONIZING RADIATION ENHANCES BREAST CANCER CELL MIGRATION AND LOCAL INVASION

BACKGROUND/OBJECTIVES: It is estimated that >90% of cancer-related deaths are associated with the development and growth of tumour metastases. Metastasis occurs when cancer cells dissociate from the primary tumour, escape into the lymphatics or bloodstream, and establish secondary masses in other tissues. Radiation therapy is an efficacious cancer treatment where a large overall dose is given to patients in small fractions (1.8-2.5Gy). The ability of tumour cells to migrate and invade is conducive to the metastatic potential of the cells, and emerging evidence suggests that tumour cell migration can be enhanced by clinically relevant doses of ionizing radiation in vitro. Ionizing radiation is thought to induce epithelial-mesenchymal transition (EMT) in tumour cells that survive radiation treatment, leading to enhanced migration. However, published in vitro studies have used endpoint assays to assess radiation-induced migration/invasion of cell line cultures, with little consideration of the proportion of cells that have survived the radiation dose. We hypothesize that tumour cells that survive radiation therapy have a higher propensity to migrate, invade, and metastasize to secondary sites independent from EMT and radiation-induced changes in the solid tumour microenvironment.

METHODS: Breast cancer cell lines stably expressing green fluorescent protein are seeded into multi-well plates and treated with different radiation doses in a single administration using a radiation-attenuator insert. Using the IncuCyte ZOOM system, irradiated cells are imaged in real-time over 72h to facilitate single cell tracking of individual tumour cells to quantify changes in migration over time. Viable breast cancer cells are co-cultured with conditioned medium collected from 2.3Gy irradiated MDA-MB-231 breast cancer cells, imaged in real-time and single cell tracked to monitor changes in migration.

RESULTS: Our results indicate increases in tumour cell migration with as low as 2.3Gy. The metastatic and fully mesenchymal cell lines MDA-MB-231 and MDA-MB-231 LM2-4 treated with 2.3Gy of ionizing radiation migrated a greater total distance and displaced further from the point of origin compared to untreated cells. Epithelial-like non-metastatic MCF-7 cell line irradiated with 2.3Gy did not exhibit increases in total distance migrated, although a higher proportion of cells observed a greater displacement distance from the point of origin ($p<0.05$). Conditioned media from 2.3Gy treated tumour cells, when co-cultured with untreated breast cancer cells, enhanced migration and displacement compared to tumour cells co-cultured with untreated conditioned media.

CONCLUSIONS: I have demonstrated that ionizing radiation doses as low as 2.3Gy is sufficient to enhance the migratory phenotype of both non-metastatic and metastatic breast cancer cell lines. By quantifying changes in the metastatic ability of tumour cells treated with clinically relevant doses of radiation both in vitro and in vivo, our findings will help to determine whether there is a need for administration of targeted secondary therapy after (or during) radiation therapy to minimize tumour cell dissemination.

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ADAM YU | GRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: | JACQUELINE QUANDT

HIGH YIELD PRIMARY MICROGLIA DISTINGUISH SUBTLE PHENOTYPICAL AND FUNCTIONAL DIFFERENCES RELEVANT TO DISEASE ONSET AND PROGRESSION IN NEURODEGENERATION

AUTHORS:

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BACKGROUND/OBJECTIVES: Microglia are the resident myeloid-derived immune cell found in the central nervous system (CNS) and are important in the neuromodulatory, neurotrophic, and neuroimmune response to disease. Microglia fall under scrutiny in attempts to characterize how their activation and temporal regulation of inflammatory markers may influence damage, repair, and overall neuronal and axonal health related to disease progression. Microglial cultures serve as excellent tools to compare factors that influence their phenotype and associated function for phagocytosis and cytokine production under inflammatory or reparative settings in the CNS. Our objective for this study was to establish a simple protocol to culture microglia from primary cortical neural progenitor cells with comparable characteristics to their in vivo counterparts.

