PATHOLOGY DAY 2019

ABSTRACT BOOK 2019

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www.pathology.ubc.ca

Message **from the** Head

Pathology Day is a 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 allows us to recognize outstanding contributions in service and research 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.

We have again invited two outstanding individuals to participate in the program this year, highlighting academic excellence and continuing in the tradition of having leaders in their disciplines speak at Pathology Day. Dr. Blake Gilks will give the James Hogg Lecture, while Dr. Andrew Beck is our Keynote Speaker.

I wish to extend my sincere thanks to members of the committee responsible for organizing the event, including Dr. Corree Laule, Dr. Tony Ng, Dr. Sophia Wong, Dr. Will Lockwood, Dr. Fatemeh Derakhshan, Lise Matzke, Brennan Wadsworth, Cristina Low, and Adeline Chan, as well as all the other individuals whose efforts make the day a success.
Hoping you have a wonderful Pathology Day!

Dr. Don Brooks
Interim Department Head

Acknowledgements

Pathology Day is a team effort and we would like to extend our thanks to everyone who contributed to the 2019 edition.

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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:

Agatha Jassem
Aleksandra Stefanovic
Amal El Naggar
Anuli Uzozie
Ayesha Vawda
Blake Gilks
Bojana Rakic
Bradley Coe
Bruce Verchere
David Farnell
David Schaeffer
Ed Pryzdial
Emily Thompson
Gerry Krystal
Hamid Masoudi
Helene Cote
Honglin Luo
Inna Sekirov
Jacquie Quandt
Jeff Terry
Karina Rodriguez-Capote
Karla Bretherick
Katherine Serrano
Lauren Tindale
Lenka Allan
Lien Hoang
Lik Hang Lee
Linda Hoang
Mari DeMarco
Maria Issa
Martin Wale
Mateus De Camargo Barros
Meng Wang
Miguel Imperial
Muhammad Morshed
Nevio Cimolai
Peter Schubert
Peter van den Elzen
Ramesh Saeedi
Renata Scopim Ribeiro
Serena Singh
Shazia Masud
Suzanne Vercauteren
Tyler Smith
Vicky Monsalve
Wan Lam
Willian Schreiber

We would also like to thank our student and resident volunteers:
Amy Nagelberg, Anam Liu, Anna Wilhelm, Basile Tessier-Cloutier, Forouh Kalantari, Guangze Zhao, Haisle Moon, Julia Naso, Lisa Decotret, Mike Steel and Muntadhar Al Moosawi.

Finally, sincere thanks to the staff who kindly assisted with technical and administrative support throughout the day and our photographer: Aileen To, Debbie Bertanjoli, Heather Cheadle, Jennifer Xenakis, Joanne Wouterse, Julie Ho, Mayumi Shimada and Tamsin Tarling.

Committee 2019

Corree Laule

Donald Brooks

William Lockwood

Tony Ng

Sophia Wong

Lise Matzke

Brennan Wadsworth

Fatemeh Derakhshan

KEYNOTE SPEAKER

ANDREW H. BECK, MD PHD

Co-Founder & CEO, PathAI

TITLE: “Artificial Intelligence for Pathology: from Discovery to AI-Powered Companion Diagnostics”

Pathology Day will take place on **Friday, May 24, 2019**. Oral presentations and guest speakers will be at the **Paetzold Auditorium & Atrium, 1st Floor, Jim Pattison Pavilion**, Vancouver General Hospital while the poster session and awards reception will be at **ICORD, Blusson Spinal Cord Centre,** 818 West 10th Ave.

JAMES HOGG LECTURER

DR. BLAKE GILKS, MD

TITLE: "Being wrong: lessons learned from research, teaching, clinical service and administration"

DR. PETER WATSON MB BCHIR

TITLE: "Solutions to improve the research biobanking pipeline"

GUEST SPEAKER

DR. MARIANNE SADAR, PHD

TITLE: "From the bench to the bedside: the journey to developing a new therapy"

DR. SHO C, PDF

TITLE: "Granzyme B: A novel target for pemphigoid diseases"

PATHOLOGY DAY 2019
MAY 24, 2019

PATHOLOGY laboratory medicine
FACULTY OF MEDICINE
UNIVERSITY OF BRITISH COLUMBIA

CONFERENCE OUTLINE

7:45 am	BREAKFAST
7:55 am	OPENING REMARKS – DR. DON BROOKS, INTERIM DEPT HEAD

+ ORAL PRESENTATIONS BY STUDENTS & RESIDENTS

GRAD STUDENTS Paetzold Lecture Theatre		RESIDENTS Multipurpose Room		RESIDENTS JPPN 1411 Taylor Fidler	
8:10 am – 8:25 am	Derek Wong	8:10 am – 8:25 am	Muntadhar Al Moosawi	8:10 am – 8:25 am	Kyra Berg
8:25 am – 8:40 am	Emily Button	8:25 am – 8:40 am	Bhupinder Johal	8:25 am – 8:40 am	Ellen Cai
8:40 am – 8:55 am	Jessica Pilsworth	8:40 am – 8:55 am	Eric McGinnis		

9:00 am – 9:20 am	GUEST SPEAKER: DR. MARIANNE SADAR Paetzold Lecture Theatre *"From the Bench to the Bedside: the Journey to Developing a New Therapy"*
9:20 am – 9:50 am	POSTER POWER PITCH SESSION Paetzold Lecture Theatre
9:50 am – 10:05 am	BREAK (ATRIUM)
10:05 am – 11:05 am	JAMES HOGG LECTURE: DR. BLAKE GILKS Paetzold Lecture Theatre *"Being Wrong: Lessons Learned From Research, Teaching, Clinical Service and Administration"*

+ ORAL PRESENTATIONS BY STUDENTS & RESIDENTS

GRAD STUDENTS Paetzold Lecture Theatre		RESIDENTS Multipurpose Room		RESIDENTS JPPN 1411 Taylor Fidler	
11:15 am – 11:30 am	Amy Nagelberg	11:15 am – 11:30 am	Lynne Li	11:15 am – 11:30 am	Raymond Yip
11:30 am – 11:45 am	Emily Kamma	11:30 am – 11:45 am	Victor Yuen	11:30 am – 11:45 am	Basile Tessier-Cloutier
11:45 am – 12:00 pm	Rachel Cederberg	11:45 am – 12:00 pm	Adrian Levine	11:45 am – 12:00 pm	Mike Steel

12:10 pm – 2:10 pm	POSTER SESSION & LUNCH ICORD
2:20 pm – 2:40 pm	GUEST SPEAKER: DR. PETER WATSON Paetzold Lecture Theatre *"Solutions to Improve the Research Biobanking Pipeline"*

+ ORAL PRESENTATIONS BY STUDENTS & RESIDENTS

GRAD STUDENTS Paetzold Lecture Theatre		RESIDENTS Multipurpose Room		RESIDENTS JPPN 1411 Taylor Fidler	
2:50 pm – 3:05 pm	Austin Taylor	2:50 pm – 3:05 pm	Jennifer Pors	2:50 pm – 3:05 pm	Sam Au
3:05 pm – 3:20 pm	Alberto Delaidelli	3:05 pm – 3:20 pm	Fatemeh Derakhshan	3:05 pm – 3:20 pm	Lisa Borretta
3:20 pm – 3:35 pm	Yasir Mohamud	3:20 pm – 3:35 pm	~~Jenny Chu~~ - cancelled	3:20 pm – 3:35 pm	Julia Naso
3:35 pm – 3:50 pm	Angela Mo	3:35 pm – 3:50 pm			

3:55 pm – 4:15 pm	PDF GUEST SPEAKER: DR. SHO HIROYASU (DR. GRANVILLE'S LAB) Paetzold Lecture Theatre *"Granzyme B: a Novel Target for Pemphigoid Diseases"*
4:15 pm – 4:30 pm	BREAK (ATRIUM)
4:30 pm – 5:30 pm	KEYNOTE SPEAKER: DR. ANDREW BECK Paetzold Lecture Theatre "Artificial Intelligence for Pathology: from Discovery to AI-Powered Companion Diagnostics"
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Brennan J Wadsworth[1,2], Kevin L Bennewith[1,2]

65 SAMUEL SHAO HUAN WENG [graduate student]

SENSITIVE IDENTIFICATION OF PROTEOLYTIC PROTEOFORMS IN LIMITED SAMPLES

Samuel S. H. Weng[1,2,5], Fatih Demir[3,5], Enes K. Ergin[1,2], Sabrina Dirnberger[3], Anuli Uzozie[1,2], Domenic Tuscher[3], Lorenz Nierves[1,2], Janice Tsui[1,2], Pitter F. Husegen[3,4], Philipp F. Lange[1,2]

66 ANNA-CATHARINA WILHELM [graduate student]

THE EFFECT OF GRANZYME B-CLEAVED THROMBOSPONDIN 2 ON ENDOTHELIAL CELL PHENOTYPE AND FUNCTION

Anna-Catharina Wilhelm[1,2], Matt Zeglinski[1,2], David Granville[1,2]

67 DAPHNE WU [graduate student]

IDENTIFICATION OF BIOMARKERS TO PREDICT PLATELET TRANSFUSION OUTCOME

Daphne Wu[1,2], Dana Devin[1,2]

68 TIM XUE [graduate student]

ENTEROVIRAL INFECTION IN MOUSE BRAIN LEADS TO AMYOTROPHIC LATERAL SCLEROSIS-RELATED PROTEIN PATHOLOGIES AND MOTOR DYSFUNCTION

Yuan Chao Xue[1,2], Gabriel Fung[1,2], Yasir Mohamud[1,2], Eric Deng[1,2], Huitao Liu[2,3], Jingchun Zhang[1,2], Ralph Feuer[4], Neil Cashman[5], Honglin Luo[1,2]

69 JOYCE ZHANG [graduate student]

MODELLING INITIATION EVENTS OF SEROUS OVARIAN CANCERS WITH ORGANOID CULTURES AND SINGLE CELL SEQUENCING

Joyce Zhang[1,2], Dawn Cochrane[1], Kieran Campbell[1], James Hopkins[1], Genny Trigo[1], Germain Ho[1], Winnie Yang[1], Maya DeGrood[1], Sorab Shah[1,2], David Huntsman[1,2]

70 GUANGZE ZHAO [graduate student]

COXSACKIEVIRUS B3-INDUCED CLEAVAGE OF DESMOSOME PROTEINS ENHANCES DEGRADATION OF GAMMA-CATENIN AND BENEFITS WNT/BETA-CATENIN SIGNALING PATHWAY

Guangze Zhao[1,2], Huifang M. Zhang[1,2], Ye Qiu[1,2], and Decheng Yang[1,2]

71 HANQI (WAYNE) ZHAO [graduate student]

CHARACTERIZATION OF COLD-STORED PLATELETS IN VITRO

Hanqi (Wayne) Zhao[1,2], Katherine Serrano[1,2,3], Peter Schubert[1,2,3], Elena Levin[2,3], Brankica Culibrk[2,3], Zhongming Chen[2,3], and Dana V.Devine[1,2,3]

72 ADAM ZIADA [graduate student]

PROPOSAL TO INVESTIGATE THE EFFECTS OF INTERRUPTIONS IN HIV THERAPY ON MITOCHONDRIAL AGING

Adam S. Ziada[1,2], Nancy Yang[1,2], Beheroze Sattha[1,2], Hélène C.F. Côté[1,2,3], and The CIHR Team Grant on Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

73 JOHN AIRD [postdoctoral fellow]

MISMATCH REPAIR PROTEIN IMMUNOHISTOCHEMISTRY IN CASES OF RECURRENT GYNECOLOGIC AND GASTROINTESTINAL MALIGNANCIES: IS THERE A NEED FOR REPEAT STAINING?

John Aird[1,2], Mike Steel[1], Robert Wolber[2,3], Lien Hoang[1,2], David Schaeffer[1,2]

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74 JOHN AIRD [postdoctoral fellow]

PROGNOSTIC ASSESSMENT OF IMMUNOGENICITY IN AMPULLARY CARCINOMA POINTS TOWARDS AN IMMUNOMODULATORY THERAPEUTIC OPTION

John Aird[1,2,3], Steve Kalloger[2,3], Christine Chow[2], Daniel Renouf[3,4], and David Schaeffer[1,2,3]

75 WAI HANG (TOM) CHENG [postdoctoral fellow]

CHIMERA REPETITIVE MILD TRAUMATIC BRAIN INJURY INDUCES CHRONIC BEHAVIORAL AND NEUROPATHOLOGICAL PHENOTYPES IN WILD-TYPE AND APP/PS1 MICE

Wai Hang Cheng[1], Kris M. Martens[1], Asma Bashir[1], Honor Cheung[1], Sophie Stukas[1], Ebrima Gibbs[2], Dhananjay R. Namjoshi[1], Emily B. Button[1], Anna Wilkinson[1], Carlos J. Barron[1], Neil R. Cashman[2], Peter A. Cripton[3], Cheryl L. Wellington[1]

76 SHO HIROYASU [postdoctoral fellow] ORAL PRESENTATION

GRANZYME B: A NOVEL TARGET FOR PEMPHIGOID DISEASES

Sho Hiroyasu[1,2,3], Valerio Russo[1,2,3], Matthew R. Zeglinski[1,2,3], Hongyan Zhao[1,2,3], Yoan Machado[4,5], Anika Kasprick6, Chiharu Tateishi[7], Wataru Nishie[8], Angela Burleigh[9], Peter A. Lennox[10], Nancy Van Laeken[10], Nick J. Carr[10], Richard I. Crawford[2,9], Hiroshi Shimizu[8], Daisuke Tsuruta[7], Christopher M. Overall[4,5], Ralf J. Ludwig[6], and David J. Granville[1,2,3]

77 ANULI UZOZIE [postdoctoral fellow]

INTEGRATED PROTEOME, PHOSPHO-PROTEOME AND N TERMINOME CHARACTERIZATION OF PEDIATRIC PATIENT AND MATCHED ENGRAFTED LEUKEMIA

Anuli Uzozie[1,3], Janice Tsui[1], Christopher Maxwell[2,3], James Lim[2,3], Gregor Reid[2,3], Philipp Lange[1,3]

78 MATTHEW MASOUDI [research associate]

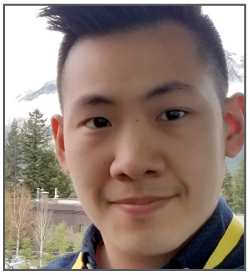
TRANSLATING RESEARCH INTO PRACTICE: INVESTIGATING THE IMPACT OF ALZHEIMER'S DIAGNOSTICS IN CANADA (IMPACT-AD)

Mari L. DeMarco[1,2], Matthew Masoudi[3], Colleen Chamber[4], Robin Hsiung[5], Howard Feldman[5,6], John R. Best[6], Howard Chertkow[7], Serge Gauthier[8], Jason Karlawish[9]

79 VEENA LIN [undergraduate student - poster without presenter, Al Rohet Hossain will bring in the poster]

DIFFERENTIAL EXPRESSION OF NUP98-ERBB4-PSEN1-NRG1 (NEPN) SIGNALING AXIS AS POTENTIAL BLOOD-BASED BIOMARKERS FOR VIRAL MYOCARDITIS

Veena Lin, Paul J. Hanson[1,2], Bruce M. McManus[1,2]



DEREK WONG

LOSS OF ATAXIN-1-LIKE (ATXN1L) DESTABILIZES THE TUMOUR SUPPRESSOR CAPICUA (CIC) PROMOTING DYSREGULATION OF THE CELL CYCLE

SUPERVISOR: STEPHEN YIP

AUTHOR(S): Derek Wong[1], Kohl Lounsbury[2], Amy Lum[3], Veronique LeBlanc[4], Jungeon Song[4], Susanna Chan[4], Suganthi Chittaranjan[4], Marco Marra[4], and Stephen Yip[1]

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BACKGROUND/OBJECTIVES:
Aberrations in the tumour suppressor Capicua (CIC) have recently been implicated as a negative prognostic factor in multiple cancers through the regulation of several oncogenic E-twenty-six (ETS) transcription factors (ETV1/4/5) and the mitogen activated protein kinase (MAPK) pathway. Loss of CIC protein has also been shown to increase metastatic potential and confer treatment resistance to MAPK inhibition in mouse models. ATXN1L, a protein involved in the neurodegenerative disease SCA1 has been shown to be an interactor of CIC. However, the role and mechanism of CIC-ATXN1L interaction in regulating CIC function remains unclear.

METHODS:
CIC and ATXN1L knockout (KO) cell lines were generated using CRISPR/Cas9 gene editing technology followed by gene expression profiling using Affymetrix microarray and differential expression (DE) analysis. DE analysis was also performed on patient lower grade glioma (LGG), stomach adenocarcinoma (STAD), and prostate adenocarcinoma (PRAD) RNAseq data publicly available through The Cancer Genome Atlas (TCGA) using samples which harbored aberrations in CIC or ATXN1L. Lastly, functional in vitro experiments were performed using CIC and ATXN1L KO cell lines to assess the effects of ATXN1L loss on CIC stability and function.

RESULTS:
DE analysis of CIC KO and ATXN1L KO cell lines resulted in 110 and 299 shared differentially expressed genes in human embryonic kidney (HEK) and normal human astrocytes (NHA), respectively. Gene set enrichment analysis of our KO cell lines converged upon activation of the MAPK pathway and dysregulation of central nervous system development. Interestingly, MAPK activation was not found in our transcriptomic analyses of LGG, STAD, and PRAD TCGA cohorts. Instead, shared differentially expressed genes were enriched for gene sets related to cell cycle regulation such as E2F targets, mTOR, and myc upregulation, and Rb downregulation. Using translation inhibition, degradation of CIC protein was found to occur more rapidly in ATXN1L KO cell lines. Lastly, CIC binding to the promoter regions of target genes was found to also be impaired in cell lines expressing a mutant CIC construct lacking the ATXN1L interaction domain.

CONCLUSIONS:
CIC and ATXN1L were found to be functional interactors that regulate similar gene sets both in our in vitro cell systems and in TCGA patient data. On the transcriptomic level, loss of either interactor appears to lead to dysregulation of the MAPK pathway in cell lines, whereas, loss of either CIC or ATXN1L in LGG, STAD, and PRAD TCGA cohorts lead to dysregulation of the cell cycle. The dysregulation of similar gene sets was found to be a result of ATXN1L's role in promoting CIC protein stability and facilitating CIC repression of CIC target genes. Our data suggests that CIC-ATXN1L may be general regulators of the cell cycle and that loss of either interacting partner results in convergent transcriptomic changes.

EMILY BUTTON

HIGH-DENSITY LIPOPROTEIN DEFICIENCY IN TRANSGENIC ALZHEIMER'S DISEASE MICE INCREASES GLOBAL AMYLOID PATHOLOGY AND NEUROINFLAMMATION AS WELL AS VASCULAR AMYLOID AND INFLAMMATION

SUPERVISOR: CHERYL WELLINGTON

AUTHOR(S): Emily B. Button[1,2], Guilaine K. Boyce[3], Anna Wilkinson[1,2], Sophie K. Stukas[1,2], Arooj Hayat[1], Jianjia Fan[1,2], Brennan J. Wadsworth[2], Jerome Robert[1,2], Kris M. Martens[4], and Cheryl L. Wellington[1,2]

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BACKGROUND/OBJECTIVES:
Alzheimer's disease (AD) is defined by amyloid beta (Aβ) plaques and neurofibrillary tangles and characterized by neurodegeneration and memory loss. The majority of AD patients also have Aβ deposition in cerebral vessels known as cerebral amyloid angiopathy (CAA), microhemorrhages, and vascular co-morbidities, suggesting that cerebrovascular dysfunction contributes to AD etiology. Promoting cerebrovascular resilience may therefore be a promising therapeutic or preventative strategy for AD. Plasma high-density lipoproteins (HDL) have several vasoprotective functions and are associated with reduced AD risk in some epidemiological studies and with reduced Aβ deposition and Aβ-induced inflammation in 3D engineered human cerebral vessels. Mice genetic made deficient in apoA-I, the primary protein component of HDL, have reduced plasma HDL-cholesterol levels, increased CAA and increased cognitive dysfunction, whereas overexpression of apoA-I from its native promoter in liver and intestine has the opposite effect and lessens neuroinflammation. Similarly, acute peripheral administration of HDL can reduce CAA and Aβ levels in the brain. Here we expand upon the known effects of plasma HDL described above to investigate the interaction of HDL, amyloid, and astrocytes on the cerebrovasculature in AD model mice. Astrocytes, the most common glial cell of the brain, help to support neurons and cells of the blood-brain barrier and act as a bridge between them.

METHODS:
APP/PS1 mice have 2 mutant human transgenes causing them develop brain Aβ plaques similar to those seen in people with AD. APP/PS1 mice deficient or hemizygous for apoa1 were aged to 12 months. Plasma lipids, amyloid plaque deposition, Aβ protein levels, protein and mRNA markers of neuroinflammation, and astrogliosis were assessed using ELISA, qRT-PCR, and immunofluorescence. Contextual and cued fear conditioning was used to assess behavior.

RESULTS:
Mice with a complete apoA-I deficiency had robustly reduced plasma HDL-cholesterol levels compared to those with hemizygous apoA-I expression. In APP/PS1 mice, complete apoA-I deficiency increased total and vascular Aβ deposition in the cortex but not the hippocampus compared to APP/PS1 littermate controls hemizygous for apoA-I. Protein levels of the vascular related inflammatory markers platelet derived growth factor receptor b (PDGRFβ), glial fibrillary acidic protein (GFAP) and intercellular adhesion molecule (ICAM-1) were elevated in apoA-I-deficient APP/PS2 mice. Loss of apoA-I in APP/PS1 mice also increased vascular specific immunofluorescent staining of GFAP and ICAM-1 were elevated in apoA-I-deficient APP/PS1 mice. No behavioral changes were observed.

CONCLUSIONS:
ApoA-I-containing HDL can reduce amyloid pathology, neuroinflammation, and vascular specific inflammation in APP/PS1 mice. Together, this work suggests that low levels of plasma HDL can be detrimental to brain health in the context of AD.

JESSICA PILSWORTH

GENOMIC CHARACTERIZATION OF ADULT-TYPE GRANULOSA CELL TUMOURS REVEALS MUTATIONS IN EPIGENETIC REGULATORS

SUPERVISOR: DAVID HUNTSMAN

AUTHOR(S): Jessica Pilsworth[1,2], Dawn Cochrane[2], Samantha Neilson[2], Bahar Moussavi[2], Anniina Färkkilä[3,4], Hugo Horlings[2], Satoshi Yanagida[5], Yi Kan Wang[2], Ali Bashashati[2], Winnie Yang[2], Janine Senz[2], Jacqueline Keul[6], Adele Wong[7], Esther Oliva[7], Stefan Kommoss[6], Friedrich Kommoss[8], Sohrab Shah[9], David Huntsman[2,10]

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BACKGROUND/OBJECTIVES:
Adult-type granulosa cell tumours (AGCT) are the most common sex cord-stromal tumour and represent 3-5% of all ovarian cancers. AGCTs are derived from granulosa cells, which surround the oocyte. Most patients are diagnosed at an early stage and have indolent tumours. However, one-third of patients relapse, typically 4-7 years after initial diagnosis, leading to mortality in 50% of these relapsed patients. Surgery is the first-line treatment for both primary and relapsed tumours, but there are currently no effective treatments for patients with unresectable or advanced stage tumours. Our research team previously discovered the somatic missense mutation (c.402C>G; pC134W) in the transcription factor FOXL2 in 97% of AGCTs. This discovery has been developed into a diagnostic biomarker, but our ability to understand the biology of the mutation, pathogenesis of the tumour and develop treatments has been hindered by a lack of appropriate model systems. My project aims to profile the genomic landscape of AGCT and develop a relevant model system to study the impact of FOXL2 and secondary mutations on tumour development. We hypothesize that additional mutations besides FOXL2 are responsible for the clinical variability of AGCT and could represent treatment opportunities.