METHODS: Mixed Lymphocyte Reaction (MLR) based bioreactor systems were used to produce uIA1 using either allogenic human peripheral blood mononuclear cells (PBMC) or H-2 disparate murine splenocytes. Each MLR reaction well contained a total of 2 x 106 cells (single donor for resting or equal numbers for disparate donors for MLR). Cell free MLR conditioned media were collected at Day 5 as optimal human- or murine-source uIA1 preparations. Proliferation of human resting T cells (CD3+, CD8+ and CD4+) and murine lymphocytes in a secondary MLR was assessed using CFSE Cell Proliferation Kit at Day 10. CD4+ Th17 (proinflammatory) and Foxp3+ Treg (tolerogenic) subsets were also determined at Day 10.

RESULTS: One well of a 6 well plate yielded 4.2E105 microglia cultured accordingly to a previously established protocol in comparison to the 2.2E106 cells yielded when GM-CSF was added to the culturing media. Harvested microglia are >99% pure by flow cytometry (leukocyte marker CD45lo, myeloid marker CD11b+ and dendritic marker CD11c-), with low expression of antigen presenting molecules MHC class I and IIlo and negligible/low levels of T cell co-stimulatory molecules CD40-, CD80lo and CD86lo. LPS altered cellular morphology and enhanced expression of CD11b, CD40, CD80, CD86 and MHC class I in microglia while decreasing CD39 expression, a protein which functions in microglial migration. IFN-γ increased CD40, CD45, CD80, and MHC class I levels significantly ($p<0.05$). IFN-γ significantly decreased rates of phagocytosis in GM-CSF cultured microglia ($p<0.05$). LPS significantly upregulated CCL2, IL-6, IL-10, and TNF-α release. The microglial cell line, BV-2, did not model IL-6 and IL-10 cytokine secretion when treated with LPS or IFN-γ in comparison to our GM-CSF microglial cultures.

CONCLUSIONS: This method provides a reliable and simple means to obtain highly purified microglial cultures in high yield. Cells can be used to model mechanisms that drive differential states of activation in vitro and can be correlated to phenotype and function *in vivo*. Increases in co-stimulatory molecule expression and more subtle regulation of CD39 will provide insight into complex and diverse roles for microglia in biology and disease.

DHANANJAY NAMJOSHI	POST-DOCTORAL FELLOW	POSTER PRESENTATION
SUPERVISOR:	CHERYL WELLINGTON	

LINEAR HEAD KINEMATICS PREDICTS DURATION OF UNCONSCIOUSNESS AND SEVERITY OF AXONAL INJURY IN THE CHIMERA (CLOSED HEAD INJURY MODEL OF ENGINEERED ROTATIONAL ACCELERATION) MOUSE MODEL OF CONCUSSION

BACKGROUND/OBJECTIVES: Traumatic brain injury (TBI) is a leading worldwide cause of death and disability with a cost to society of over $76B USD per year. TBI is often called the most complex pathology of the most complex organ. We have developed an innovative rodent TBI platform, called CHIMERA (Closed Head Injury Model of Engineered Rotational Acceleration) that integrates head kinematics with functional and neuropathological outcomes. We previously showed that mice subjected to two mild, closed-head TBIs with an impact energy of 0.5 J using CHIMERA faithfully replicate several functional and neuropathological features of human TBI including repeatable human-like head kinematics (Namjoshi et al 2015). However, much remains to be learned about the full potential of this nascent technology to faithfully replicate human TBI and provide a transformative platform for future studies in aging and disease models. The present study was conducted to address two aims: 1) to assess head kinematics and acute neurological and histopathological outcomes following single TBI with increasing impact energy dose and 2) to determine the relationship between impact energy dose-dependent changes in head kinematics and post-TBI outcomes.

METHODS: Male C57Bl/6 mice at 4 months of age were subjected to a single closed-head TBI using impact energies of either 0 (sham) or 0.1, 0.3, 0.4, 0.5, 0.6 or 0.7 J. Head kinematics were assessed with high-speed videography (at 9000 fps). Duration of loss of righting reflex (LRR) was recorded immediately after TBI. Axonal injury was assessed with silver stain at 2 d post-TBI.

RESULTS: Mice subjected to single TBI showed an impact energy dose-dependent increase in several parameters of linear head kinematics (displacement, velocity and acceleration), LRR duration and silver uptake in white matter regions. Further analyses revealed a significant positive correlation of the all three linear head kinematic parameters with LRR duration ($R2 > 0.41$ and $p < 0.02$) as well as the degree of silver uptake in the optic tracts ($R2 > 0.46$ and $p < 0.02$).

CONCLUSIONS: Our present data indicates that linear head kinematics may be able to predict the duration of unconsciousness and severity of axonal injury following single closed-head TBI in mice using CHIMERA.

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NICK SUNDERLAND | HEMATOPATHOLOGY FELLOW | POSTER PRESENTATION

SUPERVISOR: TYLER SMITH

DIRECT ORAL ANTICOAGULANTS: VALIDATION OF SPECIFIC QUANTITATIVE ASSAYS AMENABLE TO RAPID CLINICAL TESTING

AUTHORS:

Nick Sunderland[1], Dan Holmes[1,2], Tyler Smith[1,3]

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BACKGROUND/OBJECTIVES: The direct oral anticoagulants (DOACs) are an increasingly clinically used class drugs used for atrial fibrillation, orthopedic surgery and prevention and treatment of venous thromboembolism. The drugs were designed and licenced to be used without active monitoring, however situations of renal or hepatic insufficiency, extremes of weight, guidance on clinical management during acute bleeding events, urgent surgery or overdoses, and the imminent availability of new reversing agents, make reliable tests for the degree of drug effect highly desirable. Historically standardization has been hampered by a lack of gold standard, with PTT and PT/INR being unreliable for routine use. The goal of our study was to validate dilute thrombin time and anti-Xa assay derived drug levels based on mass spectroscopy data, as well as confirm DOACs effects on locally obtained measurements of PTT, PT/INR and other routine tests.

METHODS: Our study looked at 128 adult outpatients on steady state dabigatran etexilate, rivaroxaban, or apixaban (42, 37, and 49 respectively) over a 2 year period. Patients age varied from 36-90. Specific drug levels were obtained by mass spectroscopy and verified by reference laboratory measurements. These were compared to routine coagulation parameters (PTT, PT/INR) as well as more specific tests such as dilute thrombin time (dTT) as well as anti-Xa calibrated for heparin and with drug specific calibrators, at two different local tertiary hospitals, with both photo-optical and mechanical detection methods. A number of other parameters were concurrently evaluated including fibrinogen (FIB), antithrombin (AT), protein C (PC), protein S (PS), and factor VIII using multiple dilutions, and DRVVT for assessment of any interfering lupus anticoagulant. Patient weight, eGFR and dose and time since last dose were recorded where available.

RESULTS: Correlation of MS derived dabigatran levels with dTT showed a high degree of correlation (R2=0.96), as did rivaroxaban and apixaban with drug-calibrated anti-Xa levels (R2=0.98, R2=0.96 respectively). Dabigatran was also compared to PTT/PT showing minimal effect on PT even at high doses, but showed variable dose-dependent prolongation of PTT. Rivaroxaban showed moderate dose-independent prolongation of PTT, with no effect on TT and no/borderline effect on PT/INR. No significant trends in levels of AT, PC or FIB were seen, with dose related increased PS activity for dabigatran only. There was no significant trend in drug levels with weight, eGFR or time since administration. Evaluation of heparin-calibrated anti-Xa compared to drug-specific anti-Xa and drug levels is in progress.