METHODS:
We performed whole genome sequencing on ten AGCTs and their matched normal blood. From this analysis, we designed a custom amplicon panel to perform targeted sequencing on a cohort of over 200 formalin-fixed paraffin-embedded AGCTs collected internationally. For our model system, we used density centrifugation to isolate primary granulosa cells from follicular fluid collected from healthy women undergoing oocyte retrieval and used lentiviral delivery to incorporate select mutations identified from our genomic analysis.

RESULTS:
We have successfully developed a model system expressing fluorescently-tagged FOXL2 (wildtype or C134W mutant) and human telomerase (previously identified from our genomic analysis) in primary granulosa cells. Additionally, our whole genome and targeted sequencing analysis on a cohort of over 200 AGCT patients from six countries revealed that two epigenetic regulators, KMT2D and KDM5C, were recurrently mutated with various missense and truncating mutations in 83 (41%) and 26 (13%) of 203 patients, respectively. To study KMT2D truncating mutations, we have cloned CRISPR guide RNAs targeting KMT2D into lentiviruses and are testing their knockout efficiency in the AGCT-derived cell line KGN.

CONCLUSIONS:
Our large genomic study revealed mutations in epigenetic modifiers, suggesting that epigenetic dysregulation may play a role in the development of AGCT. We plan to knockout KMT2D in our primary cell models (wildtype or mutant FOXL2) to study the impacts on cell viability. Through this project, we will gain insight into the interaction between mutant FOXL2 and epigenetic regulators including KMT2D, which is the first step in developing biologically-informed therapeutics.

MUNTADHAR AL MOOSAWI

IMPLEMENTATION AND CLINICAL IMPLICATION OF HEPCIDIN ASSAY IN BRITISH COLUMBIA

SUPERVISOR: **ANDRE MATTMAN**

AUTHOR(S): Muntadhar Al Moosawi[1], Grace van der Gugten[3], Andre Mattman[2]

AFFILIATION(S): [1]Hematological Pathology, Dept of Pathology and Laboratory Medicine, UBC; [2]Medical Biochemist, St. Paul's Hospital; [3]Assay Development Scientist, St. Paul's Hospital

BACKGROUND/OBJECTIVES:
Hepcidin is a 25-peptide hormone produced by hepatocytes and acts as the main regulator of iron homeostasis. By acting on ferroportin, hepcidin controls the main efflux of cellular iron into plasma. High plasma hepcidin concentration results in the internalization of ferroportin, and trapping of iron inside enterocytes, macrophages and hepatocytes. Signals to hepcidin are mediated by intracellular iron stores, transferrin-bound circulating iron (Tf-Fe2) and inflammation. Hepcidin testing has proven beneficial in the investigation of various clinical conditions including confirmation of genetic disorders of iron regulation and the anemia of inflammation. However, the methodology for measuring plasma hepcidin concentration is complex and a reliable assay for hepcidin-25 has not been widely available.

METHODS:
A liquid chromatography–tandem mass spectrometry (LC-MS/MS) method with multiple reaction monitoring was developed and validated for quantification of serum or plasma hepcidin concentration at St. Paul's Hospital. Calibrator and internal standard solutions were prepared from lyophilized peptides purchased from Peptides International and stored in "lo-bind" tubes. Sample preparation was performed with solid phase extraction into 96-well plates. Precision, linearity, limit of quantitation and bias as compared to a reference standard were evaluated. The initial assessment of the assay included testing samples from three subjects with suspected genetic iron dysregulation. Respective samples were from a proband with a suspected novel hereditary hemochromatosis phenotype, and two patients with suspected iron refractory iron deficiency anemia (IRIDA).

RESULTS:
The method coefficient of variation was <5.5% and <10% in the high (45 ng/mL) and low (3.8 ng/mL) serum pools. Limit of Quantitation was 1.0 ng/mL. Method linearity up to 133 ng/mL was verified. Bias at 2.4 ng/mL and 10.5 ng/L was +1.2 and +1.6 ng/mL, respectively. The literature reference interval for hepcidin is <0.5 to 15.4 ng/mL. Patient results were as shown:

Patient	Ferritin (ug/L)	Hemoglobin (g/L)	Hepcidin (ng/mL)	Interpretation
Iron deficient Proband 1 (15 yo male)	4	82	<1	Not IRIDA
Iron deficient Proband 2 (69 yo female)	117	117	22	IRIDA
Hemochromatosis proband (44 yo female)	2453	132	33	Novel form of hemochromatosis
15 yo males	30 - 100	Normal	14 and 15	Normal control

Result of the hemochromatosis proband was suggestive of ferroportin mediated hereditary hemochromatosis but ferroportin genetic testing was normal. Genetic testing is in progress for the iron deficient proband 2 and for hemochromatosis proband.

CONCLUSIONS:
We have made available a hepcidin method to facilitate the investigation of suspected disorders of iron metabolism. This development enables local testing as opposed to testing in the Netherlands as had previously been done for such cases. Future development is to investigate the utility of this test in other patient groups such as anemia of inflammation. This later work will be centered in Vancouver Island Health Authority (VIHA) where, incidentally, a hepcidin method has also been established.

BHUPINDER JOHAL

FREQUENCY OF POSITIVE ANTIBODY SCREENS ON PATIENTS WITH SERIAL GROUP AND SCREENS EVERY 3 DAYS (PASSES 3D STUDY)

SUPERVISOR: ANDREW SHIH

AUTHOR(S): Bhupinder Johal[1], Ann Tran[1], Lawrence Sham[2], Monika Hudoba[1], Andrew Shih[1]

AFFILIATION(S): [1]Dept of Pathology, UBC, Vancouver Coastal Health Authority; [2]MLT, Vancouver Coastal Health Authority

BACKGROUND/OBJECTIVES:
Pre-transfusion compatibility testing includes the group and screen, to determine the patient's blood group and to determine if the patient has had a sensitizing event (such as red blood cell (RBC) exposure from transfusion and/or pregnancy) to identify alloantibodies in an antibody detection test (ADT). Identification of alloantibodies is key to finding crossmatched compatible blood for transfusion, as transfusion of RBCs with antigens for which the patient has cognate alloantibody(ies) produces an immune reaction to destroy the transfused RBCs. A group and screen must therefore be performed within 3 days of transfusing crossmatched blood, based on Health Canada regulations. Unique to VCH Authority, the inpatient pre-printed transfusion order forms for RBC ordering for transfusion allow for serial group and screens every 3 days to ensure that there is always in-date pre-transfusion testing for the provision of the crossmatched blood. This occurs even though inpatients not requiring transfusion during their hospitalization period will likely not have any changes in their ADT. This can lead to unnecessary iatrogenic anemia and testing. This population also allows the study of serological status during hospitalization with minimal confounding factors that could result in extensions of group and screens for crossmatched blood similar to outpatients.

This purpose of this study is to assess inpatients at VGH who have serial ADTs every 3 days (or sooner) to determine the rate of positive ADTs in patients who are transfused and those who are not transfused. As a secondary outcome, we will also determine the rate of transfusion for patients who do have serial ADTs every 3 days.

METHODS:
All adult (age >18) inpatients who have serial group and screens every 3 days (or less), admitted for greater than 10 days with 2 or more ADTs will be enrolled in our retrospective cohort study. Our primary outcome is the frequency of positive ADTs in adult patients, stratified by patients with and without sensitizing events during hospital admission. Data will be abstracted from two sources from the timeframe January 2015 to December 2018. These sources will be the transfusion medicine laboratory data and retrospective chart review including the admitting diagnosis, length of stay, number of transfusions during hospital stay, and the change in ADT test during hospital stay.

RESULTS:
Preliminary results to be presented at Pathology Day.

CONCLUSIONS:
We hypothesize that the rate of positive ADTs will be near zero for inpatients with serial tests with no transfusions during their inpatient stay; and that transfusion rates will be low (<10%) for patients with serial ADT testing. If our hypothesis is correct, we anticipate a possible change in our serial screen inpatient orders for patients admitted to VGH. Barriers to extending group and screens for crossmatched blood for inpatients include physician education regarding uncrossmatched blood, potential increased use of emergency red cells, and considerations regarding current lab information systems being created to outdate group and screens indiscriminately after three days.

ERIC MCGINNIS

EVALUATION OF ACTIVATED PARTIAL THROMBOPLASTIN TIME COAGULATION WAVEFORMS GENERATED BY ACL TOP SERIES COAGULATION ANALYZERS CAN HELP IDENTIFY PATIENTS WITH ACQUIRED FACTOR VIII INHIBITORS

SUPERVISOR: TYLER SMITH

AUTHOR(S): Eric McGinnis[1], Steven K.W. Wong[2], and Tyler W. Smith[3]

AFFILIATION(S): [1]UBC; [2]St. Paul's Hospital; [3]Vancouver General Hospital

BACKGROUND/OBJECTIVES:
ACL TOP instruments measure activated partial thromboplastin time (aPTT) turbidimetrically and generate coagulation waveforms (CWs), which graphically represent change in light absorbance over time. Isolated prolonged aPTT results pose challenges for clinical laboratories because they are frequently caused by a lupus anticoagulant (LA), which is generally not associated with bleeding but often requires laboratory investigation to exclude uncommon disorders that are associated with bleeding, including acquired inhibitors of coagulation factors (particularly FVIII). Early identification of factor inhibitors is of critical importance, as delayed recognition can result in significant bleeding-related morbidity and mortality. We sought to identify features in the CW to differentiate LAs from factor inhibitors and facilitate rapid initial testing to identify patients at risk of severe bleeding.

METHODS:
We retrospectively assessed CWs collected at two tertiary care centers from ACL TOP 700 CTS instruments using SynthASil aPTT reagent, including normal controls and patients with LA (or probable LA; PLA), acquired FVIII inhibitors, congenital intrinsic pathway factor deficiencies, and medical anticoagulation. Initial examination of CWs identified the presence of a double peak in the first derivative curve (DP1D) as potentially useful to differentiate factor inhibitors from LAs. CWs were inspected for DP1D and numeric parameters of CW first and second derivative (2D) curves were used to compute receiver operating characteristic (ROC) curves.

RESULTS:
182 CWs were assessed, including 60 LA/PLA (6 of which showed DP1D) and 21 FVIII inhibitors (17 of which showed DP1D). In cases with prolonged aPTT (>38 seconds, n=143), DP1D had 81.0% sensitivity and 89.3% specificity for FVIII inhibitors. Among other CW parameters, 2D minimum demonstrated the highest area under the ROC curve for identification of FVIII inhibitors (0.892; sensitivity 88.9%, specificity 82%).

CONCLUSIONS:
CW assessment for DP1D and derivative curve parameters readily available on ACL TOP instruments appears to be of value in differentiating acquired factor inhibitors from other causes of prolonged aPTT. Incorporating CW analysis in routine laboratory practice may prove useful in directing initial investigations of unexplained prolonged aPTT and facilitate early diagnosis of acquired hemophilia.

KYRA BERG

RE-EXAMINING THE 1 MM MARGIN AND SUBMUCOSAL DEPTH OF INVASION: A REVIEW OF 216 MALIGNANT COLORECTAL POLYPS

SUPERVISOR: DAVID SCHAEFFER

AUTHOR(S): Kyra B. Berg[1], Jennifer J. Telford[2], Laura Gentile[3], and David F. Schaeffer[4]

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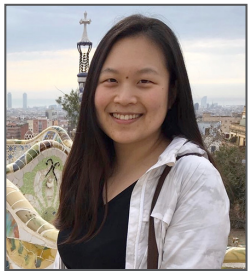
BACKGROUND/OBJECTIVES:
Malignant polyps have a risk of lymph node metastases between 9-24%, but patients who are negative for certain pathologic poor prognostic factors have the potential to be treated with polypectomy alone. Pathologic positive margin is an indication for surgery, but the definition in the literature has varied from tumor at cautery to 2 mm, with the currently most accepted standard at less than or equal to 1 mm. Submucosal depth is a prognostic factor that has received much attention in the literature but has only been officially implemented in Japan, again with variation in the cut-off value in the literature from 1000 to 2000 micrometers.

METHODS:
We identified 216 malignant polyps through a Colon Screening program, and reviewed all malignant polyps with either pathologic positive margin as the sole, or with no high risk features, with the objective of examining current margin cut-offs, submucosal depth, and width of carcinoma relative to rates of residual carcinoma and lymph node metastases.

RESULTS:
A positive margin cut-off of tumor at cautery showed significantly increased rates of lymph node metastases ($p=0.04$) compared to a margin of greater than zero millimeters, and polyps with a margin of greater than zero millimeters had no risk of residual carcinoma. A submucosal depth of greater than 2000 micrometers had an increased risk of lymph node metastases compared to less than 2000 micrometers ($p=0.01$).

CONCLUSIONS:
Refinement of and strict adherence to these cut-offs has the potential to reduce the number of surgeries required in patients with malignant polyps, a group that continues to grow significantly, in part due to the introduction of colon screening programs.



ELLEN CAI

TRANSFORMATION OF OVARIAN LOW-GRADE SEROUS NEOPLASM TO HIGH-GRADE CARCINOMA: A RARE PHENOMENON AND REPORT OF 4 CASES

SUPERVISOR: LIEN HOANG

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AFFILIATION(S): [1]Dept of Anatomical Pathology, Vancouver General Hospital and UBC, Vancouver, BC; [2]Genetic Pathologic Evaluation Center, Vancouver, BC

BACKGROUND/OBJECTIVES:
The transformation of LGSN to high-grade carcinoma is exceedingly rare (with only 6 cases reported to date) and the molecular events which lead to such transformation are unknown. The vast majority of LGSN do not bear TP53 mutations. BRAF (V600E) mutations in LGSN have been reported to be associated with better prognosis. Our objective was to assess p53, BRAF and WT1 immunohistochemistry and to perform NGS panel in our cases of LGSN with transformation to high-grade carcinoma in order to better understand the molecular events underlying this rare phenomenon.

METHODS:
We identified 4 cases where LGSN transformed to a high-grade component. H&E slides were reviewed and immunohistochemistry was performed. p53 was scored as wild-type, null or overexpressed. BRAF and WT1 was scored as positive or negative. Targeted DNA sequencing with an NGS panel (120 hotspots and 17 exons in 33 known solid cancer genes) was performed on the 3 of the 4 cases.

RESULTS:
Patient ages for cases 1 to 4 were 46, 44, 69 and 70 years, respectively. The first two cases exhibited a high-grade carcinoma arising from a LGSN. In these two cases, WT1 was positive and BRAF was negative, in both the low-grade and high-grade components. In case 1, p53 was wild-type in both the low-grade and high-grade areas. NGS panel did not identify any hotspot mutations for case 1. In case 2, p53 was wild-type in the low-grade area but mutated (null) in the high-grade area, and this is corroborated by NGS panel, which identified a nonsense point mutation in TP53in the high-grade component. In case 3, the LGSN had transformed to a carcinosarcoma with a homologous sarcomatous component. p53 was mutated (overexpressed) in the both the LGSN and carcinosarcomatous components. The same TP53and KRASmissense point mutations were identified in both components. Again, BRAF was negative and WT1 was positive. In case 4, LGSN had transformed to an undifferentiated carcinoma which had metastasized to the bowel. The tissue for case 4 was not available for immunohistochemical studies and NGS analysis.

CONCLUSIONS:
Transformation from LGSN to high grade carcinoma is rare, with only 4 cases occurring over a 20 year span at our institution. Abnormal p53 was observed in 2 of 3 cases in the high-grade component, and this was corroborated by NGS analysis. No cases displayed abnormal BRAF. The NGS panel did not identify any other molecular events underlying the transformation of LGSN to high-grade carcinoma in our study.

AMY NAGELBERG

IDENTIFICATION OF CANDIDATE EGFR-COOPERATING DRIVERS IN LUNG CANCER USING A SLEEPING BEAUTY INSERTIONAL MUTAGENESIS SCREEN

SUPERVISOR: **WILL LOCKWOOD**

AUTHOR(S): **Amy L. Nagelberg[1,2], William W. Lockwood[1,2]**

AFFILIATION(S): **[1]Dept of Integrative Oncology, BC Cancer Agency, Canada; [2]Dept of Pathology & Laboratory Medicine, UBC, Canada**

BACKGROUND/OBJECTIVES:
Lung cancer (LC) is the leading cause of cancer mortality worldwide. Targeted therapies— such as tyrosine kinase inhibitors (TKIs) that inhibit mutant forms of the Epidermal Growth Factor Receptor (EGFR) — have improved life expectancy for LC patients carrying specific mutations. Still, significant challenges remain. Many tumors harbor mutations in uncharacterized genes or genes that are non-actionable due to difficulty in the development of a suitable drug, and even those that initially respond to TKIs eventually succumb to resistance and recurrence of LC. Therefore, discovery and characterization of genes that cooperate with EGFR is critical to the development of combination-based therapies, which will help to combat TKI resistance. Identification of true LC drivers from the thousands of passenger mutations often contained in patient tumors is difficult using sequencing and *In vitro* screening technologies; bioinformatic analyses of clinical tumors do not prove causality and in vitro screens are not necessarily representative of i*n vivo* tumorigenesis. Thus, we have utilized a transposon-mutagenesis system, *Sleeping Beauty* (SB), to screen for novel EGFR-cooperating drivers in a genetically engineered mouse model of LC.

METHODS:
Mice were bred such that mutant EGFR expression and SB transposition were induced upon administration of doxycycline in their diet through a TetO/Cre-lox system. Once tumors formed, 231 lesions from 23 mice were harvested; SB-insertion sites were amplified, barcoded, and sequenced. Genes with a statistically significant frequency of the SB transposon insertions($p<0.0001$) are candidate drivers of tumorigenesis. The SB insertion pattern across samples was used to predict the function of the candidate drivers (oncogene versus tumor-suppressor).

RESULTS:
Using this approach, 526 candidate LC driving genes were identified (458 candidate tumor suppressors and 68 candidate oncogenes). Over 200 of these have not been previously characterized in LC. Pathway analysis on these genes has confirmed enrichment in key oncogenic pathways, including the YAP/TAZ, Wnt, and MAPK signaling pathways. We have overlaid this data with human genomic data from The Cancer Genome Atlas (TCGA) to identify genes frequently altered in human LC. Genes with alterations in human LC (n=388) are currently undergoing systematic validation using a competitive CRISPR-Cas9 knockout screen in specialized LC precursor cells.

CONCLUSIONS:
Both novel candidates and well-characterized LC drivers were identified in an unbiased functional in vivo screen. Further validation of the role of these genes in tumorigenesis will aid in our understanding LC and ultimately the development of novel therapeutics to combat this devastating disease.

EMILY KAMMA

CHARACTERIZING THE CLINICAL AND IMMUNOPATHOLOGICAL ALTERATIONS IN A NOVEL MOUSE MODEL OF PROGRESSIVE MULTIPLE SCLEROSIS

SUPERVISOR: JACQUELINE QUANDT

AUTHOR(S): Emily Kamma[1], Pierre Becquart[1], Jasmine Hui[1], Cecily Bernales[2], Mary Encarnacion[2], Fabio Rossi[2], Carles Vilariño-Güell[2], Jacqueline Quandt[1]

AFFILIATION(S): [1]Dept of Pathology and Laboratory Medicine, UBC; [2]Dept of Medical Genetics, UBC

BACKGROUND/OBJECTIVES:
Multiple sclerosis (MS) is a neurodegenerative disease of the central nervous system that causes disability, fatigue, vision and cognitive disturbances. Pathologically it is characterized by inflammation, demyelination, oligodendrocyte damage, and disability secondary to axonal damage and loss. 15% of patients present with primary progressive MS (PPMS), where disease worsens without recovery, and more than half of those with MS ultimately progress. While many MS disease modifying therapies treat relapsing MS, there are no therapies for progressive MS. Nuclear receptor subfamily 1 group H member 3 (NR1H3) is a regulator of immune and glial cell function and controls transcriptional regulation of lipid and cholesterol homeostasis and inflammation. The NR1H3 R415Q mutation was identified in two Canadian families linked with a severe, rapidly progressing form of PPMS. We developed a novel model of progressive MS carrying this NR1H3 mutation. The objective of this study is to use the NR1H3 mouse to characterize the course and the immunopathological processes driving risk and progressive disease in the experimental autoimmune encephalomyelitis (EAE) model of MS.

METHODS:
Wild-type (WT), heterozygous (HET), and homozygous (HOM) mice for a NR1H3 R413Q mutation were analyzed by whole transcriptome RNA sequencing of spleen, liver, brain, and spinal cord to identify differentially regulated genes. Serum was screened using Mesoscale, ELISA and flow cytometry to assess immune profiles. NR1H3 mice from each genotype were immunized to induce chronic progressive EAE. The mice were followed for 50 days and scored for disability, spasticity, and weight loss. Immunohistochemistry was performed on spinal cord sections to characterize hallmarks of inflammatory neurodegeneration including immune cell infiltration, activated microglia (Iba-1), gliosis (GFAP), demyelination (MBP), axonal damage and loss (SMI-32), and foamy lipid-containing macrophages or microglia (Oil red O stain+).

RESULTS:
Transcriptome analysis showed significant differences in CD5 antigen-like (Cd5l) and other gene expression in the spleen of NR1H3 HOM mice compared to WT. ELISA showed CD5L levels in the serum of NR1H3 HOM mice tended to be lower compared to HET and WT. NR1H3 mice had increased IL-17A and IL-16 serum levels compared to WT. NR1H3 EAE had greater clinical disease severity than WT EAE indicated by higher average disease score, spasticity, weight loss, and earlier onset; this difference was most significant in male mice. Histologically, NR1H3 EAE showed increased microglia activation, gliosis, demyelination, axonal loss, and lipid accumulations in the spinal cord compared to WT EAE.

CONCLUSIONS:
The NR1H3 mutation dysregulates the immune system, altering proteins and cytokines that are key mediators of inflammation and injury in MS and EAE. This contributes to a more severe clinical and histopathological EAE phenotype associated with progression. Studies are ongoing to further validate the NR1H3 mouse as a valuable model to study the pathophysiological processes of progression in MS and as a tool to identify novel therapeutic targets and approaches.