CONCLUSIONS: Previous studies have looked at various aspects of DOAC administration including effects on routine coagulation parameters; however methods and instruments used vary and do not always apply to local patient populations and have not been compared to mass spectroscopy. MS derived drug levels show excellent correlation to locally preformed dTT and calibrated anti-Xa levels. Validation of readily available coagulation tests to document drug levels will allow better understanding of drug dynamics and more informed clinical care decisions. Limitations of the study were lack of patients with severe renal insufficiency or those in emergency situations. Development of a clinical guideline regarding DOACs and reversal agent use is an expected future initiative from this data.

OMAIR ARSHAD | DIRECTED STUDIES STUDENT | POSTER PRESENTATION

SUPERVISOR: HÉLÈNE CÔTÉ

EXPLORING THE BIOLOGICAL CORRELATES OF CELL-FREE DNA IN THE CONTEXT OF HIV INFECTION

BACKGROUND/OBJECTIVES: HIV infection and antiretroviral treatment are associated with mitochondrial dysfunction, cytotoxicity, and chronic inflammation; processes that can contribute to the accelerated biological aging seen in HIV-infected (HIV+) persons. Cell-free DNA is an emerging prognostic biomarker in many fields of medicine but remains relatively unexplored in the context of HIV. Generally, increased cell-free nuclear (cf-nDNA) and mitochondrial (cf-mtDNA) DNA levels are associated with poor health outcomes, with cf-mtDNA being particularly proinflammatory. Our objective was to quantify plasma cf-mtDNA, cf-nDNA, and the inflammatory marker interleukin-6 (IL-6) in participants of the CARMA cohort. We then explored factors associated with these measures.

METHODS: Our observational cross-sectional study included 99 HIV+ (aged 4-66y) and 103 HIV-uninfected (HIV-; 2-78y) participants. Fresh whole blood (WB) was centrifuged at 14000xg and the plasma filtered (0.45 microns) to fully remove cells and platelets. MtDNA and nDNA levels were measured in filtered plasma and WB via multiplex qPCR. Plasma IL-6 was quantified by ELISA and categorized as high (≥2pg/mL) and low (<2pg/mL). Univariate analyses were conducted by Student's t-test, Mann-Whitney U-test, and by correlations. Factors showing some univariate association ($p<0.15$) were further investigated through analysis of covariance (ANCOVA) models.

RESULTS: Within all participants, cf-mtDNA was a median six times higher than cf-nDNA, but the two were highly correlated ($n=202$, $rho=0.78$, $p<0.0001$). No correlation was seen between cf-mtDNA and leukocyte mtDNA content. Age was negatively correlated with cf-mtDNA ($n=202$, $rho=-0.31$, $p<0.0001$) and cf-nDNA ($n=202$, $rho=-0.26$, $p<0.0001$) but not with leukocyte mtDNA content. Among pediatric participants (<18y, n=75), HIV infection was associated with higher cf-mtDNA ($p=0.03$) and cf-nDNA ($p=0.05$), and the same was true among adults (≥18y, n=127), both for cf-mtDNA ($p=0.05$) and cf-nDNA ($p=0.04$). High IL-6 was associated with increased cf-mtDNA ($p=0.008$) and cf-nDNA ($p=0.001$). In the ANCOVA ($n=202$), age ($beta\leq-0.29$, $p<0.0001$), high IL-6 ($beta\geq0.24$, $p\leq0.0004$), and HIV infection ($beta=0.18$, $p\leq0.007$) were independently associated with higher cf-mtDNA and cf-nDNA, respectively. Among HIV+ participants, 20% had detectable HIV plasma viral load (pVL). Total leukocyte count was positively correlated with both cf-mtDNA ($n=95$, $r=0.23$, $p=0.03$) and cf-nDNA ($n=95$, $r=0.24$, $p=0.02$). CD4+ T cell count was also positively correlated with both cf-mtDNA ($n=98$, $rho=0.23$, $p=0.02$) and cf-nDNA ($n=98$, $rho=0.25$, $p=0.01$), but having a detectable HIV pVL was not. In an ANCOVA within the HIV+ group (n=98), only age ($beta\leq-0.23$, $p=0.01$) and higher CD4+ T cell count ($beta\geq0.15$, $p\leq0.03$) were independently associated with higher cf-mtDNA and cf-nDNA.

CONCLUSIONS: Younger age, HIV infection, and higher IL-6 levels are associated with higher cf-mtDNA and cf-nDNA, implicating possible links between these and aging, HIV infection, and inflammatory processes.

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ANGELA CHENG | SUMMER STUDENT | POSTER PRESENTATION

SUPERVISOR: BLAKE GILKS, ANTHONY KARNEZIS

HPV-INDEPENDENT VULVAR SQUAMOUS CELL CARCINOMA HAS A SIGNIFICANTLY WORSE PROGNOSIS THAN HPV-ASSOCIATED CARCINOMA

AUTHORS:

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BACKGROUND/OBJECTIVES: Vulvar squamous cell carcinoma (VSCC), the most common vulvar malignancy, can be subdivided based on HPV status into two molecularly distinct diseases. Some studies indicate that patients with HPV-associated tumours have a better prognosis. HPV-associated SCCs in the head and neck (HNSCC) have a better prognosis than HPV-independent tumours, and immunohistochemistry (IHC) for the p16 tumour suppressor – a surrogate marker for HPV – has become standard of care in HNSCC to inform patient prognosis.

The objectives of this study are to: 1) determine whether p16 is an accurate surrogate marker of HPV status in VSCC and 2) determine whether HPV status is prognostic in VSCC.

METHODS: This is a retrospective analysis of patients with vulvar squamous cell carcinoma diagnosed at the British Columbia Cancer Agency and Vancouver General Hospital between 1985-2005. VSCCs were first reviewed by two pathologists (CBG and ANK), and their HPV status was assessed based on morphological criteria (n=201). Whole sections of representative formalin fixed paraffin embedded blocks from each case were selected for p16 IHC (Ventana CINtec antibody E6H4) using the Ventana Discovery automated stainer according to the manufacturer's protocol. Stained slides were classified as HPV-associated when both nuclear and cytoplasmic staining was observed in a "block" pattern, i.e: moderate to intense staining of all cells in at least the basal third of the epithelium. Slides were scored by pathologists blinded to the H&E prediction of HPV status. Cases with discrepancy between HPV status assigned by H&E and p16 IHC were selected for HPV PCR molecular testing.

After successfully subclassifying 201 VSCC cases, a retrospective multivariable survival analysis was performed using Cox proportional hazard models and Kaplan-Meier plots.

RESULTS: Using the three assessment methods – H&E morphology, p16 IHC and HPV PCR – we were able to subclassify HPV status in 201 cases. HPV status by H&E morphology and p16 IHC was concordant in 162 cases (81%), discordant in 34 cases (17%). For the 34 discordant cases, HPV PCR agreed with p16 IHC in 32 cases (94%). For 5 indeterminate cases with discrepant HPV status by morphology, p16 IHC and HPV PCR, final HPV status was based on additional patient criteria. Of 199 informative cases, the sensitivity, specificity, positive and negative predictive values of p16 IHC as a marker of HPV-associated VSCC were 100%, 98%, 98% and 100%.
Through retrospective multivariable analysis, HPV-independent VSCCs were observed to have a worse disease specific survival and overall survival compared to HPV-associated VSCCs.

CONCLUSIONS: Morphological criteria alone are not sufficient to accurately subclassify VSCC based on etiology. p16 IHC is an accurate surrogate biomarker of HPV status in VSCC. HPV status by p16 IHC stratifies VSCC into two prognostically different diseases, with HPV-associated tumours showing improved prognosis like in HNSCC. Our results support the use of p16 IHC in clinical practice of VSCC patients.