RACHEL CEDERBERG

INTERLEUKIN-5 DRIVES THE EXPANSION OF LUNG B-1 B CELLS AND RESTRICTS PULMONARY METASTASIS

SUPERVISOR: KEVIN BENNEWITH

AUTHOR(S): Rachel A. Cederberg[1,2], Alvina So[3], S. Elizabeth Franks[1], Jenna Collier[1,2], Brennan J. Wadsworth[1,2], Michael R Hughes[4], Kelly M. McNagny[4], Kevin L. Bennewith[1,2,5]

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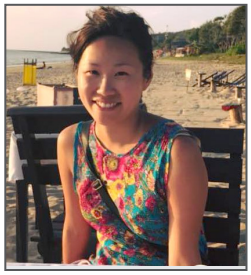
BACKGROUND/OBJECTIVES:
The use of immunotherapy to treat lung cancer is becoming increasingly common, highlighting the importance of the immune system in the lung tumor microenvironment. The lungs are host to a variety of immune cell subsets, including B cells, which are a population of immune cells that secrete antibodies and can act as antigen-presenting cells in the lungs. Despite the presence of B cells in solid tumors and their prevalence in the lung, the role of B cells in lung cancer is controversial and largely undefined. Additionally, various research groups have demonstrated that B cells can both hinder and support tumor growth. We have found that transgenic mice which over-express the cytokine interleukin-5 (IL-5) have an expansion of lung B cells, as well as a decrease in pulmonary metastasis. Therefore, we hypothesize that B cells may decrease tumor growth in the lungs.

METHODS:
We used IL5Tg transgenic mice that over-express interleukin-5 (IL-5) to study the impact of B cells on tumor growth in the lungs. IL-5 is also an important cytokine for the immune cell population eosinophils, therefore IL5Tg mice also have a systemic increase in eosinophils. Because of this, we also used ddGATA/IL5Tg double-transgenic mice (excess IL-5 but no Eo) to specifically study B cells. Lewis Lung carcinoma (LLC) cells were injected intravenously (IV) to seed the lungs. Circulating immunoglobulins (Ig) were quantified by ELISA. Flow cytometry was used to quantify immune cell subsets in the lungs, as well as immunoglobulin binding to tumor cells, and histological analysis was used to measure tumor burden.

RESULTS:
Naive IL5Tg and ddGATA/IL5Tg mice had an increased total number of lung B cells, but this increase was attributed to an increase in CD11b+ B cells. These CD11b+ B cells co-expressed IgM, indicating that they are a population of B-1 B cells. B-1 B cells isolated from IL5Tg mice also expressed higher levels of the apoptosis-inducing cell surface molecule FasL, which has been shown to mediate tumor cell lysis. IL5Tg mice injected IV with LLC cells had an increase in the total number of lung-infiltrating conventional B cells and B-1 B cells compared to naive mice, whereas there was no change in B cell subsets between naive and LLC IV injected WT mice. Importantly, IL5Tg mice had a decrease in LLC lung tumor burden compared to WT mice. Both circulating IgM and IgG2b were elevated in IL5Tg mice compared to WT, and we found that serum IgM from IL5Tg mice was able to bind to the surface of LLC tumor cells. We are currently investigating whether binding of IgM to tumor cells can induce tumor cell cytotoxicity.

CONCLUSIONS:
We have found that excess IL-5 drives the expansion of lung B-1 B cells. A decrease in lung tumor burden in IL5Tg mice suggests that IL-5 may promote anti-tumorigenic immune cell activities in the lungs. Illuminating the specific role of B cells in lung cancer growth will help deconvolute the complex interplay between host immune cells and malignant cells and could reveal new avenues for immunotherapy development.



LYNNE LI

NOT IN KANSAS ANYMORE: CLINICAL SPECTRUM OF MYCOBACTERIUM KANSASII INFECTION IN BRITISH COLUMBIA FROM 2006-2018

SUPERVISOR: **INNA SEKIROV**

AUTHOR(S): Lynne Li[1], Trevor Hird[2], Cindy Zheng[2], Inna Sekirov[2]

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BACKGROUND/OBJECTIVES:
Mycobacterium kansasii is considered as more pathogenic amongst non-tuberculous *Mycobacterium* species (NTM). Epidemiologically, there is significant geographic variation in the extent of *M. kansasii* disease. In regions of Slovakia and Poland. Previous studies demonstrated an association with urban areas, mining industry and disruption of soil .

Diagnosing NTM disease including M. kansasii is challenging both clinically and microbiologically. We aimed to characterize the clinical spectrum, and severity of M. kansasii disease in British Columbia, and to describe patterns of drug susceptibility.

METHODS:
Retrospective chart review was conducted on patients with positive M. kansasii cultures from 2006 to 2018. Variables of interest included baseline and follow-up imaging, clinical history, treatment and outcomes. Imaging consistent with NTM disease included findings showing bronchiectasis, nodular or cavitary lesions. Severe comorbid illness denotes patient required intensive care, or palliative care due to underlying conditions.

Student's T-test and Fisher's exact test were used for statistical analyses.

RESULTS:
There were 48 cases of *M. kansasii* infection between 2006 and 2018, of which 43 had clinical information available. Between the treated and untreated cases, there were no statistically significant differences in respiratory and constitutional symptoms, radiographic findings of NTM disease or underlying lung disease (Table 1). Eighteen patients (38%) had other NTM organisms isolated as part of initial cultures or in follow-up specimens.

Twelve patients (28%) underwent treatment. Of the 8 patients with known follow-up, 6 reported clinical improvement, 2 died during treatment for NTM disease, 1 did not report improvement post-treatment, and 1 one was always asymptomatic (Table 2).

The most common reasons for not treating *M. kansasii* infection were absent or improved symptoms (10 of 21), or severe comorbidities precluding therapy (7 of 23). The latter trended towards significance (p=0.07) as compared to treated group. All three isolates with susceptibility testing results available were sensitive to rifampin. Empiric anti-mycobacterial regimens were chosen for the remaining patients without susceptibility testing.

CONCLUSIONS:
M. kansasii infection in B.C. is uncommon and few diagnosed patients receive therapy. Isolates tested to date are susceptible to rifampin, but most clinicians choose treatment regimens empirically.

VICTOR YUEN

AUDIT AND FEEDBACK INTERVENTIONS ASSOCIATED WITH LOWER MORTALITY IN A RETROSPECTIVE ANALYSIS OF CLINICAL OUTCOMES FROM AN ANTIMICROBIAL STEWARDSHIP PROGRAM

SUPERVISOR: JENNIFER GRANT

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AFFILIATION(S): [1]UBC, Vancouver, BC, Canada; [2]Vancouver Coastal Health, Vancouver, BC, Canada

BACKGROUND/OBJECTIVES:
Inappropriate use of antimicrobials results in unnecessary costs, *Clostridium difficile* infections, longer length of hospital stay (LOS), adverse drug events, and antimicrobial resistance. As such, an antimicrobial stewardship program should measure these and other outcomes for quality improvement of patient care. The objective of this study was to assess the feasibility of specific patient-centred outcome measures that would be possible targets for ongoing prospective analysis.

METHODS:
We reviewed the electronic medical records of patients admitted to the internal medicine and family practice services at our tertiary referral centre from June to August 2018. Patients who received audit and feedback interventions were matched to controls by age, gender, antibiotic, and indication. The following outcomes were collected: acceptance of stewardship intervention, ICU admission, represcription, bloodstream infection with any antibiotic-resistant organisms, fungemia, renal toxicity, neutropenia, readmission, and mortality. Linear regression was used for analysis of numerical outcomes and logistic regression was used for analysis of categorical outcomes.

RESULTS:
The 31 patients in the intervened group and the 31 patients in the control group were similar with respect to their baseline characteristics of age (median 83.5 years, IQR 73.75-90), gender (71% males), Charlson Comorbidity Index (median score 8, IQR 6-10), and pre-intervention LOS (median 2 days, IQR 0-5). Twenty-three interventions (74%) were accepted. Compared to the control group, the intervened group had significantly lower 30-day mortality (Odds Ratio 0.31, p=0.0485), adjusted for comorbidity score and pre-intervention LOS. Other outcomes were not significantly different between the two groups.

CONCLUSIONS:
Audit and feedback interventions were associated with significantly lower 30-day mortality. Ultimately, mortality represents the most objective clinical outcome measure and is readily extractible from electronic medical records for analysis and inclusion in dashboard metrics and quality improvement cycles.

ADRIAN LEVIN

A DEEP LEARNING APPROACH FOR AUTOMATED BREAST CANCER NODAL STAGING

SUPERVISOR: STEPHEN YIP

AUTHOR(S): Adrian Levine[1], Jasleen Grewal[2], Colin Schlosser[2], Steve Jones[2], Stephen Yip[1]

AFFILIATION(S): [1]Pathology and Laboratory Medicine, UBC, Vancouver, BC, Canada; [2]Canada's Michael Smith Genome Sciences Centre, Vancouver, BC, Canada

BACKGROUND/OBJECTIVES:
Over the past decade, convolutional neural networks (CNN), a form of deep learning, have made unprecedented progress in the ability of computers to analyze images, including in several medical specialties. In pathology, 32 teams competed in the CAMELYON 2016 competition to identify metastatic breast cancer in whole slide images (WSI) of lymph nodes, and the top performers used CNNs to achieve comparable accuracy to expert pathologists. The follow up CAMELYON 2017 competition extended this to a more clinically realistic scenario, in which teams were tasked with nodal staging of simulated patients with 5 lymph nodes each. In this presentation we present our submission to the ongoing CAMELYON 2017 leaderboard.

METHODS:
Our study used the CAMELYON 2016 and CAMELYON 2017 data sets. The CAMELYON 2016 set contains 399 WSIs, scanned at 40x magnification, with exhaustive annotations on all tumor containing slides. The CAMELYON 2017 training set contains 100 simulated patients, each consisting of 5 WSIs with slide level labels (macrometastasis, micrometastasis, isolated tumor cells, or negative) for a total of 500 slides. Fifty of these slides have exhaustive annotations of tumor regions. The CAMELYON 2017 test set contains an addition 100 patients with 5 slides each. For the pixel level identification of tumor vs. non-tumor, a modified fully-convolutional version of the popular InceptionV3 CNN was used. WSIs are typically around 100,000-200,000 pixels in height and width, which is far too large to be processed in their entirety. Rather, the CNN was trained on 882,000 small image patches of 768 x 768 pixels, which were extracted from all 399 CAMELYON 2016 slides and 163 CAMELYON 2017 slides, including the 50 with tumor annotations. Using the trained classifier, heatmaps of pixel-level tumor probability were generated for the CAMELYON 2017 slides. The largest region of connected tumor pixels was then extracted from each heatmap and 26 quantitative image features were calculated. The resulting feature vector was used to train a random forest classifier for classification of each slide as negative, or containing isolated tumor cells, a micrometastasis, or a macrometastasis. 215 slides were used to train this classifier, with the remaining 285 training slides used as a validation set. Patient level pN staging was done according to the AJCC criteria, using the slide level classifications of each patient's 5 slides.

RESULTS:
The CAMELYON 2017 test metric is the Cohen's kappa for staging patients into 5 classes - pN0, pN0i+, PN1mi, PN1, and PN2. Furthermore, the accuracy for classifying individual slides can be evaluated as an internal validation metric. Our best model achieved 0.915 accuracy for slide level classification, and a kappa of 0.922 for patient level nodal staging. On the CAMELYON 2017 test set, we achieved a kappa of 0.8331.

CONCLUSIONS:
Deep learning is an effective method for the automated analysis of lymph node dissection specimens. Ongoing challenges remain in identifying isolated tumor cells and micrometastases. This technology is likely to have a significant impact on pathology practice in the future.

RAYMOND YIP

TERMINAL ILEUM IS THE MOST SENSITIVE SITE FOR THE HISTOLOGIC DIAGNOSIS OF GRADE 4 GRAFT VERSUS HOST DISEASE (GVHD) IN THE LOWER GI TRACT AND IS A HARBINGER OF POOR OUTCOME

SUPERVISOR: HUI-MIN YANG

AUTHOR(S): Raymond Yip[1], David F. Schaeffer[1,2], Hui-Min Yang[1,2]

AFFILIATION(S): [1]UBC, Vancouver, BC, Canada; [2]Vancouver General Hospital, Vancouver, BC, Canada

BACKGROUND/OBJECTIVES:
The site of the GI tract where biopsy is most likely to be diagnostic of graft versus host disease (GvHD) remains a controversial topic. The rectosigmoid colon is the preferential site of biopsy for assessment of GvHD as recent reports have indicated equal or higher sensitivity of detection compared to other sites of the lower GI tract, and biopsies can be obtained via a less invasive flexible sigmoidoscopy procedure. It is also known that while histologic grade 1-3 GvHD have correlated poorly with patients' symptoms and the overall clinical grade, patients with histologic grade 4 acute GvHD tend to have more severe clinical manifestations and a poor prognosis.

METHODS:
In our study, we examined cases of lower GI biopsies in the past 2 years at a tertiary centre in patients status post stem cell transplantation for the evaluation of graft versus host disease, which included a complete colonoscopy with ileal intubation and biopsies. The site of biopsies and the severity of GvHD were collected from pathology reports.

RESULTS:
In 6 of 22 cases, a histologic diagnosis of severe GvHD was rendered. All of these 6 patients showed histologic grade 4 GvHD in terminal ileal biopsies, while only half (3 of 6 patients) showed histologic grade 4 GvHD elsewhere in the GI tract, including the sigmoid colon. One third of patients (2/6) showed no GvHD or rare non-specific enterocyte apoptosis. Significantly, 4 of the 6 patients with histologic grade 4 GvHD diagnosed in ileal biopsies died from complication of severe GI GvHD. 15 of 16 patients without grade 4 GI GvHD are alive at follow-up, and one patient died from respiratory failure from pulmonary GvHD.

CONCLUSIONS:
In our study cohort, grade 4 GI GvHD was best detected in terminal ileal biopsies, while colonic biopsies alone (including the sigmoid colon) would have detected only 50% of severe GI GvHD cases. While the histologic diagnosis of GI GvHD with the proper clinical correlate typically prompts the clinicians to initiate steroid therapy, given the poor prognosis of histologic grade 4 GvHD in the terminal ileum, the detection of this finding may also serve to inform the clinicians that escalation or modification of treatment may need to be considered.

~~JENNY CHU~~ - CANCELLED

POPULATION-BASED SCREENING FOR BRAF V600E IN METASTATIC COLORECTAL CANCER (MCRC) REVEALS TRUE PROGNOSIS

SUPERVISOR: JONATHAN LOREE

AUTHOR(S): Jenny E. Chu[1], Benny Johnson[2], Van K. Morris II[3], Kanwal Pratap Sing Raghav[3], Lucas Swanson[4], Howard John Lim[5], Daniel John Renouf[5], Sharlene Gill[5], Robert Wolber[6], Aly Karsan[1,5], David F. Schaeffer[7], Scott Kopetz[3], Jonathan M. Loree[5]

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BACKGROUND/OBJECTIVES:
BRAFV600E (BRAF) mutations portend poor prognosis in metastatic colorectal carcinoma (mCRC) and patients may die before ascertainment. Since 2014, Vancouver Coastal Health (VCH) has performed reflex hereditary screening of CRCs with BRAF and mismatch repair (MMR) immunohistochemistry (IHC). We evaluated this BRAF mutated population-based cohort ($BRAF_{POP}$) to establish the true prognosis of BRAF mutations in mCRC.

METHODS:
We reviewed all mCRC patients from VCH between 4/2014 and 5/2018 for BRAF by IHC (VE1 antibody). Overall survival (OS) from stage IV diagnosis was compared to mCRCs with next generation sequencing (NGS) determined BRAF mutations ($BRAF_{NGS}$) from BC Cancer and MD Anderson. $BRAF_{NGS}$ OS did not differ by center (p=0.77). All statistical tests were two-tailed.

RESULTS:
$BRAF_{POP}$ (n=64) and $BRAF_{NGS}$ (n=255) had similar baseline characteristics of gender (56% female; 51 % female, P=0.42) and tumour sidedness (67% right sided; 66% right sided, P=0.97). $BRAF_{POP}$ patients had an older median age at diagnosis (72 vs 61 years, P<0.0001); were less likely to be metastatic at the time of diagnosis (63% vs 78%, P=0.0083); and were less likely to have mucinous or signet ring cell histology (14% vs 27%; P=0.031). $BRAF_{POP}$ patients had worse OS than $BRAF_{NGS}$ patients (6.0 vs 20.7 months, HR 2.5, 95% CI 1.6 – 3.9, P<0.0001). Median OS for all BRAF mutated patients was 17.9 months. Both groups had worse OS than wild type patients (39.8 months, P<0.0001). 52 (81%) of $BRAF_{POP}$ patients were referred to oncology, 40 (63%) received chemotherapy, and 12 (19%) had NGS BRAF testing. BRAFPOP patients who had NGS testing showing BRAF mutations had OS comparable to other $BRAF_{NGS}$ patients (P=0.89) and better OS than $BRAF_{POP}$ patients that never had NGS testing (HR 0.37, 95% CI 0.18-0.76, P=0.030).

Patients with BRAF mutations and MMR deficiency (dMMR) (n=40) had worse OS than patients with proficient MMR (pMMR, n=202) (HR 1.6, 95% CI 1.0-2.5, P=0.011). This was driven by $BRAF_{POP}$ dMMR patients (HR 1.9, 95% CI 0.9-4.0, P=0.036) as no difference was seen by MMR in $BRAF_{NGS}$ patients (HR 1.3, 95% CI 0.8-2.2, P=0.30).

CONCLUSIONS:
Current estimates of prognosis for mCRC with BRAFV600E mutation likely underestimate its impact due to referral bias for NGS testing. BRAF mutants with dMMR are associated with worse prognosis than pMMR. This appears driven by $BRAF_{POP}$ patients.

MICHAEL STEEL

CYTOHISTOLOGICAL DIAGNOSIS OF PANCREATIC SEROUS CYSTADENOMA: A MULTIMODAL APPROACH

SUPERVISOR: DAVID F. SCHAEFFER

AUTHOR(S): Michael Steel[1], Samarth Rao[2], Julie Ho[3], Fergal Donnellan[2,4*], Hui-Min Yang[1*], David F. Schaeffer[1,4*]

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BACKGROUND/OBJECTIVES:
Serous cystadenomas (SCA) are benign pancreatic cystic neoplasms (PCN) that, despite multiple investigational approaches, present diagnostic challenge. Notwithstanding the promise of molecular diagnostics, these tests have limited accessibility in day-to-day surgical pathology practice. Recent evidence suggests that alpha-inhibin immunohistochemistry (IHC) is a helpful adjunct in the biopsy diagnosis of lesions clinicopathologically suspicious for SCA.

METHODS:
We retrospectively reviewed 22 fine needle aspirates/biopsies from 14 patients (mean age 65 years, 47-83 years) with pancreatic multicystic lesions radiologically suspicious for SCA (6 body, 2 head, 4 tail, 1 neck, 1 uncinate; cyst size: mean 3.7 cm, 2.0-7.6 cm) as well as ten pancreatic resection specimens with morphological confirmation of SCA; alpha-inhibin IHC was performed on all cell blocks, biopsy slides, and representative resection specimen slides. Where available, cyst fluid was also analyzed for VEGF-A and CEA levels.

RESULTS:
59% (13/22) of fine needle aspirate/biopsy specimens contained epithelial cells strongly positive for alpha-inhibin. Selecting for specimens that exhibited microscopically distinct strips of cuboidal epithelium, the alpha-inhibin strong positivity rate increased to 73% (8/11), compared to 45% (5/11), in those that did not. VEGF-A values were supportive of false-negative inhibin IHC in 3 cases, and true-negative inhibin IHC in 1 case. We additionally observed an 80% alpha-inhibin IHC sensitivity in those with resection confirmed SCA.

CONCLUSIONS:
This study postulates a multimodal diagnostic algorithm to confirm pancreatic SCA which may help to decrease unnecessary follow-up endoscopy or surgical resection, and would decrease the cost and morbidity in patients with this otherwise benign condition.

AUSTIN TAYLOR

PROHORMONE CONVERTASE 1/3 DEFICIENCY IN BETA CELLS INCREASES DIABETES SUSCEPTIBILITY IN MICE

SUPERVISOR: BRUCE VERCHERE

AUTHOR(S): Austin J. Taylor[1,2], Yi-Chun Chen[1,3], C. Bruce Verchere[1,2,3]

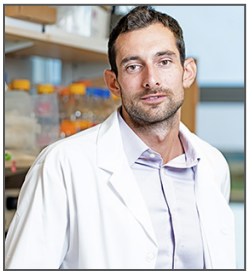
AFFILIATION(S): [1]BC Children's Hospital Research Institute; [2]Department of Pathology & Laboratory Medicine, UBC; [3]Department of Surgery, UBC

BACKGROUND/OBJECTIVES:
An estimated 11 million Canadians live with diabetes or prediabetes. Although type 1 and type 2 diabetes have different pathologies, both diseases include a relative insufficiency of insulin. Insulin is synthesized in pancreatic beta cells from its precursor proinsulin, which is cleaved by prohormone convertases 1/3 (PC1/3) and 2 (PC2) to generate mature insulin. In patients with diabetes, proinsulin processing to mature insulin is impaired, and elevated proinsulin:insulin ratios are observed in the circulation of patients with type 1 or 2 diabetes. It is unknown whether the beta-cell prohormone processing impairments observed in diabetes contribute to or are secondary consequences of beta-cell failure. Given the key role of PC1/3 in insulin synthesis, we hypothesized that beta-cell PC1/3 deficiency would result in beta-cell dysfunction and glucose intolerance.

METHODS:
The cre-lox recombination system was used to generate a mouse model of beta-cell specific PC1/3 deletion. Male and female $PC1/3^{flox/flox}$ $Ins1^{cre/+}$ (PC1/3 beta-KO), $PC1/3^{flox/+}$ $Ins1^{cre/+}$, $PC1/3^{+/+}$ $Ins1^{cre/+}$, and $PC1/3^{flox/flox}$ $Ins1^{+/+}$ mice were fed either a standard chow or high fat diet and monitored for changes in body weight, fasting glycemia. PC1/3 deletion in beta cells was confirmed by western blot and immunohistochemistry. Glucose tolerance tests were administered at 12 and 26 weeks of age, and fasting plasma proinsulin was analyzed by ELISA at 10 and 24 weeks of age. Pancreatic beta-cell area was analyzed by immunohistochemistry at 30 weeks of age. Mass spectrometry and transmission electron microscopy of islets isolated from 7-week-old mice were used to assess proinsulin processing and insulin granule morphology.

RESULTS:
Beta-cell PC1/3 deletion increased fasting plasma proinsulin in both male ($PC1/3^{flox/flox}$ $Ins1^{+/cre}$ vs $PC1/3^{+/+}$ $Ins1^{+/cre}$: 2931±942 vs 14±3 pM, $p < 0.0001$) and female mice, but did not influence fasting glycemia or body weight up to 30 weeks of age. PC1/3 beta-KO mice displayed a trend toward increased pancreatic beta-cell area ($PC1/3^{flox/flox}$ $Ins1^{+/cre}$ vs $PC1/3^{+/+}$ $Ins1^{+/cre}$:0.92% vs 0.63%, $p = 0.11$). PC1/3 beta-KO islets lacked mature insulin granules and by mass spectrometry were found to have elevated proinsulin peptides and decreased mature insulin peptides. There was no difference in glucose tolerance at 12 weeks of age and a trend towards glucose intolerance at 26 weeks of age in PC1/3 beta-KO male mice. Some male PC1/3 beta-KO mice developed diabetes after 20 weeks of high fat diet duration (3/5 PC1/3 beta-KO vs 0/4 $PC1/3^{+/+}$ $Ins1^{cre/+}$ mice) while female PC1/3 beta-KO mice fed high fat diet did not develop diabetes.