HANNAH DREKSLER	SUMMER STUDENT	POSTER PRESENTATION
SUPERVISOR:	MARI DEMARCO	

INVESTIGATION OF A MASS SPECTROMETRIC APPROACH FOR DETECTION AND QUANTITATION OF ALPHA-SYNUCLEIN IN PRECLINICAL MODELS

BACKGROUND/OBJECTIVES: Neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease are associated with pathogenic protein misfolding and aggregation. alpha-Synuclein is an intrinsically disordered protein that can form neurofibrillary tangles and/or Lewy bodies, which are proteinaceous aggregates characteristic of Parkinson's disease pathogenesis. Mouse models are commonly used in the investigation of alpha-synuclein pathology; however, existing strategies to investigate alpha-synuclein provide only relative quantitation. Our goal is to develop a quantitative mass spectrometric assay for detection and quantitation of alpha-synuclein in mouse brain homogenate (Mo-BH).

Concentrations of specific isoforms of α-synuclein in mouse brain homogenate, as measured by a robust mass spectrometric assay, can improve characterization of disease pathology. The results of this study will help us identify specific alpha-synuclein signatures to be investigated as potential in vivo diagnostic biofluid markers for individuals with cognitive impairment.

METHODS: Wild-type recombinant mouse alpha-synuclein was used in the initial design of the mass spectrometric method. Mo-BH was analyzed from mice that had been injected with recombinant mouse alpha-synuclein fibrils in order to initiate Parkinson's disease pathology. The Mo-BH was digested with trypsin and analyzed on a Waters Quattro Micro tandem mass spectrometer.

RESULTS: Using recombinant mouse alpha-synuclein, we have identified three alpha-synuclein peptides with m/z 1928.0, 1505.8, 1325.7 at retention times of 4.75, 4.68, 3.97 minutes, respectively. For confirmation of the tryptic peptide sequences, we have identified three product ions from each peptide. Time course digestion analysis at 0.5, 1, 2, 4, 6, and 24 hours revealed that the digestion asymptote was reach by 2 hours.

CONCLUSIONS: Preliminary results indicate that LC-MS/MS is a promising strategy for quantitation of alpha-synuclein in preclinical models of Parkinson's disease.

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JESSICA QUE | SUMMER STUDENT | POSTER PRESENTATION

SUPERVISOR: STEPHEN YIP

DEVELOPMENT AND IMPLEMENTATION OF A IDH1/2 GENOTYPING ASSAY FOR CLINICAL BRAIN TUMOUR SPECIMENS

AUTHORS:

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BACKGROUND/OBJECTIVES: Diffuse gliomas are the most common type of primary brain tumours and are associated with high morbidity and mortality. Recent genome sequencing studies have revealed that recurrent mutations in the isocitrate dehydrogenase 1 and 2 genes (IDH1/2) are present in a large proportion of diffuse gliomas – particularly low grade astrocytomas and oligodendrogliomas (WHO II/III), and secondary glioblastomas (WHO IV). IDH1/2 mutations can distinguish diffuse gliomas from non- neoplastic mimickers including vasculitis, encephalitis, demyelinating disease, or reactive gliosis. Moreover, low grade gliomas that are wildtype for IDH1/2 have found to exhibit aggressive clinical behaviour similar to glioblastoma and this information can lead therapeutic change. Hence, there is a strong need to accurately identify the genotype status of IDH1/2 in gliomas.

The R132H mutation-specific IDH1 antibody is used to detect the most frequent subtype of IDH mutations. While it is a powerful screen of brain biopsies, it does not detect the remaining 5% of IDH1/2 mutations. The purpose of this study is to develop and validate a real-time PCR clinical assay for the detection of the entire spectrum of IDH1/2 mutations in formalin fixed paraffin-embedded (FFPE) tissues of brain tumour surgical resection and biopsy specimens.

METHODS: Validation of the analytical performance of the real-time PCR clinical assay was performed by assessing accuracy, precision, sensitivity, specificity, reportable range, and reference range. We used a combination of artificial gene fragments and clinical brain tumour (gliomas and medulloblastomas) samples with known IDH1/2 status for assay validation.

RESULTS: Validation of the analytical performance of the IDH assay was performed by assessing accuracy, precision, sensitivity, specificity, reportable range, and reference range. We used a combination of artificial gene fragments and clinical brain tumour (gliomas and medulloblastomas) samples with known IDH1/2 status for assay validation.

CONCLUSIONS: Validation of the analytical performance of the IDH assay was performed by assessing accuracy, precision, sensitivity, specificity, reportable range, and reference range. We used a combination of artificial gene fragments and clinical brain tumour (gliomas and medulloblastomas) samples with known IDH1/2 status for assay validation.

SABA VAFAEI-NODEH	SUMMER STUDENT	POSTER PRESENTATION
SUPERVISOR:	JIRI FORHLICH	

EFFECTS OF NIACIN, STATINS, OR A COMBINATION OF BOTH ON PLASMA LIPOPROTEIN(A) CONCENTRATION

BACKGROUND/OBJECTIVES: Lipoprotein (a) (Lp[a]) is an independent and causal risk factor for cardiovascular disease (CVD). Currently, Lp(a) lowering treatments include nicotinic acid (niacin) and statins. However, many contradictory findings have been reported for the effects of these treatments in reducing plasma Lp(a) levels and subsequent CVD risk. Here, we aim to verify whether plasma Lp(a) levels can be reduced significantly with niacin monotherapy, statin monotherapy, or niacin in combination with statins and compare the phenotype of responders and non-responders.

METHODS: A retrospective analysis of 1256 patient charts was completed. Subjects attended the Healthy Heart Program at St. Paul's Hospital, Vancouver, B.C. Patients (n=150) with repeated Lp(a) measurements were identified. The earliest and most recent Lp(a) measurements were documented (units = mg/L) as well as patient demographics, clinical, and laboratory data.

Patients were divided into two groups. Group 1 (n=74) took lipid-lowering medication or increased their current dose. Group 1 was further divided into Subgroups A, B, and C based on the type of lipid lowering therapy, i.e. niacin monotherapy, statin monotherapy, and niacin in combination with statins, respectively. Group 2 (n=76) included patients who did not take lipid-lowering medication nor made changes to their current dose. Statistical analysis was done using Wilcoxon signed rank test ($p \leq 0.05$).

Further statistical analysis on Group 1 compared the phenotype of responders (whose Lp(a) level decreased) and non-responders (whose Lp(a) level did not change or increased) to treatment in subgroups A, B, and C.

RESULTS: Plasma Lp(a) levels decreased by 1.2% in Group 1. Specifically, there was a 7.6% decrease, 8.7% increase, and 8.2% decrease in Lp(a) in Subgroups A (n=24), B (n=35), and C (n=15), respectively. In Group 2 (n=76), there was a 5.5% increase in Lp(a). The percent change in all groups and subgroups was not significant ($p>0.05$). In Subgroup C, there was a significant correlation ($p<0.05$) between gender and response to treatment – males were less responsive. None of the responders in the three subgroups had diabetes, however, its prevalence in non-responders was between 10-20%. The difference was not significant, most likely, due to a small sample size.

CONCLUSIONS: Our findings indicate that niacin monotherapy, statin monotherapy, and niacin in combination with statins do not reduce plasma Lp(a) levels significantly ($p>0.05$). Upon examining the phenotype of responders and non-responders in each subgroup, we identified a significant correlation between gender and response to treatment in Subgroup C; men were less likely to respond to a combination of niacin and statins. However, due to our small sample size, further investigation should be conducted to determine the relationship between phenotype and response to Lp(a) treatment.