CONCLUSIONS:
Despite severe impairments in insulin synthesis, PC1/3 deficient beta cells can compensate remarkably well to regulate glycemia. In states of increased beta-cell secretory demand (insulin resistance associated with aging or high fat diet), PC1/3 deficiency in beta cells results in glucose intolerance and diabetes. Our data suggest that impaired PC1/3-mediated prohormone processing in beta cells increases diabetes susceptibility.



ALBERTO DELAIDELLI

A LINK BETWEEN SMALL NON CODING RNAS AND MRNA TRANSLATION ELONGATION: THE LET7-EEF2K AXIS IN PEDIATRIC TUMOR ADAPTATION TO NUTRIENT DEPRIVATION

SUPERVISOR: **POUL SORENSEN**

AUTHOR(S): **Alberto Delaidelli[1,2], Gian Luca Negri[1], Simran Sidhu[1], Brian Cho[1], Gabriel Leprivier[3], Volker Hovestadt[4], Stefan Pfister[5], Michael Taylor[6], Marcel Kool[5], and Poul H Sorensen[1,2]**

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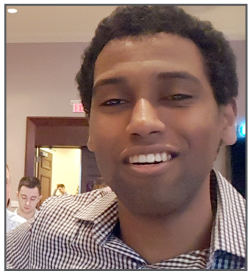
BACKGROUND/OBJECTIVES:
Every day, 43 children in North America are diagnosed with cancer, the number one cause of death by disease for children in the Western world. Medulloblastoma (MB) and neuroblastoma (NB) are the most common pediatric solid cancers and the leading cause of childhood related cancer deaths. Genetic amplifications of the *MYC* and *MYCN* oncogenes are the strongest independent predictor of adverse outcome in *MB* and *NB*, underscoring a dire need for novel and more effective therapeutic approaches. Tumors are continually exposed to acute changes in the microenvironment, including limited nutrient availability. The let-7 family of small non-coding RNAs (miRNAs) is known to inhibit tumor progression and regulate metabolism by targeting and degrading several cellular mRNAs, including *MYC* and *MYCN*. Indeed, let-7 miRNAs are frequently repressed in several cancer types, including in *MYC-driven MB* and *NB*. We previously reported that the mRNA translation elongation regulator eukaryotic Elongation Factor-2 Kinase (eEF2K) is a pivotal mediator of cancer cell adaptation to nutrient deprivation. Our preliminary data indicate that the eEF2K 3' untranslated region (UTR) harbors a potential binding site for let-7 miRNAs. In addition, eEF2K mRNA and let-7 miRNA expressions negatively correlate in MB and NB, suggesting a potential regulation of the former by the latter. We therefore hypothesized that let-7 down-regulation induces eEF2K expression, thereby supporting MYC-driven MB and NB adaptation to nutrient deprivation and tumor progression.

METHODS:
Effects of eEF2K pharmacological and genetic inhibition on MB and NB cell death were evaluated *In vitro* by flow cytometry, while cellular mRNA translation rates were studied by polysome fractionation and Click chemistry. The ability of let-7 to degrade eEF2K mRNA was assessed by let-7 miRNAs transfection into MB cells, followed by RT-PCR and Western Blotting for eEF2K. Binding of let-7 to the eEF2K 3'UTR was validated by luciferase reporter assay. Finally, NB xenograft mouse models were used to confirm *In vitro* observations.

RESULTS:
Inhibition of eEF2K significantly reduces survival of *MYC/MYCN-amplified NB* and *MB* cell lines under nutrient deprivation, hindering their mRNA translation rates. Let-7 miRNAs transfection decreases eEF2K mRNA and protein levels (by ~40-50%). Down-regulation of luciferase activity by let-7 miRNAs is impaired upon mutation of the let-7 binding site on the eEF2K 3'UTR. Knockdown of eEF2K determines a twofold growth decrease of *MYCN-amplified NB* xenografts when mice are kept under calorie restriction diets.

CONCLUSIONS:
Let-7 miRNAs degrade the eEF2K mRNA by binding to its 3'UTR, indicating that let-7 repression in *MYC-driven NB* and *MB* is partially responsible for increased eEF2K levels. Moreover, the let-7-eEF2K axis constitutes a critical mechanism for MYC-driven *MB* and *NB* adaptation to acute metabolic stress, representing a promising therapeutic target. Future therapeutic studies will aim to combine eEF2K inhibition with caloric restriction mimetic drugs, as eEF2K activity appears critical under metabolic stress conditions.



YASIR MOHAMUD

ENTEROVIRUS INFECTION INITIATES NON-CANONICAL AUTOPHAGY TO BYPASS LYSOSOMAL CLEARANCE AND FACILITATE VIRAL PATHOGENESIS

SUPERVISOR: HONGLIN LUO

AUTHOR(S): Yasir Mohamud, Junyan Shi, Hui Tang, Yuan Chao Xue, Huitao Liu, Honglin Luo

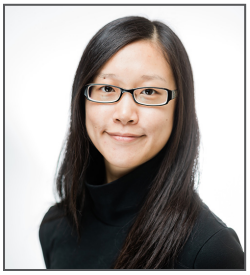
AFFILIATION(S): [1]BC Children's Hospital Research Institute; [2]Department of Pathology & Laboratory Medicine, UBC; [3]Department of Surgery, UBC

BACKGROUND/OBJECTIVES:
Enteroviruses are a genus of medically important human pathogens that cause a broad spectrum of pathology. The pathologies triggered by enteroviruses can range from flu-like symptoms to major tissue damage within the central nervous, endocrine, and cardiovascular systems. As intracellular parasites, enteroviruses must hijack the host cellular machinery to propagate. One important example is the autophagy pathway that is responsible for the proper recycling and clearance of cellular waste including misfolded proteins, damaged organelles, and invading pathogens. Previously, our group has identified the cellular autophagy machinery to be usurped by enteroviruses in order to facilitate viral replication, however the precise mechanisms remain unclear. Our preliminary findings have shown that viral proteases target essential components of the canonical autophagy pathway for degradation. Therefore, we hypothesize that enterovirus uses a non-canonical autophagy pathway to promote viral pathogenesis.

METHODS:
To investigate the mechanism by which enterovirus hijacks the autophagy pathway, we used a methodical approach to silence essential components of the canonical autophagy pathway. The canonical pathway is organized into 4 major complexes. Chemical inhibitors, RNA interference as well as the CRISPR-Cas9 system were used to silence and/or inactivate components of the canonical autophagy pathway including complex 1 (FIP200, ATG13, ULK1/2), complex 2 (BECN1, VPS34), complex 3 (WIPI2, ATG9), and complex 4 (ATG3, ATG5, ATG16). Enterovirus induced autophagy was monitored by quantifying LC3-II protein levels as a marker of autophagosome formation. In vitro cleavage assays and site-directed mutagenesis were used to confirm targeting of autophagy proteins by viral proteases.

RESULTS:
We demonstrate that genetic silencing and/or inhibition of the canonical autophagy pathway disrupts its normal function in cells (i.e. starvation-induced autophagy is abolished in the absence of FIP200, ULK1/2, BECN1) . However, components of complex 1, 2, and 3 are dispensable for enterovirus induced autophagy (as measured by LC3-II formation). Furthermore, we discovered novel substrates of enteroviral protease 3C that are components of the canonical autophagy pathway (ULK1/2, UVRAG, WIPI2, ATG4A). Our results also confirm that components of complex 4 are required for viral-induced autophagy.

CONCLUSIONS:
Enterovirus hijacks the host autophagy process to disrupt its canonical recycling function and promote viral pathogenesis. It accomplishes this feat by targeting essential components of the canonical autophagy pathway for degradation. Our findings in this study provide a strong rationale of a potential therapeutic benefit in targeting the autophagy-virus interface.



ANGELA MO

LOSS OF FBXO11 FUNCTIONS DRIVES ACUTE MYELOID LEUKEMIA

SUPERVISOR: ALY KARSAN

AUTHOR(S): Angela Mo[1,2], Linda Chang[1,2], Gerben Duns[1,2], Rawa Ibrahim[1], Rod Docking[1], Patricia Umlandt[1], Jeremy Parker[1,2], Aly Karsan[1,2]

AFFILIATION(S): [1]Genome Sciences Centre, British Columbia Cancer Agency; [2]Department of Pathology and Laboratory Medicine, University of British Columbia

BACKGROUND/OBJECTIVES:
Acute myeloid leukemia (AML) is the most common adult leukemia (~80% of cases), and AML patients have a 5-year survival rate of <30%. However, there has been little advancement in therapeutic options for patients. We found frequent somatic mutations in ubiquitin proteasome system genes by exome and RNA-seq of 140 clinical AML samples, with recurrent inactivating mutations in FBXO11, which codes the substrate-recognizing component of the SKP1-CUL1-F-BOX (SCF) ubiquitin E3-ligase complex. 11.4% of patients had loss-of-function mutations in SCF complex genes. AML cells also had lower FBXO11 mRNA expression compared to normal hematopoietic stem/progenitor cells (HSPC) ($P<0.0001$). Preliminary data from a mouse marrow transplant model showed that knockdown of Fbxo11 by shRNA cooperated with AML1-ETO fusion protein expression to initiate AML. AML1-ETO/shFbox11 AMLs were serially transplantable, indicating the presence of leukemic stem cells (LSC). LSCs tend to be quiescent and thus more chemoresistant. Knockdown of FBXO11 by shRNA in normal human CD34+ HSPCs reduced differentiation in vitro while maintaining a stem/progenitor phenotype. We hypothesize that FBXO11 depletion in HSPC promotes LSC self-renewal and drives leukemogenesis through dysregulated expression of its ubiquitination targets.

METHODS:
We performed quantitative tandem mass spectrometric analysis of FBXO11 co-immunoprecipitating proteins in FBXO11 CRISPR/Cas9 knockout (KO) and control clones from the K562 myeloid cell line to identify FBXO11-regulated ubiqutination targets and processes.

RESULTS:
Several proteins implicated in leukemogenesis were identified as FBXO11 targets. The top target, ABCF1 (normalized protein enrichment score = 0.87, 1 is max), was recently shown to regulate METTL3 expression, which is required for maintaining leukemic state, and to enhance protein synthesis. We measured protein synthesis in the K562 CRISPR clones by using flow cytometry to detect incorporation of O-propargyl-puromycin into new peptides, and accordingly, found increased global protein synthesis in clones with FBXO11 loss. Ingeuinity Pathway Analysis of FBXO11 targets recurrently identified glutathione metabolism-related pathways, which are frequently dysregulated in AML cells expressing the CD34 stem-cell marker. Inhibition of glutathione redox reactions in K562 CRISPR clones by piperlongumine treatment resulted in increased sensitivity in clones with FBXO11 loss (reduced cell counts, $p<0.001$, increased apoptosis measured by Annexin V staining, $p = 0.024$).

CONCLUSIONS:
FBXO11 loss likely has pleiotropic effects that contribute to leukemogenesis. We identified novel candidate targets of FBXO11. With the commonality of SCF-FBXO11 perturbations in AML, this could lead to development of new and widely applicable therapeutic options. Excitingly, piperlongumine has been undergoing clinical trials for other cancers, and there has been budding interest in the role of METTL3 as a transcriptopmic epigenetic regulator in leukemogenesis. We will perform more validation work on the mass spectrometry results and repeat the above experiments in CD34+ human cord-blood-derived cells.

~~KEVIN GOWING~~ - CANCELLED

MUCINOUS CYSTIC NEOPLASM OF THE PANCREAS WITH CLASSIC HISTOLOGIC FINDINGS OF TYPE 1 AUTOIMMUNE PANCREATITIS IN A PATIENT WITHOUT IGG4-RELATED DISEASE

SUPERVISOR: HUI-MIN YANG

AUTHOR(S): Kevin Gowing[1], David F. Schaeffer[1], Hui-Min Yang[1]

AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia, Canada

BACKGROUND/OBJECTIVES:
Mucinous cystic neoplasm is a cyst forming epithelial neoplasm characterized by mucinous epithelial lining and subepithelial ovarian-like stroma. It occurs predominantly in middle aged females and is a precursor to invasive adenocarcinoma. IgG4-related pancreatitis is an autoimmune disease in the spectrum of IgG4-related systemic disease and can present as a pancreatic mass. There is no known association between the two entities in the currently available literature. Here, we present a case of mucinous cystic neoplasm of the pancreas associated with an IgG4 plasma cell rich inflammatory response with histologic features compatible with IgG4 pancreatitis.

METHODS:
A 41-year-old female was found to have a non-enhancing 1.1 cm cystic lesion in the body of her pancreas while undergoing magnetic resonance imaging performed for melanoma surveillance. At the time, a differential of mucinous cystadenoma, side branch IPMN, and pancreatic pseudocyst were raised. Interval enlargement and septation were noted on repeat MRI scans and the patient underwent a distal pancreatectomy.

RESULTS:
Gross examination of the pancreas showed a 4.3 cm pancreatic mass with a centrally-located 3.4 cm cystic lesion, confirmed to be a mucinous cystic neoplasm (MCN) on microscopic examination. The pericystic tissue showed extensive fibrosis with a prominent chronic inflammatory infiltrate rich in plasma cells. Foci of storiform fibrosis and multifocal prominent obliterative phlebitis/arteritis were also identified. An IgG4 immunohistochemical stain showed focally increased numbers of IgG4 plasma cells (75 in one hpf in the "hot spot" areas) and focal IgG4 to IgG ratio of approximately 80%. Of note, the findings of AIP type 1 appear to surround the mucinous cystic neoplasm.

CONCLUSIONS:
To our knowledge, this is the second reported case of pancreatic MCN associated with AIP-like changes in a patient without established IgG4-RD. Awareness of AIP-like changes around pancreatic neoplasms may be helpful in preventing misdiagnosis in needle biopsies and aspiration specimens.

FATEMEH DERAKHSHAN

IMMUNE CHECKPOINT INHIBITOR STAINING TO SEPARATE BENIGN FROM MALIGNANT MESOTHELIAL PROLIFERATIONS

SUPERVISOR: ANDREW CHURG

AUTHOR(S): Fatemeh Derakhshan[1], Diana Ionescu[2], Andrew Churg[1]

AFFILIATION(S): [1]Department of Pathology, Vancouver General Hospital and University of British Columbia; [2]Department of Pathology, British Columbia Cancer Agency Vancouver, BC, Canada

BACKGROUND/OBJECTIVES:
The differentiation of malignant mesothelioma (MM) from reactive mesothelial proliferations (RMP) can be very difficult. Expression of immune checkpoint inhibitors is upregulated in a variety of malignant tumors, but the usefulness of immune checkpoint inhibitors in separating MM from RMP is not known. Here we examined immunohistochemical expression of 3 immune checkpoint inhibitors: PDL-1, CD47, and IDO1 (indoleamine 2, 3-dioxygenase 1), using 41 malignant mesotheliomas and 20 RMPs.

METHODS:
Tumor cell staining was scored for diffuseness and intensity using a system with a maximum score of 12 and a cutoff score of 3 or more for a positive result.

RESULTS:
For CD47 all 17/17 evaluable cores scored less than 3 for the epithelioid component of RMP; however, the spindle cells of RMP scored ≥3 in 10/20 cases. Twelve of 26 epithelioid MM and 7/10 sarcomatoid MM scored ≥3. For PDL-1 both the epithelioid and spindle components of RMP scored less than 3 (score 0 in 19/20, score 1 in 1/20 in the epithelial compartment). In 4/27 epithelioid mesotheliomas and 9/12 sarcomatoid mesotheliomas PDL-1
stain scored ≥3. IDO1 showed very focal staining in 2/27 epithelioid mesotheliomas and 1/6 sarcomatoid mesotheliomas and no staining in any RMP.

CONCLUSIONS:
We conclude that immune checkpoint inhibitor immunohistochemical staining can be useful in separating MM from RMP. PDL-1 staining works well with sarcomatoid mesotheliomas and is specific but insensitive for epithelioid mesotheliomas. CD47 staining is accurate and moderately sensitive for epithelioid mesotheliomas; however, CD47 is probably unsuitable for sarcomatoid mesotheliomas. IDO1 staining is too insensitive for practical use.

JENNIFER PORS

MESONEPHRIC NEOPLASMS OF THE GYNECOLOGIC TRACT ARE AGGRESSIVE TUMORS ASSOCIATED WITH DISTANT METASTASIS AND POOR PROGNOSIS

SUPERVISOR: LIEN HOANG

AUTHOR(S): Jennifer Pors[1], Sheila Segura[2], Daniel J. Fix[2], Kay Park[2], Brooke Howitt[4], David Kolin[5], Blake Gilks[1,3], Lien Hoang[1,3]

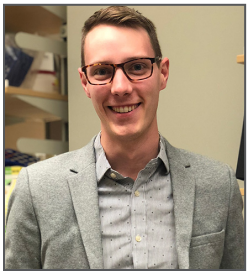
AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, Vancouver General Hospital and University of British Columbia, Vancouver, British Columbia, Canada; [2]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, New York, USA; [3]Genetic Pathology Evaluation Center, Vancouver, British Columbia, Canada; [4]Department of Pathology, Stanford University School of Medicine, Palo Alto, California, USA; [5]Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, USA

BACKGROUND/OBJECTIVES:
Mesonephric carcinomas are rare neoplasms of the female genital tract, which are thought to be derived from remnants of the male (Wolffian) system. Due to their rarity, little is known about the clinical behavior these rare tumors. Small studies have demonstrated mixed findings – some suggest these tumors are more indolent than Mullerian-derived gynecologic neoplasms, while others conclude that they are more aggressive. To date, no large studies have examined the clinical behaviour features of these lesions. In this multi-institutional study, we examined the clinicopathologic features of a large cohort of mesonephric neoplasms of the uterine cervix and uterine corpus. These clinical features will be compared with a matched group of patients with usual type endocervical carcinoma and endometrial endometrioid adenocarcinomas.

METHODS:
Eighty-two cases of mesonephric carcinoma were retrieved from the files of the Anatomical Pathology Departments at Vancouver General Hospital, Memorial Sloan Kettering, Stanford University Hospital, and Brigham and Women's Hospital. Additional cases were obtained from the BC Cancer Registry Database. The hematoxylin and eosin slides were reviewed by a subspecialty trained pathologist (LH, KP, DK, BH), and the cases were included if they showed the histologic and immunohistochemical features of mesonephric carcinomas. Tumors were subclassified into mesonephric carcinomas of the cervix (n = 34) and mesonephric-like carcinomas of the uterus (n = 48). The following features were recorded: age at diagnosis, presenting symptom, biopsy result and date, tumor size, FIGO stage, type and date of surgery, radiation or chemotherapy received, the presence or absence of recurrence, and the status at last follow-up.

RESULTS:
The mean age at diagnosis was 56 and the most common presenting symptom was vaginal bleeding. Mesonephric carcinoma was diagnosed on biopsy in only 24% of cases (15 of 63). Cervical mesonephric carcinoma was detected on PAP test in 13% of cases (4 of 30). At presentation, 57% (17 of 30) of mesonephric carcinomas, and 63% (29 of 46) of mesonephric-like carcinomas presented at advanced stage (FIGO stage II-IV) ($p>.05$). 45% of cases (13 of 29) of mesonephric carcinoma, and 65% of cases (30 of 46) of mesonephric-like carcinoma were associated with recurrences ($p>.05$). When these tumors recurred, the most common site was the lungs (54% or 7 of 13 cervical; 52% or 16 of 30 uterine). Follow-up for all mesonephric neoplasms ranged from 8 days to 123 months; 43% (32 of 75) had no evidence of disease, 32% (24 of 75) were alive with disease, and 25% (19 of 75) died of disease.

CONCLUSIONS:
In this multi-institutional study, we examined the clinicopathologic features of 34 cervical mesonephric and 48 uterine mesonephric-like carcinomas. Our results demonstrate that mesonephric neoplasms are clinically aggressive and difficult to diagnose at biopsy. The majority of patients with mesonephric neoplasms presented at advanced stage, and developed distant metastasis, most commonly to the lungs.



BASILE TESSIER-CLOUTIER

PROTEOMIC ANALYSIS OF TRANSITIONAL-LIKE CARCINOMA VARIANT OF TUBO-OVARIAN HIGH-GRADE SEROUS CARCINOMA

SUPERVISOR: **DAVID HUNTSMAN**

AUTHOR(S): Basile Tessier-Cloutier[1,3], Dawn R Cochrane[2], Anthony N Karnezis[2,4], Shane Colborne[12], Jamie Magrill[2], Aline Talhouk[2], Jonathan Zhang[2], Samuel Leung[2], Christopher S. Hughes[12], Anna Piskorz[5], Angela S. Cheng[6], Kendall Greening[2], Andreas du Bois[7], Jacobus Pfisterer[8], Robert A. Soslow[9], Stefan Kommoss[10], James D. Brenton[5], Gregg B Morin[12,13], C. Blake Gilks[3], David G. Huntsman[2] and Friedrich Kommoss[11]

AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; [2]BC Cancer, Department of Molecular Oncology, Vancouver, BC, Canada; [3]Department of Anatomical Pathology, Vancouver General Hospital, Vancouver, BC, Canada; [4]Department of Pathology and Laboratory Medicine, UC Davis Medical Center, Sacramento, California, United States; [5]Cancer Research UK Cambridge Research Institute, Li Ka Shing Centre, Cambridge, UK; [6]Genetic Pathology Evaluation Centre, University of British Columbia, Vancouver, Canada; [7]Kliniken Essen Mitte, Essen, Germany; [8]Gynecologic Oncology Center, Kiel, Germany; [9]Memorial Sloan Kettering Cancer Center, New York, NY, United States; [10]Tübingen University Hospital, Tübingen, Germany; [11]Institute of Pathology, Medizin Campus Bodensee, Friedrichshafen, Germany; [12]Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, BC, Canada; [13]Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada

BACKGROUND/OBJECTIVES:
The current WHO classification does not separate transitional cell-like carcinoma of the ovary (TCC) from conventional tubo-ovarian high-grade serous carcinoma (HGSC), although some evidence suggests a better prognosis for patients with TCC. The immunohistochemical (IHC) markers applied to date do not distinguish between TCC and HGSC. Therefore, we sought to compare the proteomic profiles of conventional HGSC and TCC to identify proteins enriched in TCC. Prognostic biomarkers in HGSC have proven elusive and our aim was to identify biomarkers of TCC variant as a way of reliably and reproducibly identifying patients with a favorable prognosis and better response to chemotherapy compared to those with conventional HGSC.

METHODS:
Quantitative global proteome analysis was performed on archival material of 12 cases of TCC and 16 cases of HGSC using SP3-CTP, a recently described protocol for full proteome analysis from formalin-fixed paraffin-embedded tissue. From the proteomic analysis, the potential TCC biomarkers were tested by IHC on tissue microarrays containing 88 samples of TCC and 237 of HGSC from Canada, Germany and the United States.

RESULTS:
We identified 323 proteins that were significantly enriched in TCC over HGSC. Unsupervised co-clustering precisely separated TCC from HGSC based on protein expression. Pathway analysis showed that proteins associated with cell death, necrosis and apoptosis were highly expressed in TCCs, while DNA homologous recombination, cell mitosis, proliferation and survival and cell cycle progression pathways had reduced expression. In agreement with the proteomic analysis, IHC expression for those proteins was stronger in TCC compared to HGSC ($p<0.0001$).