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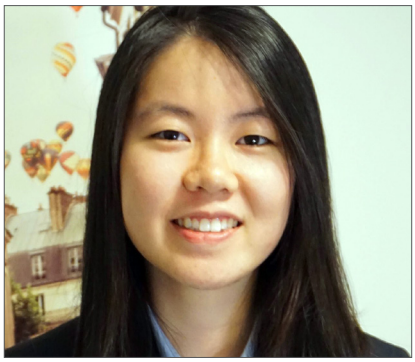

MAYANNE ZHU | UNDERGRADUATE STUDENT | POSTER PRESENTATION

SUPERVISOR: HELENE COTE

LEUKOCYTE MTDNA CONTENT DYNAMICS IN HIV-EXPOSED UNINFECTED CHILDREN EXPOSED TO COMBINATION ANTIRETROVIRAL THERAPY (CART) IN UTERO: A COHORT STUDY

AUTHORS:

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BACKGROUND/OBJECTIVES: ~35M people live with HIV globally, of which 48% are women and 10% children. Maternal cART during pregnancy along with postnatal 6-weeks infant prophylaxis has been successful in preventing vertical HIV transmission. However, *in utero* exposure to cART drugs, many of which can cross the placenta, could have long term effects on HIV-exposed uninfected (HEU) infants. Some cART drugs can cause mitochondrial toxicity and affect mitochondrial DNA (mtDNA) quantity and quality. Previous studies among HEU infants exposed to cART *in utero* have been inconsistent, with some reporting increased blood mtDNA levels at birth compared to HIV-unexposed uninfected (HUU) infants, while others report a decrease. Our objective was to compare HEU and HUU infant blood mtDNA content at birth and over the first 3y of life, and investigate any relationship to cART exposure.

METHODS: Peripheral blood mtDNA content was measured by qPCR in 324 HEU (0-3y, of whom 214 had ≥2 blood samples) and 308 HUU children (0-3y, each with a single blood sample). Univariate analyses were conducted to investigate associations between mtDNA content at birth and: infant sex, gestational age (GA) at birth, birth weight, small for GA (yes vs. no), maternal age at birth, ethnicity (Aboriginal, Black, Others vs. White), smoking ever during pregnancy (yes vs. no), duration of in utero cART exposure, and type of cART (AZT+3TC+NFV vs. AZT+3TC+LPV/r vs. other). Important factors ($p<0.05$) were included in multivariable analyses. Cross-sectional comparison of mtDNA content was also performed by Mann Whitney, after grouping data into the following age bins: 0-3d, 3d-6w, 6w-6m, 6m-1.5y and 1.5-3y.

RESULTS: A total of 114 HEU (23 with GA<37w), and 79 HUU (9<37w) had a birth sample. In a multivariable model of mtDNA at birth that included GA, maternal smoking during pregnancy, and HEU/HUU status, lower GA was the only factor independently associated with higher mtDNA content ($p<0.001$). In a separate model that investigated maternal cART parameters in HEUs only (n=114), neither duration nor type of in utero cART exposure were associated with mtDNA content at birth. HEU mtDNA content tended to increase during the prophylaxis period (birth to 6w, $p=0.057$), followed by a gradual decrease up to 3y of age. In contrast, HUU infants (n=305) did not show such changes. Two group comparisons between HEU and HUU within each age period suggested that HEU children had significantly higher mtDNA content compared to HUU children at every time point examined, an effect that persisted after adjusting for GA at 3d-6w and 6w-6m. GA data was too sparse to evaluate later age bins. A sensitivity analysis that included only term births revealed that both lower GA at birth ($p=0.044$) and HEU status ($p=0.025$) were independently associated with higher birth mtDNA content.

CONCLUSIONS: HEU infants had higher mtDNA content at birth but this effect was related to lower GA in that group. The increase in mtDNA content from birth to 6w suggests an effect of AZT prophylaxis on mtDNA. The persistently higher mtDNA content among HEUs may be due to an adaptive mitochondrial biogenesis process in response to *in utero* stresses.

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