CONCLUSIONS:
Using global proteomic analysis, we are able to separate TCC from conventional HGSC. Follow up studies will be necessary to confirm that these molecular and morphologic differences are clinically significant.

SAM AU

BLADDER CANCERS POST-BRACHYTHERAPY SHOW VARIANT MORPHOLOGIES AND MOLECULAR SUBTYPES

SUPERVISOR: PEYMAN TAVASSOLI

AUTHOR(S): Sam Au[1], Mira Keyes[2], Peter Black[3], Carlos F. Villamil[4], Isabel Serrano[2], Peyman Tavassoli[1]

AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; [2]Department of Radiation Oncology, BC Cancer Agency, Vancouver, BC, Canada [3]Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; [4]Department of Pathology, BC Cancer Agency, Vancouver, BC, Canada

BACKGROUND/OBJECTIVES:
The long term increased risk of secondary malignancy is a well-documented late effect of radiation therapy. However, the incidence, time interval, anatomic site and histopathology are not well studied. As our knowledge of molecular subtyping of urothelial carcinoma (UC) has advanced, we sought to characterize the secondary malignancies in a cohort of patients from 1998-2016.

METHODS:
Among a total of >6000 patients who have undergone brachytherapy, a cohort of 72 patients have been diagnosed with secondary bladder cancer. Slides were reviewed, and site and pathologic features were recorded. Individual slides were stained for GATA3 and CK5/6 and classified as luminal and basal subtypes.

RESULTS:
The average time interval between brachytherapy and developing cancer was 6.3 years (range 1-17). The most common sites of involvement were the bladder neck (26%), trigone (25%) and lateral wall (24%). 70% (51/72) of cases in this cohort were high-grade, and 25% (18/72) were muscle-invasive. 57% (41/72) of cases were non-invasive. 19% of cases showed variant morphology, including small cell carcinoma (n=3), sarcomatoid carcinoma (n=1), squamous cell carcinoma (n=2), UC with divergent differentiation (n=6), nested variant (n=1) and adenocarcinoma arising from the prostatic urethra (n=1), Molecular subtyping by immunohistochemistry demonstrated that 72% of cases were luminal. All of the papillary tumors and all of the non-invasive cases expressed luminal immunophenotype. The basal subtype cases were all high-grade and invasive, whereas 60% of the luminal cases were high-grade and 29% were invasive. Among the variants, squamous cell carcinoma and UC with squamous differentiation were classified as basal subtype. Nested variant and UC with glandular differentiation were classified as luminal subtype. Small cell carcinoma and sarcomatoid carcinoma were negative for both markers.

CONCLUSIONS:
Bladder neck and trigone were among the common sites of involvement, with an average time interval between brachytherapy and developing bladder cancer of 6.3 years. The majority of bladder cancers post-brachytherapy in this cohort were of high grade and low stage at diagnosis, most of them demonstrating luminal immunophenotype. A significant number of variant morphologies are seen, each demonstrating a specific immunophenotype.

LISA BORRETTA

LOSS OF CD34 STAINING IN DISORDERS OF COLLAGEN ALTERATION OTHER THAN MORPHEA

SUPERVISOR: RICHARD CRAWFORD

AUTHOR(S): Lisa Borretta[1] and Richard Crawford[1]

AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada

BACKGROUND/OBJECTIVES:
It is well known that the sclerotic collagen in morphea is characterized by the loss of CD34+ dendritic stromal cells. To date, the status of these CD34+ cells has not been investigated in other disorders of collagen alteration. Our aim was to determine the distribution of CD34+ dendritic stromal cells in three other common disorders of collagen degeneration: lichen sclerosus (LS), granuloma annulare (GA), and necrobiosis lipoidica (NLD).

METHODS:
Skin lesions from 5 patients with LS, 5 patients with GA and 3 patients with NLD were evaluated for their distribution of CD34+ dendritic stromal cells. We assessed unaffected areas of the dermis in each biopsy as an internal negative control.

RESULTS:
CD34 stromal staining was absent in all areas of affected collagen that were detected on hematoxylin and eosin (H&E) staining in all cases of LS and GA. In our cases of NLD, there were areas that appeared unaffected on H&E that were found to have complete loss of CD34. Additionally, there were areas that appeared altered on H&E that had a reduction, but not a complete loss, of CD34 stromal staining.

CONCLUSIONS:
CD34 staining is lost not only in morphea, but also in other disorders of collagen degeneration, and as such may serve as a useful diagnostic adjunct in these conditions as well, specifically in early evolving disease or non-representative biopsies. CD34 staining may be particularly useful for the early detection of NLD, as some areas that were not detectably abnormal on H&E were found to have complete loss of CD34 staining. As both morphea and LS result in zones of complete loss of CD34+ stromal cells, CD34 staining is not useful in distinguishing these two conditions.

JULIA NASO

INTRATUMORAL HETEROGENEITY IN PD-L1 IMMUNOREACTIVITY IS ASSOCIATED WITH VARIATION IN NON-SMALL CELL LUNG CARCINOMA HISTOTYPE

SUPERVISOR: **PEYMAN TAVASSOLI**

AUTHOR(S): Julia R. Naso[1], Kate Zhu[2], Diana N. Ionescu[2], Gang Wang[2] and Chen Zhou[2]

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BACKGROUND/OBJECTIVES:
Accurate assessment of PD-L1 levels in non-small cell lung carcinoma (NSCLC) samples is complicated by intratumoral heterogeneity. We aimed to (i) establish whether intratumoral PD-L1 variation is associated with differences in local histotype, (ii) identify histotypes associated with a risk of under- or over-scoring PD-L1 levels in a tumor, and (iii) estimate the frequency of clinically significant discordance in PD-L1 levels between intratumoral histotype areas.

METHODS:
We reviewed 167 NSCLC resection specimens clinically tested for PD-L1 using the 22C3 pharmDx assay.

RESULTS:
Multiple histotypes were present in 41% of immunostained sections. Solid and squamous histotypes had significantly higher PD-L1 levels than other histotypes, both when grouping samples by predominant histotype and when histotype areas within a tumor were compared (p-values <0.02). Lepidic areas had significantly lower PD-L1 levels than other histotypes areas within the same tumor (p-values <0.002). Discordance between intratumoral histotype areas at a clinically relevant threshold (1% or 50% PD-L1 tumor proportion score) was present in 35% of cases with multiple histotype areas. The lepidic histotype was most frequently involved in discordance.

CONCLUSIONS:
Intratumoral heterogeneity in PD-L1 is associated with variation in histotype. Over-representation of solid or squamous areas may risk over-scoring PD-L1 levels in a tumor, whereas over-representing lepidic areas may risk under-scoring PD-L1 levels. The frequent discordance observed between histotype areas within the same tumor supports the notion that histotype representation should be considered when selecting tissue for PD-L1 testing.

LOÏC CALOREN

DRUG-INDUCED MTDNA CONTENT CHANGES CAN BE MODULATED BY MITOCHONDRIAL TELOMERASE IN A CELL CULTURE MODEL OF HIV ANTIRETROVIRAL EXPOSURE

SUPERVISOR: HÉLÈNE COTÉ

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BACKGROUND/OBJECTIVES:
Combination Antiretroviral Therapy (cART) is lifelong for people living with HIV. Certain cART drug classes such as nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) are known to have mitochondrial toxic effects. However, less is known about the mitochondrial effects of more recently developed drug classes such as integrase strand transfer inhibitor (InSTI). Mitochondrial toxicity could play a role in the accelerated aging experiemced by people living with HIV. Human telomerase reverse transcriptase (hTERT) translocates to the mitochondria in response to oxidative stress, where it protects mitochondrial DNA (mtDNA) but its activity may be inhibited by NRTIs. Our objective was to explore the effects of intermittent exposure to select cART regimens on mitochondrial DNA content, and determine whether telomerase modulates mitochondrial drug effects.

METHODS:
Three alternate lengthening of telomere (ALT) human fibroblast cell lines with different telomerase capabilities (no telomerase [-hTERT], wild type telomerase [+hTERT +MS], and mutated telomerase missing the mitochondrial signal so that it cannot translocate to the mitochondria [+hTERT -MS]) were exposed to 9 different cART regimens at pharmacological concentrations (1X Cmax) in 0.1% DMSO. Each treatment was administered in five cycles consisting of 3 days of exposure, followed by 4 days of recovery. At the end of the exposure and recovery phases for cycles 1, 3 and 5, cell counts and viability were determined, and mtDNA copy number per cell was assayed using multiplex monochrome qPCR, and normalized to that of the 0.1% DMSO control culture.

RESULTS:
Cells exposed to stavudine (d4T), a drug known for depleting mtDNA content, showed decreased mtDNA after each treatment and levels rebounded during the recovery phase. –hTERT cells failed to rebound while +hTERT –MS cells experienced the greatest mtDNA decline during exposure. In contrast, the InSTI dolutegravir (DTG) seemed to generally increase mtDNA content, and elicited some of the greatest changes in mtDNA content, particularly in –hTERT and +hTERT –MS cells. Overall, by the fifth cycle's exposure, mtDNA content was generally decreased relative to the first cycle, both immediately post-exposure and after the recovery period. This effect was most pronounced in +hTERT –MS and –hTERT cells, and was much attenuated in +hTERT cells which maintained relatively stable mtDNA content throughout the experiment.

CONCLUSIONS:
cART induced changes in mtDNA content are drug-dependent, occur with old and new drugs, and appear modulated by hTERT. Repeated cycles of exposure seem to exacerbate these effects while mitochondrial hTERT may confer protection against such mtDNA fluctuation. Further investigations and additional independent replicates are required to determine the consistency of these findings. Future work will examine whether cyclic exposure to cART, and the mtDNA fluctuations triggered by it, promoted the clonal expansion of mtDNA mutations.

HONOR CHEUNG

APOA-1 ATTENUATES ACUTE VESSEL-ASSOCIATED ASTROGLIOSIS IN MICE AFTER TRAUMATIC BRAIN INJURY

SUPERVISOR: CHERYL L. WELLINGTON

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BACKGROUND/OBJECTIVES:
ApoA-1 is the largest protein constituent of circulating high density lipoproteins (HDL), which have a well established role in maintaining peripheral vascular health. HDL is crucial for reverse cholesterol transport, a process in which excess cholesterol from blood vessels are reabsorbed and transported to the liver for further degradation, preventing atherosclerosis. Furthermore, ApoA-1 plays an anti-inflammatory role in inhibiting trans-endothelial migration of immune cells by lowering integrin production. Intriguingly, increasing evidence suggest that ApoA-1's vessel protective properties also provide resilience to vessels in the brain, as it reduces vessel associated plaque load in Alzheimer's Disease mice. As vascular injury is nearly universal after traumatic brain injury (TBI), this study was designed to test the hypothesis that ApoA-I deficiency would worsen acute vascular and inflammatory outcomes in mice after Closed-Head Impact Model of Engineered Rotational Acceleration (CHIMERA) TBI.

METHODS:
Male and Female ApoA-1 KO and WT mice were randomized to sham or single moderate TBI of 2.5J at 3-4 months of age. Animals were assessed for acute neurological deficits using the loss of righting reflex (LRR) immediately after injury, and Neurological Severity Score (NSS) tasks at 2hr or 2d post injury. To assess blood brain barrier damage, animals were injected with Evan's blue that circulated for 4 hours before sacrifice. All mice were harvested at 6hr or 2d post injury for histological and biochemical analysis.

RESULTS:
Injured ApoA-1 KO have higher NSS scores at 2d relative to WT-TBI. TBI resulted in increased astrocyte area and increased number of vessel-associated astrocytes in the entorhinal cortex and anterior cortex of ApoA-1 KO with significant genotype and Injury effects, but not genotype x In-jury interaction at 2d. Intriguingly, we observed an increase in %Area of CD31+ endothelial cells in injured ApoA1-KO animals relative to WT controls. TBI at 2.5J did not seem to produce extensive blood brain barrier damage assessed by Evan's Blue and IgG extravasation.

CONCLUSIONS:
Preliminary results of this on-going study support a vasoprotective role for apoA-I in attenuating acute TBI-induced astrogliosis. Further, the increase in endothelial % area may suggest a compensatory angiogenesis response in injured ApoA1-KO animals. Additional experiments are required to confirm these findings and assess chronic time points.

ZOE GIDDEN

DYSREGULATION OF GENE EXPRESSION AND IMMUNOPHENOTYPE IN A MOUSE MODEL OF PROGRESSIVE MULTIPLE SCLEROSIS BASED ON AN NR1H3 MUTATION LINKED TO DISEASE IN FAMILIES

SUPERVISOR: JACQUELINE QUANDT

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BACKGROUND/OBJECTIVES:
Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disorder that involves the degeneration of neurons as well as the lipid-rich myelin sheath that covers axons, which are ultimately lost. Disease-modifying therapies are not effective once disease progresses, which is characterised by increasing disability over time without remissions. Through genetic analysis of several families with MS, a mutation in the nuclear receptor (NR)1H3 gene was found to greatly increase the risk of developing progressive MS (Wang, 2016). NR1H3 codes for liver X receptor alpha (LXRA), a key contributor to lipid metabolism and homeostasis. LXRs help control the expression of CD5L, a protein that is implicated in both lipid homeostasis and immune regulation, as well as several other immune and myelin-related genes. We have developed a novel mouse model of progressive MS through incorporation of this human NR1H3 Arg415Gln mutation. We hypothesize NR1H3 mice will show altered gene expression compared to wild-type littermates and we predict that identifying these genes will help us to identify genes and processes that are linked to disease progression.

METHODS:
Gene expression levels were assessed using whole transcriptome analyses of spleens of 3 month old heterozygous and homozygous NR1H3 as well as wild type mice. An online tool (Zhang et al. 2014) was used to compare the relative expression of NR1H3 as well as the identified dysregulated immune genes in several glial as well as neuronal cell types in the mouse cerebral cortex. Levels of immune-related proteins were examined in sera from mutant and wildtype mice through immunoblotting and ELISA. The immune profiles of spleen cells from mice of each genotype were labelled with fluorescent antibodies and identified using flow cytometry.

RESULTS:
Whole transcriptome analyses of the spleen showed over 20 genes are dysregulated in homozygous NR1H3 mice compared to wild type. While these genes were associated with immunity, several are also expressed in microglia, astrocytes, neurons or oligodendrocyte progenitor cells and are altered through development or disease. Top dysregulated genes included CD5L and C6, where expression is reduced in the spleen of mice with one copy of the mutated NR1H3 gene and even further reduced in mice homozygous for the mutation. Similarly, CD5L protein was reduced in the sera of homozygous mice compared to wild type controls. Flow cytometric analysis of spleen populations showed that homozygous NR1H3 mice had increased granulocytes (p-value = 0.040) and decreased levels of F4/80 positive macrophages. Notably, macrophages also had far lower levels of the reparative or non-inflammatory “M2-associated” markers CD163 (p-value = <0.0001) and CD209 (p-value = <0.01).

CONCLUSIONS:
Initial findings in this novel mouse model suggest that the disruption of pathways associated with immunomodulation and repair is likely to contribute to worsened disease and disability. Ongoing studies will further address the impact of the NR1H3 mutation and may ultimately highlight novel areas for therapeutic intervention to limit progression and disability in MS.

DANIEL LI

IMPROVING THE WORKFLOW OF THE CLINICAL FLUORESCENT IN-SITU HYBRIDIZATION ASSAY – ONE COUNT AT A TIME

SUPERVISOR: STEPHEN YIP

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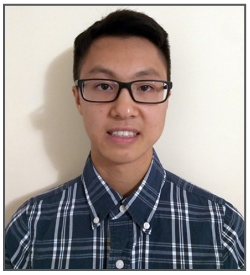
BACKGROUND/OBJECTIVES:
Fluorescent in situ hybridization (FISH) is a high-volume test in the clinical Cancer Genetics and Genomics laboratory and is the preferred technique to identify gene amplification, deletion, and structural rearrangement events in cancer specimens. FISH provides information essential for diagnostic, prognostic and therapeutics; however, it is beset by its low throughput workflow due to its heavy reliance on manual recording and calculating individual fluorescence signals.

Our project explores the implementation of an automated FISH workflow at CGL. The goals of this project are to increase the efficiency of the FISH assay, digitize the FISH probe database, and improve record-keeping for quality control.

METHODS:
Our automated FISH counter aims to replace the current workflow with an all-in-one Microsoft Excel solution and an integrated ergonomic one-handed keyboard. The Excel component consists of a worksheet template page and a digital database including all the information on typical signal patterns and cut-offs for actively used FISH probes at CGL. The new workflow allows the technologist to select a probe then automatically populates the probe information and formats itself into a printable form. Additionally, this new worksheet template will automatically alert the technologist when the probe counts are outside the laboratory's established quality metrics. This automated FISH counter will seek to improve record-keeping for quality control assessments by tracking the hybridization quality history of each.

CONCLUSIONS:
The automated FISH workflow uses the advantages of digital systems to increase the efficiency of the FISH assay. After more the 1800 lines of code, the automated FISH workflow is expected to enter clinical trials in the near future. We will continue to work closely with the FISH technologists and scientists to receive feedback and improve our design, and also to collect data on the new workflow's impact. We hope this project will reveal the streamlining potential of digitized workflows.



ALVIN LIU

IDENTIFICATION OF A NOVEL SMALL MOLECULE THAT EXPLOITS A VULNERABILITY TO REACTIVE OXYGEN SPECIES IN LUNG CANCER

SUPERVISOR: **WILLIAM LOCKWOOD**

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BACKGROUND/OBJECTIVES:
Lung cancer (LC) is the leading cause of cancer-related deaths worldwide, mainly due to the lack of effective drugs available. Through a screen of 189,290 small molecules, the compound LC Screen 3 (LCS3) was identified that selectively inhibits the growth of LC cells but not normal cells. Twenty-six LC cell lines were screened and all but two were found to be sensitive to LCS3 (IC50<5μM). To elucidate the mechanism of action of LCS3, we employed a global transcriptomic and proteomic approach.

METHODS:
49 potential binding targets of LCS3 were identified by thermal proteome profiling (TPP), which identifies protein-drug interactions through conferred protein thermal stability in drug bound states. Microarray, SILAC, and flow cytometry studies were conducted to determine the cellular responses to LCS3 and distinguish LCS3 effectors from binders. Cell proliferation assays on sensitive cell lines were performed with and without antioxidant challenge to ascertain the suspected cellular responses through observation of phenotypic rescue to LCS3-induced cytotoxicity.

RESULTS:
Transcriptome and proteome profiling by microarray and SILAC, respectively, suggest that LCS3 strongly induces reduction-oxidation (redox) imbalance. The top 4 predicted upstream transcription factors of LCS3-induced RNA expression changes are all key regulators in the response to oxidative stress (NRF2, MAFK, CEBPB and BACH1). In agreement, flow cytometry with oxidative stress sensor H2DCFDA detected reactive oxygen species (ROS) induction by LCS3 in sensitive cell lines but not in LCS3-resistant cell lines. Notably, the most resistant of the two insensitive LC cell lines, NCI-H1648, has biallelic functional loss of KEAP1, which negatively regulates NRF2-mediated cytoprotective gene expression. We confirmed NCI-H1648 has high basal expression of genes that support redox balance that are likely to confer the observed resistance to LCS3. The antioxidants N-acetylcysteine, GSH-MEE and Trolox partially rescued LCS3-induced cytotoxicity, which further implicates redox imbalance in the mechanism of LCS3-induced cell death. Of the 49 TPP candidates, 9 are enzymes involved in redox reactions including glutathione peroxidase 4 (GPX4), glutathione-disulfide reductase (GSR), thioredoxin reductase 1 (TXNRD1), peroxiredoxin 4 (PRDX4) and glutathione S-transferase omega 1 (GSTO1). We will further evaluate these 9 TPP hits through siRNA knockdown and in vitro activity assays to hone in on specific targets that may corroborate with our current model of the actions of LCS3.

CONCLUSIONS:
Through this work, we aim to use LCS3 as a tool compound to identify a novel cancer dependency that can be exploited for the benefit of LC patients with advanced tumors, for whom treatment is urgently needed.

SAMANTHA PAWER

CURRENT STATUS OF LABORATORY REFERENCE INTERVALS DURING PREGNANCY

SUPERVISOR: VILTE BARAKAUSKAS

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BACKGROUND/OBJECTIVES:
Profound changes in maternal physiology occur throughout pregnancy, which can lead to significantly altered levels of biomarkers in the blood relative to non-pregnant females. Accordingly, safe and accurate interpretation of laboratory test results in pregnant patients requires gestation-specific reference intervals (RI). Despite this, few laboratories establish or provide RIs appropriate for use during pregnancy. Our objective was to survey published literature for RI studies performed in healthy pregnant women, to identify gestation-specific RIs that may be appropriate for multicultural healthcare settings, and to characterize gaps to inform future pregnancy RI studies.

METHODS:
We searched PubMed and EMBASE databases, as well as the University of British Columbia Library Catalogue for papers from within the past 30 years that reported gestation-specific RIs. From the 80 included publications, study characteristics were compared with respect to sample size, study design, geographical location of reference groups, and partitioning used by the investigators. Pregnancy-specific RIs for chemistry, immunoassay, hematology, and trace element parameters were compiled and compared.

RESULTS:
More than half of the studies used cross-sectional designs, and most included time points before parturition. Only 8 of the studies were conducted in North America, 4 of which had sample sizes of ≥ 120 individuals. Over a third of studies were conducted in Asia, and relatively few were done with multicultural samples representative of North American populations. There is uneven representation of gestational time points, with a lack of studies reporting RIs during labour and delivery (N=4) or the post-partum period (N=15). Results from a variety of analytical platforms and vendors are represented in the literature, while several studies failed to adequately describe their analytical methods. Comparison of studies showed some agreement, and considerable variation among RIs. For example, TSH RIs varied between populations of different ethnicities, and by geographical location. As well, congruent gestational age-related trending was seen in studies of WBC RIs in Caucasian and Asian cohorts, but differed in African cohorts. On the other hand, studies consistently found pregnancy RIs were similar to non-pregnancy RIs for certain analytes (e.g. iron and platelets). Importantly, pregnancy RIs were different from non-pregnancy RIs for some analytes (e.g. lower albumin levels during gestation). For many tests, however, RIs varied considerably between studies, which made generalizing to other populations difficult.

CONCLUSIONS:
Our literature review demonstrates the difficulties laboratories and care providers have in using published RIs for local populations. We also identified gaps in our current knowledge, including the limited number of studies for pregnancy RIs, heterogeneity in RIs between populations or analytical methods, and missing information for some pregnancy time-points. These findings highlight a need for establishing RIs using large, ethnically diverse reference groups during pregnancy, labour, delivery, and the post-partum period.

CARRIE SMYTHIES

RECRUITMENT METHODS FOR PREGNANCY REFERENCE INTERVALS FOR SAFE MEDICINE (PRISM)

SUPERVISOR: VILTE BARAKAUSKAS

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BACKGROUND/OBJECTIVES:
Pregnancy creates a state of altered physiology for women, resulting in clinical ambiguity when interpreting whether laboratory test results reflect normal physiology or pathology in the labour process. The PRISM (Pregnancy Reference Intervals for Safe Medicine) study recruited healthy pregnant women, representative of the BC population, in order to establish reference intervals for laboratory tests around the time of labour and delivery. This study created, assessed, adjusted and optimized a methodology for the creation of laboratory reference intervals around the time of labour. It also constructed a system and infrastructure that can be utilized for future investigations of perinatal and neonatal values.

METHODS:
Women were pre-consented before the estimated due date or consented on site during early labour by study personnel or nurses. Eligibility was assessed using an intake questionnaire, chart review and a post-partum survey following hospital discharge.

Recruitment tracking occurred throughout the study via weekly and monthly reports of total recruitment numbers and actual eligibility updates. Ongoing adjustments were made, including increasing study personnel available to approach potential participants and supporting clinical staff in awareness and implementation of the study.

RESULTS:
Over 12 months, 408 women consented to participation. Blood was obtained from 91%; 201 samples prior to labour/C-section, 167 samples from labouring mothers. Up to 14% of samples experienced pre-analytical issues (hemolysis, underfilling, mislabelling). Of all consented women, 151 were excluded based on health history, delivery or post-partum complications. Information for 55 mothers remains missing/pending. The final cohort includes 120 pre-labour and 80 in-labour women. Average maternal age was 33.3 yrs (pre-labour group) and 31.8 yrs (in-labour group). Average gestational age was similar in both groups (39 weeks).

CONCLUSIONS:
PRISM has designed a methodology that focuses on efficiency of time and materials while maximizing recruitment of eligible participants. The methodology created by this study can be transferred to other reference interval creation, including implementation for other Gestational Age time points during pregnancy, post-partum assessments, or even into neonatology reference intervals.

PRAKRUTI UDAY

EFFECT OF POSTNATAL PRIMARY INFECTION(S) WITH HUMAN HERPESVIRUSES (HHV) ON PERIPHERAL BLOOD MONONUCLEAR CELL TELOMERE LENGTH, IN A UGANDAN COHORT OF HIV-EXPOSED UNINFECTED (HEU) AND HIV-UNEXPOSED UNINFECTED (HUU) CHILDREN

SUPERVISOR: HÉLÈNE CÔTÉ

AUTHOR(S): Prakruti Uday[1], Abhinav Ajaykumar[1], Izabelle Gadawski[1], Karen Simmons[2], Jessica Tuengel[2], Dong-Jun Zheng[2], Soren Gantt[2], Hélène Côté[1]

AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, UBC; [2]Department of Pediatrics, UBC

BACKGROUND/OBJECTIVES:
Chronic viral infections such as HIV, cytomegalovirus (CMV) and herpes simplex virus (HSV) have been associated with shorter telomere length (TL). HHV infections such as CMV, Epstein-Barr virus (EBV), HSV-1 & 2, and HHV-6A, 6B & 8 are lifelong. These can trigger immune activation which may in turn shorten lymphocyte TL, promote immuno-senescence, and alter immune responses later in life. Longitudinal peripheral blood mononuclear cell (PBMC) DNA specimens were obtained from a unique cohort of Ugandan HEU and HUU children who acquired multiple HHVs within the first 18m of life. Our objective was to characterize PBMC TL following the acquisition of multiple HHV infections.

METHODS:
Longitudinal PBMC DNA specimens were obtained for HEU and HUU infants, starting at ~2m of life and every 4m thereafter up to ~2y of age. PBMC TL was quantified using monochrome multiplex qPCR. Univariate analyses investigated the relationships between baseline (study entry), last visit, or the change in TL per month between first and last visit, and the following explanatory variables: sex, age, HIV-exposure status, breastfeeding, number of HHV infections and type of HHV infections. Factors important univariately were considered while developing multivariable models.

RESULTS:
Among 31 infants (16 HEU and 15 HUU) studied, 10 HEU/10 HUU acquired CMV, 13 HEU/3 HUU acquired EBV, 12 HEU/10 HUU acquired HHV-6 (all HSV-6B, except one HEU who acquired both HSV-6A and 6B), and 5 HEU/2 HUU acquired HSV (all but one HEU acquired HSV-1) postnatally. None acquired HHV-8. Baseline TL (median [range] age = 2 [2-4] months) was similar between HEU and HUU and showed no association with any factor investigated. At last visit for 13 HEU and 15 HUU, (12 [4-28] months), shorter TL was associated with HEU status ($p=0.005$) and not being breastfed currently ($p=0.002$), but not with number or type of HHV infections. Because HEU and breastfeeding status at last visit were highly collinear (0/13 HEU and 13/15 HUU were breastfed, $p<0.001$), these variables were included in the multivariable models in turn. In these two models, HEU infants ($p=0.005$) or infants who were not breastfed ($p=0.001$) both showed significantly shorter TL, after adjusting for age at last visit, baseline TL and number of HHV infections. Longitudinally, in a model that adjusted for baseline TL and number of HHV infections, infants with longer TL at baseline had a higher rate of TL loss per month ($p<0.001$). In two models, the latter was also independently associated with being HEU ($p=0.04$) or not breastfed ($p=0.006$).

CONCLUSIONS:
In our study, we did not detect any association between infant PBMC TL and the number or type of HHV infections they acquired. Instead, our data suggest that being HEU and/ or not being breastfed may affect the rate of TL attrition early in life. A larger sample of HEU and HUU children, breastfed and not would be required to tease apart the effects of HIV exposure and breastfeeding on TL dynamics.

DAVID YANG

DEVELOPMENT OF A TANDEM MASS SPECTROMETRY ASSAY FOR NEWBORN SCREENING FOR SICKLE CELL DISEASES

SUPERVISOR: **BOJANA RAKIC**

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BACKGROUND/OBJECTIVES:
Sickle cell diseases (SCD) are caused by genetic mutations altering the hemoglobin (Hb) peptide molecule. Mutations in the beta-globin chain produce the defective Hb S, causing significant morbidity and mortality during early childhood. Newborn screening (NBS) programs in Canada screen for hemoglobinopathies to ensure early treatment to prevent complications of SCD. The NBS laboratory in BC uses a High-Performance Liquid Chromatography (HPLC) based method on dried blood spot (DBS) cards to detect intact Hb molecules. However, this method also detects and reports roughly 200 carrier infants per year for sickle cell trait or benign Hb trait who do not require any medical intervention. More recently, a tandem mass spectrometry method using electrospray ionization (ESI-MS/MS) has been described, which allows detection of only the primary targets of SCD: Hb S/S, Hb S/C, and Hb S/beta-thalassemia (Moat et al., 2017). This project will work towards implementing this newer method for NBS hemoglobinopathy screening in BC to eliminate the detection of carriers and benign variants. The aims for this study: 1) To determine if an ESI-MS/MS based screening assay can detect clinically significant Hb peptides to distinguish between normal and abnormal samples. 2) To assess the stabilities of Hb peptides in DBS samples stored at room temperature over a two-week period.

METHODS:
A total of 135 anonymized residual DBS samples from BC Children's Hospital were analyzed. Disks (3.3 mm) from DBS samples were punched into 96-well plates and incubated for 4 hours at 37C in a trypsin digestion solution. The resulting extracts containing digested Hb peptides were analyzed on a Water's ESI-MS/MS instrument (Xevo-TQ) using a direct flow injection method. Transitions with good signal intensities for tryptic Hb peptides were qualitatively analyzed by calculating ratios between the variant Hb peptide to the wild-type Hb A peptide abundances. A reference range for a normal screening value was determined from 123 confirmed normal DBS samples. Eight abnormal DBS samples (Hb S/S, Hb A/S, Hb A/D, Hb A/C, and Hb A/E) were also analyzed to ensure good discrimination. To assess the stability of Hb peptides at room temperature, 4 DBS samples were punched on day 0 and roughly every 2 days up to 16 days (8 punches each). Intraassay coefficient of variation (CV) was calculated to evaluate the stability of associated peptides over time.

RESULTS:
Normal reference ranges expressed in mean (±2SD) for ratios of Hb F/Hb A, Hb S/Hb A, Hb D/Hb A, Hb C/Hb A, and Hb E/Hb A were determined. The method described here can distinguish between associated peptide ratios from normal and mutant Hb phenotypes. Assessing the stability, most ratios showed no significant decline in levels during the two-week period with intraassay CVs varying between 12.4% to 39.4%.

CONCLUSIONS:
The described method of ESI-MS/MS serves as preliminary data in hopes to translate this into a full validation for NBS hemoglobinopathy screening in BC. The stability results suggest that analysis of Hb peptides may provide a longer testing window for NBS compared to the current HPLC method.

FARHIA ABDULLAHI

ASSESSMENT OF MARKERS OF NEURODEGENERATION AND DEMYELINATION IN THE SERUM OF PATIENTS EXHIBITING DIFFERENT CLINICAL COURSES OF MULTIPLE SCLEROSIS AND THEIR ASSOCIATION WITH DISEASE PROGRESSION

SUPERVISOR: JACQUELINE QUANDT

AUTHOR(S): Farhia Abdullahi[1], Zoe Gidden[1], Pierre Becquart[1], Robert Carruthers[2], Anthony Traboulsee[2], Irene Vavasour[3,4], Cornelia Laule[1,3,4], Jacqueline Quandt[1]

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BACKGROUND/OBJECTIVES:
Multiple sclerosis MS is a neurodegenerative chronic inflammatory disorder that affects more than 100,000 Canadians and 2.5 million people worldwide. Symptoms include weakness, paralysis, visual disturbances, and fatigue as well as cognitive dysfunction and significantly impact on quality of life, particularly as the disease normally presents in early adulthood. Disease typically starts with relapses and remissions, yet more than half of patients ultimately progress with steady clinical worsening in the absence of any significant clinical recovery. A hallmark of the disease is disruption of the highly myelinated axons in the white matter linked to chronic inflammation, demyelination, and neurodegeneration with axonal loss. Myelin consists of primarily lipids but also proteins, and damage to or loss of either component can result in detection of these or immune reactivity (antibodies to these) in the circulation of MS patients. Several markers of myelin/axon loss/reactivity have been studied individually in MS. *The objective of this study is to utilize different assays to assess the level of myelin and axonal damage and reactivity in the serum of different MS patient subtypes, in comparison to healthy controls.*

METHODS:
We have recruited nearly 30 participants from each MS subtype: clinically isolated syndrome, relapse remitting, primary progressive, and secondary progressive MS as well as 40 age and sex matched healthy controls for comparative analysis. Serum samples will be assayed using enzyme-linked immunosorbent assay (ELISA) and the ultrasensitive single molecule array assay (SIMOA). Specifically, we are examining: antibodies to myelin lipids (gangliosides and phospholipids), antibodies to myelin proteins (myelin associated glycoprotein MAG, myelin oligodendrocyte glycoprotein MOG, myelin basic protein MBP, and proteolipid protein PLP), as well as neurofilament proteins (neurofilament heavy chain Nf-H, neurofilament medium chain Nf-M, and neurofilament light chain Nf-L).

RESULTS:
We expect that people with progressive MS subtypes have a higher level of lipid and protein reactivity, as well as greater evidence of axonal loss and free myelin components detectable in their serum, compared to healthy controls. We will link these findings of varied proportions of lipid versus protein loss or immunoreactivity to the clinical course of MS.

CONCLUSIONS:
The characterization of clinically significant markers linked to MS progression and disability will provide greater insight into the mechanism of progression. They may also serve as important and easily accessible biomarkers to identify patients at higher risk of progression. Finally, serum measurable biomarkers may assist in monitoring therapeutic efficacy as we work to develop therapies to limit disability and improve the quality of life for those living with MS.

ABHINAV AJAYKUMAR

DOLUTEGRAVIR-CONTAINING HIV COMBINATION ANTIRETROVIRAL THERAPY REDUCES CELL PROLIFERATION AND INCREASES MITOCHONDRIAL TOXICITIES IN A TELOMERASE REVERSE TRANSCRIPTASE-EXPRESSING CELL LINE

SUPERVISOR: HÉLÈNE CÔTÉ

AUTHOR(S): Abhinav Ajaykumar[1,2], Anthony Y.Y. Hsieh[1,2], Loic Caloren[1], Prakruti Uday[1], Rutuja Pattanshetti[1], Connor A.H. Thompson[3], Judy M.Y. Wong[3], Hélène C.F. Côté[1,2]

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BACKGROUND/OBJECTIVES:
The HIV integrase inhibitors (InSTIs) dolutegravir (DTG) and raltegravir (RAL) are increasingly used in first-line combination antiretroviral therapy (cART), but little is known regarding the short- and long-term toxicities of these drugs. Older nucleoside reverse transcriptase inhibitors (NRTIs, a class of HIV cART) are known to inhibit mitochondrial pol γ and affect mitochondrial function while protease inhibitors (PIs) can increase reactive oxygen species (ROS). Human telomerase reverse transcriptase (hTERT) can translocate to mitochondria, where it protects the organelle and mtDNA from ROS-induced damage. Our objective was to evaluate the potential cellular and mitochondrial toxicities of InSTI-containing cART, and determine whether hTERT modulates adverse drug effects.

METHODS:
A transformed human fibroblast cell line that utilizes the alternate lengthening of telomeres (ALT) mechanism to maintain telomeres was transduced to express either WT hTERT or mutant hTERT that cannot translocate to mitochondria. The three isogenic ALT cell lines were treated with various cART (1X C_{max}) for nine days, and then allowed to recover in media for six days. Cell viability and proliferation were determined every three days, while changes in mitochondrial mass, membrane potential, ROS and cellular apoptosis were quantified via flow cytometry on days 9 and 15.

RESULTS:
At day 9, ALT cells exposed to TDF+FTC+DTG, TAF+FTC+DTG and ABC+3TC+DTG showed significantly reduced cell proliferation compared to corresponding 0.1% DMSO control cells. Further, cells treated with DTG-containing cART showed increases in apoptosis, ROS, mitochondrial mass and membrane potential compared to cells treated with TDF+FTC+RAL or control cells. PI/r (lopinavir)-containing cART also induced apoptosis and ROS. A positive correlation between mitochondrial ROS and apoptosis was present across the three cell lines, with exposure to DTG-containing regimens resulting in the highest levels of ROS, while RAL- and EFV-based cART yielded the lowest ROS levels. There was no evidence of hTERT-mediated protection against DTG-associated toxicities, however WT hTERT did mitigate PI/r-induced effects. All observed toxicities were reversible upon drug removal.

CONCLUSIONS:
InSTIs have excellent clinical tolerance but in this model, DTG induced greater mitochondrial and cellular toxicities than RAL. Furthermore, the mechanism behind DTG toxicity appears different from that of boosted-PIs. Additional investigations are required to determine how these toxicities may affect human health, both in the short and long term.

ASMA BASHIR

MODERATE CHIMERA INJURY ELICITS DIFFERENTIAL ACUTE INFLAMMATORY RESPONSES IN HUMANIZED APOLIPOPROTEIN E3 AND E4 TARGETED-REPLACEMENT MICE

SUPERVISOR: CHERYL WELLINGTON

AUTHOR(S): Asma Bashir[1], Cathleen Gonzales[2], Wai Hang Cheng[1], Kassandra Welch[1], Honor Cheung[1], Anna Wilkinson[1], Carlos Barron[1], Kari Fonseca[3], Gayathri Ramaswamy[2], Cheryl Wellington[1]

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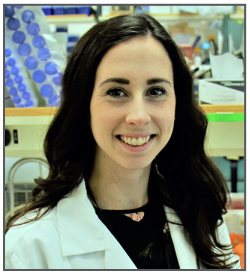
BACKGROUND/OBJECTIVES:
The CHIMERA (Closed Head Impact Model of Engineered Rotational Acceleration) is a biofidelic model of impact-acceleration head injury that replicates multiple characteristics of clinical traumatic brain injury (TBI) including elevated plasma biomarkers, grey and white matter inflammation, and microvascular injury. This study was designed to evaluate the influence of apolipoprotein E (apoE) genotype on TBI outcomes using the CHIMERA. ApoE is the main lipid carrier in the brain with important roles in microglial phenotype, cerebrovascular function and injury repair. The study was also designed to test the effects of modulating apoE activity on TBI pathophysiology using liver X receptor agonist GW3965.

METHODS:
Four-month old apoE3 and apoE4 human targeted-replacement male mice were randomized to sham or CHIMERA TBI (1x 2.2J) groups, after which they were treated with vehicle or GW3965 (subcutaneous) at 20 mg/kg 30 min post-injury. A group was sacrificed at 6h post-TBI, whereas a second group received vehicle or GW3965 every 24h until sacrifice at 7d. Outcome measurements included mortality, loss of righting reflex (LRR) latency, cytokine analysis in brain homogenates, and silver uptake by histology of fixed slices.

RESULTS:
Immediately after injury, we observed that apoE4 mice showed greater levels of respiratory apnea and fatal cardiac arrest, compared to apoE3 controls. In surviving animals, apoE3 mice had a significantly longer LRR compared to apoE4 mice. Biochemical analyses revealed a significant injury effect in cytokine levels (TNFα, IL-6, IL-1β) at both 6h and 7d time-points. Intriguingly, vehicle-treated apoE4 mice showed inhibited IL-6 and IL-1β responses following CHIMERA TBI, both of which were restored to apoE3 levels after one dose of GW3965. Silver staining in the optic tract revealed an injury effect, drug effect, and an injury-drug interaction, with the greatest evidence of silver uptake observed in GW-treated apoE4 mice subjected to TBI at 7d post-TBI.

CONCLUSIONS:
Our preliminary findings suggest apoE genotype modifies several acute outcomes after CHIMERA injury, expanding upon the translational utility of the CHIMERA platform.



LISA DECOTRET

PROTEIN TYROSINE PHOSPHATASE ALPHA CONTRIBUTES TO BREAST CANCER PROGRESSION *IN VITRO* AND *IN VIVO*

SUPERVISOR: KEVIN BENNEWITH

AUTHOR(S): Lisa R. Decotret[1,2,4], Brennan J. Wadsworth[1,4], Ling Li[2,4], Chinten J. Lim[2,3,4], Kevin L. Bennewith[1,4], & Catherine J. Pallen[2,3,4]

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BACKGROUND/OBJECTIVES:
Metastasis has been estimated to account for over 90% of cancer-related deaths. Despite great efforts, there has been limited improvements made over the past few decades outlining a critical need for early detection and intervention. Some cancer cells have acquired the ability to move throughout the body through the formation of specialized structures called invadopodia ("*invasive feet*"). Invadopodia are classified as dynamic Src-regulated, actin-based protrusions of the plasma membrane that secrete matrix metalloproteases (MMPs) to degrade the surrounding extracellular matrix (ECM). Protein tyrosine phosphatase alpha (PTPα), a widely expressed transmembrane protein, acts in normal cells to promote cell migration via the formation of similar structures called focal adhesions. However, little is known about the role of PTPα in invadopodia-mediated cancer cell motility. We propose that PTPα regulates invadopodia activity to promote the invasive motility of malignant cells.

RESULTS:
Triple-negative breast cancer cells (MDA-MB-231) depleted of PTPα showed reduced migration and invasion compared to the control, which was rescued upon reintroduction of PTPα. This suggests PTPα promotes tumour cell migration and invasion. Matrix degradation assays revealed that PTPα-depleted cells are impaired in their ability to degrade ECM. Interestingly, control and PTPα-depleted cells formed equivalent numbers of invadopodia, and PTPα co-localized with cortactin, actin, and MMP14 to punctate invadopodia-like structures. Together, these findings indicate that PTPα, present within invadopodial structures, positively regulates invadopodia-mediated ECM degradation while not affecting invadopodia formation. Furthermore, PTPa depletion decreased MMP14 cell surface expression and the localization of MMP14 to invadopodial structures outlining a possible reason for the dysfunctional invadopodia. To measure the effect of PTPα expression on tumour growth and local invasion of breast cancer cells into the surrounding mammary fat pad, control and PTPα knockdown cells were inoculated into the fourth mammary fat pad of 10-week old female immunodeficient NSG mice. We found that tumour cells depleted of PTPα produced smaller tumours with reduced invasive potential. Together, these results indicate PTPα is present within invadopodial structures and positively regulates invadopodia-mediated triple-negative breast cancer cell motility. Our research outlines PTPα as a potential therapeutic target to prevent local metastasis and limit mortality.

CONCLUSIONS:
These results have shown that PTPα is present within invadopodial structures and positively regulates invadopodia-mediated tumour cell motility. Moving forward, we aim to further extend these data using *in vivo* mouse models. Future research may reveal new mechanistic targets for therapeutic intervention to prevent cancer metastasis and limit mortality.

RACHEL DUNN

PLACENTA MITOCHONDRIAL DNA MUTATION BURDEN IN PREGNANT WOMEN WITH HIV AND THE RISK OF PREGNANCY COMPLICATIONS

SUPERVISOR: **HELENE COTE**

AUTHOR(S): Rachel A. Dunn[1,2], Adam Sherif Ziada[1,2], Izabella Gadawska[1,2], Helene C.F. Cote[1,2]

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BACKGROUND/OBJECTIVES:
There are an estimated 35 million adults living with HIV globally, approximately 52% are women, most of them of child-bearing age. Combination antiretroviral therapy (cART) is the standard treatment to limit disease progression, and while newer drugs exhibit reduced toxicity many are still associated with mitochondrial stress and accelerated aging. Several of these drugs show increased safety concerns in pregnant women with HIV, a population that is already more likely to have severe pregnancy complications. Preterm birth (PTB), the leading cause of mortality in children under 5, is up to three times more common in women living with HIV (WLWH) compared to the general population. Additionally, other pregnancy complications such as intrauterine growth restriction (IUGR) and preeclampsia (PE) are also more frequent in WLWH. The causes of PTB and complications like IUGR and PE are not fully understood, but hypoxia, oxidative stress and impaired mitochondrial function within the placenta are believed to play key roles. Several classes of HIV drugs that are core components of cART are known to increase oxidative stress and inhibit mitochondrial polymerase γ, possibly lowering its fidelity. It follows that HIV drugs may play a role in these pregnancy complications by affecting placenta mitochondrial DNA (mtDNA) and mitochondrial function. I propose to quantify placenta mtDNA mutations in cases of PTB, IUGR, and PE vs full term birth in WLWH and controls, and to identify risk factors that may increase mutations, thus may have negative effects on pregnancy. This study aims to (a) investigate the association of PTB, IUGR, or PE with increased mtDNA mutations within the placenta, and (b) identify changes in placenta mtDNA mutations associated with HIV.

METHODS:
Study samples will be drawn from pregnant women enrolled in 3 cohort studies: Children & Women AntiRetroviral therapy and Markers of Aging (CARMA-PREG), Pregnancy Study (PR.mito) and Epigenetics in Pregnancy Complications (EPIC), and who delivered between 2007 and 2017. Of the samples collected from >300 women, 149 are HIV positive women, and the rest are HIV-negative controls. Among these I will study >60 cases of PTB, >30 cases of IUGR, and >30 cases of PE, and >60 full term uncomplicated pregnancies among HIV positive and negative women. DNA will be extracted from both the maternal and fetal side of the placenta, and mtDNA mutations within the D-loop region of the mtDNA genome will be quantified using a next-generation sequencing assay previously developed in our lab. Placenta mtDNA mutational load and its association with PTB, IUGR, PE, and HIV infection will be investigated. Additional factors including age and smoking habits will be explored as possible predictors of increased mtDNA mutation.

NATALIE FIRMINO

GERMINAL CENTER HYPOXIA DEVELOPS DURING EXPOSURE TO TUMOR ANTIGENS AND MODULATES ANTI-TUMOR IMMUNE RESPONSES

SUPERVISOR: KEVIN BENNEWITH

AUTHOR(S): Natalie Firmino[1,2], Kevin L. Bennewith[1]

AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Department of Integrative Oncology, BC Cancer Research Centre

BACKGROUND/OBJECTIVES:
Anti-tumor immunotherapy, which helps the patient's immune system target and kill cancer cells, can control aggressive disease in some patients; however, these responses rely on pre-existing immune activation against the tumor. Lymph nodes (LNs) are sites of immune response development against foreign and mutated tumor antigens, and it is unknown what conditions in tumor-draining LNs (TDLNs) promote anti-tumor immunity. In response to foreign antigens, activated B cells proliferate within the germinal centers (GC) of LNs, with recent evidence suggesting some GCs can contain regions of low oxygen (hypoxia). While hypoxia in tumors causes treatment resistance and promotes metastasis, hypoxia is also essential for many normal processes, such as embryogenesis and proper wound healing. We hypothesized that tumor antigens can cause hypoxic GCs to develop in TDLNs, and that TDLN hypoxia promotes immune activation against the tumor.

METHODS:
Microscopy and flow cytometry were used to quantify hypoxia and immune cells in LNs draining orthotopic murine mammary tumors and mammary glands injected with lethally irradiated tumor cells. Immunohistochemistry was used to assess hypoxia in TDLN samples from breast cancer patients.

RESULTS:
We found that immunogenic mammary tumours could induce GCs containing hypoxic B cells in TDLNs, while hypoxia was not present in LNs from naïve, non-tumor bearing mice. Hypoxic GCs also develop in response to tumor antigens released by lethally irradiated tumor cells, and GC hypoxia is associated with the preferential development of antibody-secreting B cells over B memory cells. We generated mice with Cre-inducible stabilization of hypoxic signalling in activated GC B cells, and found that hypoxic signalling modulates serum antibody responses by B cells following exposure to tumor antigens. Hypoxia was also present in GCs in TDLNs from breast cancer patients, validating our mouse models.

CONCLUSIONS:
Our data suggest GC hypoxia reflects the extent of immune activation against tumour antigens, identifies a novel role for hypoxia in the development of anti-tumor immunity within the LN, and suggests that TDLN hypoxia may identify patients with an immune response that is primed to respond to immunotherapy.

LAUREN FORGRAVE

COMPARISON OF TDP-43 PRIMARY STRUCTURE IN FRONTOTEMPORAL LOBAR DEGENERATION AND UNAFFECTED BRAIN TISSUE

SUPERVISOR: MARI DEMARCO

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BACKGROUND/OBJECTIVES:
Frontotemporal lobar degeneration (FTLD) with aggregates of transactive response DNA-binding protein 43 kDa (TDP-43) is a form of early onset dementia (denoted as FTLD-TDP). In this most common form of FTLD, there is currently no means to diagnose the underlying TDP-43 pathology antemortem. Western blot analysis of brain tissue from FTLD-TDP cases have revealed a characteristic pattern of TDP-43-staining that suggests a range of disease-specific TDP-43 isoforms (ie. post-translational modifications including truncation). It is currently believed that much of the characteristic pattern of TDP-43-staining is due to C terminal fragments of TDP-43; however, these potentially disease-specific TDP-43 isoforms have yet to be fully elucidated. We hypothesize that sequencing TDP-43 isoforms present in the brain tissue of individuals with FTLD-TDP and absent in unaffected tissue will reveal potential disease-specific biomarkers, and aid in the development of the first biofluid diagnostic for FTLD-TDP.

METHODS:
In this pilot study, fresh frozen human frontal lobe brain tissue from an individual with FTLD-TDP and a pathologically-unaffected control were homogenized and separated into detergent-soluble and insoluble fractions for analysis. Analyses included SDS-PAGE, western blot and high-resolution mass spectrometry. For proteomic analysis, SDS-PAGE fractions were excised from the gel, with bioinformatic analysis focusing on detection of TDP-43 specific peptides and protein-level identifications in each fraction. The MaxQuant database was used to identify proteins at a 1% false discovery rate and the search included both tryptic and semi-tryptic peptides.

RESULTS:
In comparing FTLD-TDP to pathologically-unaffected brain homogenates, there were differences in TDP-43 fractionation as determined by western blot analysis, and TDP-43 proteolytic peptides detected by mass spectrometry. In the pathologically-unaffected brain homogenate, TDP-43 was observed in one of the soluble fractions; however, in the FTLD-TDP homogenate TDP-43 was identified in both the soluble and insoluble fractions. By mass spectrometric analysis of FTLD-TDP homogenate, five peptides unique to TDP-43 were identified, of which two corresponded to semi-tryptic peptides. Analysis of the pathologically-unaffected brain homogenate is underway.

CONCLUSIONS:
In this pilot study, tissue homogenization was optimized to maximize protein fractionation and isolation of TDP-43, to enable TDP-43 detection by high resolution mass spectrometry. The TDP-43 distribution visualized via western blot of pathologically-unaffected and FTLD-TDP suggests the presence of pathology-specific TDP-43 isoforms. Evidence of semi-tryptic peptides in the FTLD-TDP homogenate is consistent with the presence of truncated TDP-43 isoforms; however, unlike previous low-resolution analyses (i.e., western blot) that have suggested C-terminal fragments of TDP-43, the higher resolution mass spectrometry approach has revealed an N-terminal TDP-43 fragment. In ongoing studies, a greater number of cases of affected and unaffected tissues are being examined to corroborate this important finding.

JASMINE GILL

EVALUATION OF SERUM NEUROFILAMENT LIGHT CHAIN IN TRAUMATIC BRAIN INJURY WITHIN THE AGING POPULATION

SUPERVISOR: CHERYL WELLINGTON

AUTHOR(S): Jasmine Gill[1], Sophie Stukas[1], William Panenka[2], Robin Hsiung[3], Haakon Nygaard[3], Cheryl Wellington[1]

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BACKGROUND/OBJECTIVES:
Traumatic brain injury (TBI) is a leading cause of death and disability with nearly 70 million people affected worldwide. The elderly are highly susceptible to TBI, mainly due to falls, yet this age group is greatly understudied. TBI in the elderly coincides with the highest rates of hospitalization, emergency department visits and fatalities, and may contribute to cognitive decline. A blood biomarker such as neurofilament light (NF-L) chain, indicative of axonal damage, may facilitate diagnosis and management of TBI in this population. We hypothesize that serum NF-L will be a useful diagnostic and prognostic biomarker for TBI in persons over 60 years of age, even in subjects with comorbid neurodegeneration. Aim 1: Measure NF-L levels from randomly selected subjects from the Canadian TBI Research and Clinical Network (CanTBI) study that includes youth (60y) subjects with age-matched soft-tissue trauma controls, to determine the sensitivity and selectivity of NF-L to diagnose TBI as a function of age. Aim 2: If aim 1 shows elevated NF-L in CanTBI elderly subjects compared to controls, generate normative serum NF-L data using samples from the Canadian Health Measures Survey (CHMS) and Framingham Heart Study (FHS). Aim 3: Evaluate prognostic potential of serum NF-L following TBI in persons >60 years old in CanTBI subjects, with Glasgow Coma Scale-Extended (GOS-E) at 12 months as the primary outcome. Aim 4:To prepare for studies in elderly with neurodegeneration, measure NF-L from subjects with mild cognitive impairment (MCI) or dementia, using samples from the UBC Clinic for Alzheimer's and Related Dementias (CARD).

METHODS:
A CanTBI substudy (Vancouver General Hospital) has banked serum from TBI subjects collected within 24h, with extra samples taken on day 3 (and/or day 7) from those admitted. Of 239 recruited subjects, 72 are >60 years old. Additionally, 129 soft-tissue trauma controls are available of which 29 are >60 years old. Samples will also be retrieved from the CARD clinic, which has collected blood since 2008 and includes >1000 subjects with longitudinal cognitive and neuroimaging measures, including MCI and various dementias. Normative data will be generated using samples from the CHMS, representative of Canadians (ages 3-79y) and FHS, representative of the ethnic diversity of the USA (ages 20-79y). Outcome measures include GOS-E, neurocognitive testing, magnetic resonance imaging (MRI) and head computed tomography (CT) (as indicated). Serum NF-L analysis will be performed on the Quanterix Simoa HD-1 analyzer.

RESULTS:
We expect 1) serum NF-L will differentiate controls from TBI subjects, 2) serum NF-L levels will increase in relation to age, 3) serum NF-L (combined with outcome measures) may help identify TBI subjects >60 years who are at increased risk for complications and 4) acute serum NF-L in TBI may be elevated compared to non-injured subjects with neurodegeneration.

CONCLUSIONS:
This work will help determine the feasibility of blood biomarkers, specifically NF-L, to diagnose TBI and to aid in the development of guidelines tailored to managing TBI within the aging population.

KENDALL GREENING

CELLULAR ORIGINS OF GYNECOLOGICAL CANCERS: AN IMMUNIHISTOCHEMICAL ANALYSIS

SUPERVISOR: DAVID HUNTSMAN

AUTHOR(S): Kendall Greening[1,2], Evan Gibbard[1,2], Dawn Cochrane[2], Anthony Karnezis[1,2], David Huntsman[1,2]

AFFILIATION(S): [1]UBC Pathology and Laboratory Medicine; [2]BC Cancer Research Center

BACKGROUND/OBJECTIVES:
Ovarian and uterine cancers account for 7.7% of cancer related deaths in Canadian women, however most ovarian sybtypes arise from extraovarian tissue. High grade serous ovarian carcinoma originates from mutated fallopian tube (FT) epithelium. Yet, there is debate whether some cases arise from ovarian cortical inclusion cysts (CICs), which themselves are unknown to originate from mesothelium or FT. Endometrioid and clear cell ovarian carcinomas are known to come from endometrial (EM) tissue and are similar to EM carcinomas of the same names in the uterus. Furthermore, there is some evidence that these cancers originate from secretory or ciliated cell lineages, respectively. Thus, the cell of origin for different gynecological carcinomas is crucial to understanding their pathogenesis. H&E staining is the most common method used by pathologists to distinguish these subtypes, immunohistochemistry (IHC) for various markers is used when necessary. Our aim was to characterize the expression of known and novel IHC markers in different cells types of normal gynecological tissues and cysts of the ovary and FT, as well as in gynecological cancers. We believe that this will improve our understanding of cell of origin and may lead to the discovery of useful markers.

METHODS:
Tissue microarrays (TMAs) were constructed from normal FT, EM, ovary, cervix, CICs and paratubal cysts (PTCs) were analyzed for hysterectomy cases of women who underwent surgery for non-cancer reasons. 0.6mm cores were used for the normal TMA, and 2.0mm cores were used for the CIC/PTC array. The TMAs were stained with ER, PR, AR, FOXL2, p73, CTH, ASS1, PAX8, and CRT. The normal TMA was scored separately for each cell type (secretory, ciliated, and stromal). Scores from the CICs and PTCs were compared to EM and FT scores from the normal TMA. The epithelium of PTCs and CICs were also noted to be flat, columnar, mixed, or squamous. Additionally, novel markers discovered by single cell RNA sequencing (scRNAseq) were stained on these TMAs as well as TMAs of gynecological cancers.

RESULTS:
Notably from the normal TMA, ASS1 was specific for secretory cells in the FT and p73 was a marker of ciliated cells. FOXL2 is a specific marker stroma in all tissues. There were equal numbers of flat, columnar, and mixed CICs, whereas most PTCs were flat. For most markers, columnar CICs were more similar to FT than EM. Flat and squamous PTCs had lower expression of most markers than FT and EM. IHC staining of novel markers discovered by scRNAseq showed FAM92B, WDR16, and SNTN to be specific markers of ciliated cells and MST to be a specific marker of secretory cells. Additionally, FAM92B, MST, and SNTN were shown to have specific staining on TMAs of gynecological cancers.

CONCLUSIONS:
The staining of the normal TMA has provided a useful foundation for the comparison to other markers and TMAs, and comparison with the CIC/PTC array may provide insight into the origin of these cysts and their role in cancer pathogenesis. Further analysis of the novel markers validated through these IHC experiments may show them to be useful markers of cancers.

ANTHONY HSIEH

LYMPHOCYTE SUBSET TELOMERES ARE SHORTER IN HIV SLOW PROGRESSORS THAN IN HIV NON-SLOW PROGRESSORS

SUPERVISOR: HÉLÈNE CÔTÉ

AUTHOR(S): Anthony Y.Y. Hsieh[1,2], Beheroze Sattha[1], Nicole Bernard[3], Cecile L. Tremblay[4,5], Hélène C.F. Côté[1,2] for the CIHR Team on Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

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BACKGROUND/OBJECTIVES:
Indications of immune aging, including telomere length (TL) attrition and skewed senescent/proliferative CD8 T cell distributions, likely link HIV with premature age-related comorbidities among people stable on cART. Whether these indicators also exist in HIV slow progressors (SP) is unknown. Our objective was to characterize immune subset TL in SP to determine whether naturally controlling HIV mitigates HIV-associated immune aging. We hypothesized longer TL and fewer senescent CD8 T cells in SP than in cART-controlled HIV non-slow progressors (NSP).

METHODS:
Live PBMCs from NSP, SP, and HIV- participants in the CARMA cohort and Canadian Cohort of HIV+ Slow Progressors were matched 1:1:1 for age and sex. CD4, proliferative (CD28+) CD8, senescent (CD28-) CD8, and B cells were sorted by FACS. TL was measured in sorted subsets containing a sufficient number of cells for multiplex qPCR (~75% of all specimens). Age, sex, ethnicity, and smoking status were considered in multivariable linear models.

RESULTS:
Each group included n=55-57 participants aged 17-75y. All NSP (56/57 on cART), and 33/57 SP (2/57 on cART) were viral load undetectable, and their CD4 counts were similar. SP had shorter TL in all four cell subsets compared to both NSP ($P \leq 0.011$) and HIV- ($P \leq 0.004$) participants, after controlling for age, sex, and ethnicity. In similar models, SP CD4:CD8 ratio was lower ($P < 0.001$) and higher ($P = 0.024$) than those of HIV- and NSP participants, respectively. SP CD8 CD28+:CD28- ratio was also lower than that of HIV- participants ($P < 0.001$). Apart from B cell TL, these associations remained in sub-analyses that included only the elite controllers among the SP (n=25-28).

CONCLUSIONS:
Contrary to our hypothesis, these data strongly imply that cellular aging within lymphocyte subsets may be accelerated among SP compared to HIV+ NSP and HIV- participants. This suggests that there is an immunologic cost for naturally controlling HIV.

EMEL ISLAMZADA

DEFORMABILITY AS A BIOMARKER FOR THE QUALITY OF FRESH AND STORED RED BLOOD CELLS

SUPERVISOR: HONGSHEN MA

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BACKGROUND/OBJECTIVES:
Donated red blood cells (RBCs) is the most common form of blood product collected and manufactured by Canadian Blood Services (CBS). Blood bankers have long been aware of the significant differences in RBCs collected from different donors, and even different units from the same donor, that result in different circulation time in recipients. There is currently no method to accurately predict the circulation time of a RBC unit prior to transfusion. This capability would be extremely helpful for hematologists to manage the complex needs of transfusion recipients. Since the individuals with chronic disorders often require regular transfusions, identifying RBC units with greater circulation time could reduce the frequency of transfusions, which would reduce transfusion-related morbidities (e.g. iron- overload) and increase total blood supply.

In our lab, we have developed the Microfluidic Ratchet device that is able to sort out RBCs based on their deformability, allowing us to look at disease pathologies from a new, biomechanical perspective. We therefore hypothesize, that the distribution of RBCs after deformability-based sorting can be used as a biomarker for the quality of fresh and stored RBCs.

METHODS:
The Microfluidic Ratchet mechanism sorts RBCs based on their ability to deform and squeeze through a series of tapered funnels with opening sizes reflecting the vessel sizes encountered in blood flow. The opening sizes in the sorting matrix are gradually decreasing from 7.5 to 1.5 µm. When reaching the limiting funnel size, the cells are collected at one of the 12 distinct outlets. The RBCs are therefore separated into distinct fractions based on their deformability. The cells collected are viable, and are collected for further analysis of the fractions. The number of cells in each outlet is quantified by FACS.

RESULTS:
We validated and optimized the Microfluidic Ratchet device to sort fresh and cold-stored RBCs based on deformability. The consistency and repeatability of the device mechanism was tested with calibration beads and fresh RBCs. The device showed consistent sorting with a narrow range and good reproducibility. We then established the inter- and intra-donor variability of human RBC deformability using the Microfluidic Ratchet device. The blood samples were collected from healthy male and female donors in accordance with CBS standards, and stored for two weeks in SAGM supplemented plastic tubes. We show a donor-to-donor difference in both the initial RBC deformability, and the change in deformability with storage. We further show a significant reduction in deformability in most donors from day 0 to day 14 of storage, with a notable exception of one donor.

CONCLUSIONS:
We developed a Microfluidic Ratchet device which can sort RBCs based on their deformability. We show a significant reduction in deformability within only the first two weeks of storage in most donors, and can identify donors with very little time-dependent RBC deformability change- the potential “super-storer”.

RANA JAFARI-MINAB

IDENTIFYING ANTIBODY CORRELATES OF PROTECTION AGAINST EPSTEIN-BARR VIRUS INFECTION IN INFANTS

SUPERVISOR: PETER VAN DEN ELZEN

AUTHOR(S): Minab, Rana[1], Cohen, Jeffrey[1,2], Wei, Bu[2], McGuire, Andrew[3], Casper, Corey[4], Gantt, Soren[1,3]

AFFILIATION(S): [1]University of British Columbia, Vancouver, BC; [2]National Institutes of Health, Bethesda, MD; [3]Fred Hutchinson Cancer Research Centre, Seattle, WA; 4 Infectious Disease Research Institute, Seattle, WA

BACKGROUND/OBJECTIVES:
Epstein-Barr virus (EBV) is an oncogenic herpesvirus that persists indefinitely in the body. This virus, transmitted through saliva, infects approximately 95% of the world's population and causes malignancies such as Burkitt's lymphoma, an endemic disease that accounts for up to 70% of all pediatric malignancies in equatorial Africa. Though a preventive EBV vaccine would be highly desirable, it is not known which immune responses may prevent infection. Infection is rare in the first few months of infancy, indicating that EBV-specific maternal antibody is protective. Because maternal HIV infection impairs transplacental transfer of many antibodies, we hypothesize that HIV-exposed infants have lower levels of maternal EBV-specific antibodies.

METHODS:
A birth cohort with 32 mother-infant pairs followed from birth; 17 mothers and no infants were HIV-infected. Weekly oral swabs were collected for PCR and blood was collected every 4 months to determine when babies were infected with EBV. Binding antibody to gp350/gHgL was done using luciferase immunoprecipitations system (LIPS). Neutralizing antibody (NAb) titers, measured by direct inhibition of Raji (B cell) or HEK 293 (epithelial cell) infection by GFP-recombinant B95.8 EBV strain. Antibody-dependent cellular cytotoxicity assays (ADCC) to recombinant gp350 or gHgL antigens was measured by CD107a expression on NK effector cells (NK cell activation).

RESULTS:
EBV infection was acquired by 19 infants, with a cumulative incidence of 47.4% at 1 year (95% CI 31.3–66.6%). EBV infection occurred earlier in HIV-exposed infants (adjusted Hazard Ratio (aHR) 7.2; 95% CI 2.4–22.2; $p<0.001$) and no infection presented in HIV-unexposed infants before 6 months. Surprisingly, infant binding antibody titers to EBV envelope glycoproteins gp350 and gHgL were significantly higher in HIV-exposed infants compared with HIV-unexposed infants ($p<0.001$). Similarly, neutralizing antibody (NAb) titers, were higher in HIV-exposed infants compared to unexposed infants ($p<0.001$) and were positively correlated with gp350 and gHgL binding titers. Neither binding antibody nor NAb showed an association with risk of EBV acquisition after adjustment for maternal HIV status. In contrast, ADCC activity to recombinant gp350 or gHgL antigens, was not higher in HIV-exposed infants and showed a trend toward protection (aHR=0.1 per 1% increase in NK cell activation; 95% CI 0.0-8.6; $p=0.28$ for gp350).

CONCLUSIONS:
NAb is not protective with significantly higher levels of it in HIV-exposed infants who have earlier EBV infection. Thus, although NAb titers were not associated with risk of EBV acquisition, discordance between cell-mediated and NAb responses suggests that ADCC or other non-neutralizing antibody activities may protect against EBV infection. Our findings indicate that studies of EBV acquisition in infants have the potential to identify humoral correlates of protection using maternal antibody, and that broad systems serology approaches may be required. Future studies in this field will provide critical insights into the optimal design and evaluation of a prophylactic EBV vaccine.

KEVIN KUCHINSKI

RECOVERING INFLUENZA VIRUS GENOMES FROM WILD BIRD HABITATS FOR BETTER AVIAN FLU SURVEILLA

SUPERVISOR: NATALIE PRYSTAJECKY

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AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia, BC, Canada; [2]Public Health Laboratory, British Columbia Centre for Disease Control, BC, Canada

BACKGROUND/OBJECTIVES:
Avian influenza A viruses regularly cause severe disease outbreaks in commercial poultry flocks. Aside from being costly, these outbreaks expose agriculture workers to potentially lethal zoonotic infections. Historically, human infections by livestock influenzas have been the origin of all flu pandemics and seasonal flus, which remain among the ten leading causes of death in Canada annually. Consequently, avian flu surveillance in wild birds, the natural hosts of influenza A viruses, is a cornerstone of pandemic preparedness and disease prevention.

We are investigating the use of targeted genomic sequencing for avian influenza surveillance using environmental specimens from wild bird habitats. Since influenza A viruses are shed abundantly in bird feces, they accumulate in wetlands sediment, making this a promising specimen for genomics-based avian influenza surveillance. The objective of this study was to determine if influenza A virus genomes can be successfully recovered from these sediment specimens using targeted genomic sequencing.

METHODS:
Total RNA was extracted from wetlands sediment collected across the Lower Mainland and Fraser Valley from September to December 2016. Influenza genome fragments were captured from RNA extracts using custom-designed hybridization probes then sequenced on an Illumina MiSeq.

RESULTS:
7 influenza haemagglutinin and neuraminidase gene sequences with over 75% coverage were recovered from 4 different wetland locations. These sequences were sufficient for subtyping and phylogenetic characterization, revealing the presence of H3, H6, H11, N2, N8, and N9 subtypes in the Lower Mainland and Fraser Valley during the fall of 2016.

CONCLUSIONS:
Targeted genomic sequencing of wetlands sediment from wild bird habitats can be used for avian influenza surveillance.

HAKWOO LEE

DECIPHERING THE FUNCTIONAL MOLECULAR PROFILES OF TUMOR CLONES IN BREAST CANCER METASTASIS USING PATIENT-DERIVED XENOGRAFT MODEL

SUPERVISOR: SAMUEL APARICIO

AUTHOR(S): Hakwoo Lee[1,2], Farhia Kabeer[1,2], Ciara O'Flanagan[2,3], Samuel Aparicio[1,2,3]

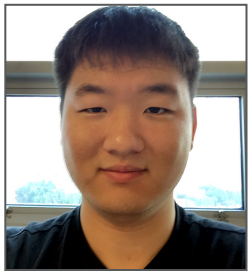
AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Department of Molecular Oncology, BC Cancer; [3]Department of Medical Genetics, University of British Columbia

BACKGROUND/OBJECTIVES:
Cancer is the leading cause of death in Canada and is responsible for 30% of all deaths. Breast cancer is the most common cancer and the second most common cause of cancer death in women in Canada. Metastasis remains the cause of >90% of cancer related mortality. Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer which shows high rate of recurrence and metastasis. Malignant cells that comprise primary tumor are heterogeneous and during disease progression selection of certain tumor cells occur as means to adapt and survive. Tumor heterogeneity can be studied by grouping cells as clones. The term “clone” refers to a group of cells related to each other by descent from a unitary origin. Understanding the mechanism of clonal dynamics and selection during cancer evolution, which is linked to the concept of fitness, is important to develop new therapeutic strategies for cancer metastasis. The molecular determinants of fitness may arise from genomic mutations and also from selection leading to stable states of gene expression. To further understand how clonal selection occurs from primary tumor and lead to metastasis fitness, it is imperative to be able to detect rare subclones that are responsible for metastasis. Thus, single-cell analysis is critical to identify rare subclones and to understand cellular heterogeneity of cancer in depth. Measuring genome and transcriptome in single-cell level will enable us to discover clonal dynamics during cancer metastasis and infer molecular determinants of fitness in metastasis. Here, we propose to investigate the clonal dynamics, genomic, and transcriptomic profiles of human breast tumor metastasis using TNBC xenograft mouse model to understand the mechanism of breast cancer progression and metastasis.

METHODS:
Tumor cells from previously established patient-derived xenograft (PDX) will be transplanted into mammary fat pad of mouse. Tumor will be removed when it reaches maximum allowed endpoint size and mice will be monitored and allowed to grow metastasis. After 16-20 weeks or when mice present clinical signs of distress from metastasis, mice will be euthanized and examined for the evidence of metastasis. We will carry out single cell whole-genome from primary tumor and metastatic cells using methods already established in the lab for single cell analysis.

RESULTS:
From Feb. 2018 to Mar. 2019, 10 different PDX lines were transplanted and 4 PDX lines showed metastasis. PDX1 developed metastatic mass in cervical and lumbar area. PDX2 developed metastatic mass in axilla and micrometastasis in lung. PDX3 developed liver metastasis and PDX4 developed micrometastasis in lung. Single cell whole-genome sequencing of PDX1 revealed monoclonal metastasis from primary tumor.

CONCLUSIONS:
We were able to identify metastatic lesions from breast cancer PDXs. Different PDXs showed different patterns of metastasis. Single cell whole genome sequencing revealed monoclonal metastasis from PDX1. Further analysis of single cell genomic and transcriptomic profile from primary tumour and corresponding metastasis is planned.



YU (MICHAEL) LI

DETERMINING THE SUBTYPE-SPECIFIC ROLE OF TUMOUR NECROSIS FACTOR RECEPTOR-ASSOCIATED FACTOR 3 IN DIFFUSE LARGE B CELL LYMPHOMA

SUPERVISOR: CHRISTIAN STEIDL

AUTHOR(S): Michael Yu Li[1,2], Shannon Healy[2], and Christian Steidl[1,2]

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BACKGROUND/OBJECTIVES:
Diffuse large B cell lymphoma (DLBCL) is the most common B cell non-Hodgkin lymphoma, which are cancers of the antibody producing cells of the immune system. Despite recent breakthroughs in disease characterization using genome-wide discovery tools, around one third of DLBCL patients receiving immuno-chemotherapeutic treatment experience relapse and are not cured. A recent study by our lab identified recurrent deletion of the tumour necrosis factor receptor-associated factor 3 (TRAF3) gene as a correlate of poor survival outcome in both the germinal center B-cell like (GCB) and activated B-cell like (ABC) subtypes of DLBCL. These subtypes are characterized by distinct gene expression profiles and survival outcomes. Biologically, TRAF3 regulates several key pathways in B cells, one of which is the non-canonical NF-kB pathway required for B cell survival. We therefore sought to determine if genetic inactivation of TRAF3 confers subtype-specific survival advantages to DLBCL cancer cells.

METHODS:
We generated TRAF3 knockout (KO) human DLBCL cell lines using CRISPR/Cas9 genome editing technology in order to assess non-canonical NF-kB pathway and toll-like receptor (TLR) signaling activation. TRAF3 status in isogenic cell line clones was validated using Sanger sequencing with primers targeting the predicted region of genomic editing and protein expression by western blotting. NF-kB activation and pro-survival protein expression was determined by western blotting of pathway components. As a readout of Toll-like receptor (TLR) signaling, cytokine production was measured using the ELISA immunoassay.

RESULTS:
Screening of CRISPR/Cas9 genome edited single cell clones revealed bi-allelic frameshift mutants and loss of protein expression of TRAF3 in genetically engineered DLBCL cell lines. TRAF3 KO cell lines had increased accumulation of NIK, a kinase critical to the activation of the non-canonical NF-kB pathway, as well as elevated nuclear localization of the NF-κB2 transcription factor complex. We also observed that TRAF3 loss increased expression of the proto-oncogenes MCL1 and activated-AKT, specifically in GCB-DLBCL. The ABC-DLBCL cell line OCI-LY3, which harbors a homozygous mutation in the TLR pathway adaptor protein MYD88, secreted more interleukin-6 (IL6) in the absence of TRAF3. We further found, consistent with the literature, autocrine IL6 signaling may increase activation of the pro-survival JAK/STAT pathway.

CONCLUSIONS:
Our findings may suggest new roles involving TRAF3 as a potential tumour suppressor in DLBCL. Ongoing work will aim to further characterize subtype-specific pathway alterations and to elucidate mechanistic targets for therapeutic intervention for patients harboring TRAF3 deletions.

ANAM LIU

ESTABLISHING A NOVEL ASSAY FOR FUTURE NORMAL TISSUE TOXICITY EVALUATION OF RADIATION COMBINED WITH DNA-PK INHIBITORS

SUPERVISOR: **ANDREW MINCHINTON**

AUTHOR(S): **Anam Nan Nan Liu[1,2], Jennifer Baker[2], Alastair Kyle[2], Sheena Liu[2], Taixiang Wang[2], Judit Banath[2], Andrew Minchinton[1,2]**

AFFILIATION(S): **[1]Pathology and Laboratory Medicine, The University of British Columbia; [2]BC Cancer Research Centre**

BACKGROUND/OBJECTIVES:
Radiation induces double-strand breaks (DSBs) which are most lethal to cancer cell survival compared to other types of DNA lesions. A crucial pathway for DSB repair is non-homologous-end-joining (NHEJ), which enables some cells to recover from radiation damage. NHEJ uses DNA protein kinase (DNA-PK) in its repair machinery, and this enzyme represents an anti-cancer target for drug development. Novel DNA-PK inhibitors effectively radiosensitize cancerous cells but normal tissues that are included in a radiation beam may also be affected, thus the toxicity to normal tissues should be evaluated. We hypothesize that an automated toxicity assay using immuno-staining can be developed. The intestine has been historically used as a model to evaluate the effects and toxicity of radiosensitizer/radioprotectors because the survival of the organ is dependent on maintaining a balanced crypt proliferation rate that is well characterized. However, methods have typically required highly subjective assessment techniques in limited regions of tissue, which is impractical and can be difficult to reproduce. We intend to develop a robust quantitative measure based on past methods using image analysis.

METHODS:
Groups of 5 female Rag2M(129S6/SvEvTac-Rag2tm1Fwa)mice were irradiated at various doses (2,5,7,10,12,15 Gy). All mice received BrdU (1000 mg/kg) and were euthanized 48 h post-irradiation. Jejunum was collected and cut open longitudinally. It was then rolled and rapidly frozen followed by embedding with OCT, sectioning and immunofluorescent staining. Tiled images of whole jejunum swirls comprising the full 5 cm length were collected for BrdU staining and imaging using a robotic microscope operated by customized ImageJ software at 0.65 µm/pixel resolution. Staining and imaging artifacts were manually removed for each image. A customized ImageJ macro was written to quantify crypt proliferation by counting the number of all pixels representing positive BrdU as a ratio to the length of jejunum analyzed. Damage was evaluated using gH2AX staining at 1,3,6 and 24h post-irradiation with the automated method described as above. Functionality was evaluated by measuring villi length at 3.5days post-irradiation with customized ImageJ macro.

RESULTS:
A minimum 2.6 cm length of jejunum was evaluated for each animal. In this 48 h dose-response study, animals treated at 10,12 and 15 Gy had reduced proliferation relative to un-irradiated control. However, the 5Gy radiation treated group exhibited an unexpected increase in proliferation while both 2 Gy and 7 Gy groups are in close proximity. Both gH2AX and villi length measurements demonstrate a decreasing trend as time and dose increase, respectively.

CONCLUSIONS:
Utilizing an automated system, we were able to examine greater lengths of tissue for each animal compared to conventional methods. Acute damage and functionality models were successfully developed. However, the spike at 5Gy complicates interpretation of proliferation model when used in the future thus optimal radiation dose range and timepoint should be explored where less ambiguous conclusions can be drawn.

JENNIFER LUU

IDENTIFYING SECRETED PROTEINS THAT PROMOTE IMMUNE EVASION DURING MALIGNANT LUNG TRANSFORMATION

SUPERVISOR: WILL LOCKWOOD

AUTHOR(S): Jennifer Luu[1,2], Fraser Johnson[2,3], Kevin Bennewith[1,3], Will Lockwood[1,2]

AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Department of Integrative Oncology, BC Cancer Research Centre; [3]Interdisciplinary Oncology Program, Faculty of Graduate and Postdoctoral Studies, University of British Columbia

BACKGROUND/OBJECTIVES:
Lung cancer is the leading cause of cancer-related deaths in Canada, with lung adenocarcinoma being the most common subtype. High mortality is partially attributed to late diagnosis; advanced stage lung cancer is often difficult to treat. To decrease mortality, earlier detection of the condition is necessary. Diagnostic biomarkers represent an sensitive method, one which is also relatively non-invasive, that addresses the need for earlier detection.

Tumor immune evasion is an area of interest in biomarker development: malignant cells can modify immune cells and the surrounding microenvironment to avoid destruction. This can be mediated through secreted proteins. Current studies have focused on secreted protein changes during advanced stages of cancer; however, pre-neoplastic and early stages are relatively unknown. Studying changes during these stages may provide valuable information for a comprehensive, diagnostic biomarker panel. My goal is to identify secreted protein changes during malignant transformation of lung epithelial cells to lung adenocarcinoma in vitro. I hypothesize that oncogenic transformation leads to secreted proteins that alter the tumor microenvironment to promote immune evasion.

METHODS:
Immortalized human bronchial epithelial cells (HBEC-3KT; HBEC) were infected with lentiviral vectors expressing clinically relevant oncogenic mutations EGFR L858R or KRAS G12V. To further promote transformation, this will be done in conjunction with a c-terminal p53 construct. Cell transformation will be confirmed with anchorage-dependent and independent growth. Anchorage-dependent growth will be assayed with clonogenic and focus formation assays; anchorage-independent growth will be assessed with soft agar assay. Then, cells will be seeded and grown to confluency in 6 mililitres standard serum-free media (KSFM containing BPE and EGF). Media will be switched to one solely containing EGF for 72 hours; this will reduce media background and maintain cell viability. Collected media will be spun at 1000 rpm, filtered with a 0.22micrometre filter, and then concentrated with a 3kDa cut-off filter to 500 microlitres. Concentrated media will be subjected to tandem mass tag mass spectrometry. Secreted proteins that show significant relative-fold change will be validated with ELISA. Validated proteins will be assessed for immune evasion in co-culture assays.

RESULTS:
HBEC cells expressing EGFR L858R or KRAS G12V show greater clonogenic potential, relative to those with the vector control. However, cells fail to show anchorage-independent growth when grown in soft agar.

CONCLUSIONS:
Oncogenic mutations EGFR L858R and KRAS G12V appear to be insufficient to confer HBEC transformation. Future work will focus on establishing a concrete, transformed phenotype in vitro to examine secreted proteins.

MADELINE MASON

ESTABLISHING THE IMMUNE PROFILE OF THE OVARIAN MICROENVIRONMENT IN ENDOMETRIOSIS-ASSOCIATED OVARIAN CANCE

SUPERVISOR: MICHAEL ANGLESIO

AUTHOR(S): Madeline Mason[1], Tayyebeh Nazeran[2], Bronwyn Gibson-Wright[3], Katy Milne[3], Julie Ho, Janine Senz, David Huntsman[1,2], Brad Nelson[3,4], Michael Anglesio[1,2]

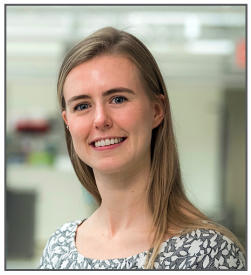
AFFILIATION(S): [1]Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, Canada; [2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada; [3]Immunotherapy Program, Deeley Research Centre, BC Cancer, Victoria, Canada; [4]Department of Medical Genetics, University of British Columbia, Vancouver, Canada

BACKGROUND/OBJECTIVES:
Endometriosis associated ovarian cancers (EAOCs) include clear cell and endometrioid ovarian carcinomas (CCOC and ENOC respectively). They arise from endometrial tissue via the malignant transformation of endometriosis. EAOCs are typically refractory to standard platinum/taxane chemotherapy, necessitating an alternative strategy. Literature of the ovarian tumor microenvironment (ME) is dominated by high grade serous carcinoma, where correlation between tumor infiltrating lymphocytes (TILs) and prognosis has been well established. Our objective is to describe the ovarian ME in women with EAOCs where our focus will be on the immune-environment as it is potentially targetable, creating the potential for alternative mechanisms of treatment.

METHODS:
Multi-color IHC is on-going for quantitative analysis of immune cell populations in CCOC and ENOC tissue micro-arrays with the aim of analyzing ~500 samples per histotype. Cell types being investigated include B-cells (CD79/CD20), macrophage (CD68/PD-L1/PD1), NK cells (CD56/CD16/PD1), cytotoxic T-cells (CD8/CD3) and regulatory T-cells (FoxP3/CD25/CD8). Data from tumor and stromal compartments are analyzed separately. This work is performed in collaboration with the Molecular and Cellular Immunology Core (MCIC), at the Deeley Research Centre in Victoria, BC. Whole-transcriptome sequencing in tumor epithelium and stromal compartments is also ongoing. Results will be used to directly cross-validate mcIHC results, as well as uncover other TME features characteristic of EAOCs.

RESULTS:
In ENOC samples, macrophage populations (both CD68+ and CD68+/PDL1+) appear restricted to the stroma (p= 0.0001 and 0.0004 respectively). This trend continues with Foxp3+/CD25-/CD8- and presumed cytotoxic T-cells (Foxp3-/CD25-/CD8+) (p= 0.0001 and 0.04 respectively). In CCOC samples Foxp3-/CD25-/CD8+ cells and Foxp3+/CD25-/CD8- are again significantly confined to the stroma (p = 0.036 and 0.009 respectively), whereas presumed regulatory T-cells (Foxp3+/CD25+/CD8-) are significantly elevated in tumor epithelium (p = 0.0032). We have generated preliminary transcriptome data; however, our cohort consisted only of CCOC samples and we are therefore unable to compare frequencies of tumor ME features with ENOC samples. Nonetheless, we were able to obtain significant results when comparing immune cell populations between tumor and stroma compartments using CIBERSORT. We found the presence of NK cells appeared to be restricted to the stroma (p = 0.017).

CONCLUSIONS:
We have been able to identify stromally restricted immune cell populations, including macrophage, NK cells and presumed cytotoxic T-cells. We have also been able to identify tumor infiltrating regulatory T-cells. However, cross-validation between transcriptome profiles and mcIHC are pending.



SARAH MORRIS

INVESTIGATING QUANTITATIVE BIOMARKERS IN POST-MORTEM HUMAN SPINAL CORD INJURY TISSUE USING ADVANCED MAGNETIC RESONANCE IMAGING AND HISTOPATHOLOGICAL TECHNIQUES

SUPERVISOR: CORNELIA LAULE

AUTHOR(S): Sarah R. Morris[1,2,3], Valentin Prevost[1,2], Piotr Kozlowski[1,2,3,4], Andrew Yung[1,2,4], Andrew Bauman[1,2,4], Zahra Samadi B.[1,5], Caron Fournier[1,5], Allan Aludino[1,6], Lisa Parker[7], Kevin Dong[1], Femke Streijger[1], G. R. Wayne Moore[1,5,8], Brian Kwon[1,6], Cornelia Laule[1,2,3,5]

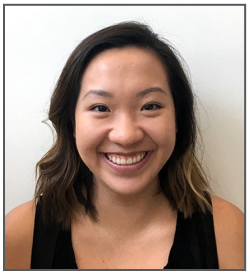
AFFILIATION(S): [1]International Collaboration on Repair Discoveries; [2]Radiology, University of British Columbia; [3]Physics and Astronomy, University of British Columbia; [4]UBC MRI Research Centre; [5]Pathology and Laboratory Medicine, University of British Columbia; [6]Vancouver Spine Surgery Institute; [7]Vancouver General Hospital; [8]Medicine, University of British Columbia, Vancouver, BC, Canada

BACKGROUND/OBJECTIVES:
Currently, the assessment of patients with spinal cord injury (SCI) relies upon a subjective clinical examination which is poorly predictive of outcome. Understanding the microstructural pathology of SCI and establishing magnetic resonance imaging (MRI) biomarkers for clinical outcomes is a vital area of research. Conventional MRI methods lack specificity to the different types of injury that can occur in SCI, so new techniques are continuously being developed, with the hope of better characterizing tissue damage. Once potential MRI biomarkers have been identified, histopathological validation is key to understanding the biological correlates for the image contrast. Two promising candidate techniques for characterizing SCI damage are inhomogeneous Magnetisation Transfer (ihMT) MRI, a measure of myelin, and Neurite Orientation Dispersion and Density Imaging (NODDI) diffusion imaging, which provides measures related to axonal health. Our goal was to investigate the myelin-sensitive ihMT and axon-sensitive NODDI MRI techniques in post-mortem human SCI and compare them quantitatively with five histological stains reflecting different aspects of spinal cord tissue damage.

METHODS:
Full-length spinal cords from six patients with acute SCI (1 female, mean age=79.3yrs (range=60-95yrs), mean time injury to death=40.8 days (range=9-112 days)) were donated to the International Spinal Cord Injury Biobank. Formalin-fixed 4.5cm sections of cord were imaged with a high-field 7 Tesla MRI scanner to obtain high resolution ihMT and diffusion data. ihMT maps were created using in-house MatLab software and diffusion data were fit with the NODDI tissue model. After MRI, the 4.5cm long blocks were cut into 15 sections using a 3D-printed mold for MRI-histology alignment, and paraffin-embedded. 5µM sections were stained with H&E (nuclei/cytoplasm), Luxol Fast Blue (LFB, myelin phospholipids), myelin basic protein, phosphorylated neurofilament (PNF, axons) and fibrinogen (blood-spinal cord barrier breakdown). LFB optical density was determined using Image Pro Premier in 20 white and grey matter regions of interest (ROI). Data was compared to corresponding ROIs on ihMT maps with Spearman correlation analysis.

RESULTS:
NODDI orientation dispersion (thought to be related to the degree of alignment of nerve fibers in white matter) showed marked reductions in areas of low ihMT. LFB optical density showed a trend-level correlation with ihMT. High magnification H&E and PNF histology images displayed axonal spheroids, and regions of low PNF corresponded to areas of reduced NODDI orientation dispersion. Fibrinogen staining revealed clear blood-spinal cord barrier breakdown.

CONCLUSIONS:
We have demonstrated the use of two advanced MRI techniques in post-mortem human SCI. We found clear histological evidence for blood-spinal cord barrier breakdown, which is a driver for secondary injury in SCI. We found correspondence between LFB, a stain for myelin phospholipids and ihMT, and between PNF axonal staining and NODDI orientation dispersion. Biomarker validation studies will enable accurate interpretation of future in vivo quantitative MRI studies in SCI.



AMY NGUYEN

PRE-ANALYTICAL AND ANALYTICAL CONSIDERATIONS FOR QUANTIFICATION OF AMYLOID-BETA IN CEREBROSPINAL FLUID BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

SUPERVISOR: MARI DEMARCO

AUTHOR(S): Amy Nguyen[1], J. Grace van der Gugten[2], and Mari L. DeMarco[1,2]

AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada; [2]Department of Pathology and Laboratory Medicine, St. Paul's Hospital, Providence Health Care, Vancouver, Canada

BACKGROUND/OBJECTIVES:
Our lab has previously developed an automated liquid chromatography tandem mass spectrometry (LC-MS/MS) assay to identify sporadic Alzheimer's disease by quantifying wild-type amyloid-beta (AB) 1-40 and AB42 as well as to diagnose familial Alzheimer's by identifying autosomal dominant AB variants in cerebrospinal fluid (CSF). As part of method development and validation, interference studies – including evaluation of endogenous and exogenous factors – were performed.

METHODS:
Mixing studies, and spike and recovery experiments were performed to investigate potential for interference from high protein content, hemolysate contamination, and immunoglobulin therapeutics (i.e., intravenous immune globulin (IVIG) and anti-AB antibodies). Analyte response under these various conditions was explore by looking at the raw signal (peak area) and signal normalized by the IS (analyte concentration). A CSF pool containing a known amount of AB was spiked with various amounts of the substances above, and AB recovery was assessed. To determine an appropriate composition for the CSF surrogate matrix used for the external calibrators, concentrations from 0-20 g/L of bovine serum albumin (BSA) were tested. The effect of total protein concentration on AB recovery was evaluated in a mixing study of two CSF pools containing 0.2 g/L and 12 g/L of total protein respectively. To investigate the effect of hemolysate on AB recovery, samples containing hemolysate by visual inspection were spiked with known quantities of AB40 and AB42. Physiologically relevant concentrations of an anti-AB antibody and IVIG, were also used to test accuracy in the presence of these immunoglobulin therapeutics.

RESULTS:
For the surrogate matrix, 1 g/L of BSA was determined to be the optimal concentration (no statistical significance on AB recovery from 0.5-2 g/L). Peak area was found to be inversely proportion to the total protein concentration over the range of 0.2-12 g/L; however, there was no statistically significance difference on the reported AB concentration from 0.2-12 g/L. The samples containing a gross amount of hemolysate were in excess of ±20% of the expected concentration of AB. For the immunoglobulins tested, there was no significant differences between peak area and AB concentration for the different concentrations ($p > 0.05$).

CONCLUSIONS:
While a CSF total protein concentration well above the reference interval of <0.45 g/L may alter AB signal intensity, this is corrected for by the IS. Based on the hemolysate interference studies, a visual acceptance criterion was established for CSF samples. Given that the LC-MS/MS assay does not include any ligand binding steps (e.g., immunoprecipitation) and uses strong denaturants during sample preparation, as well as the results of the immunoglobulin interference studies, there was no concern regarding quantification of AB peptides in individuals on immunoglobulin therapies.

LORENZ NIERVES

OPTIMIZING A PROTEOMIC WORKFLOW FOR THE ISOLATION OF CELL SURFACE PROTEINS AND TERMINI

SUPERVISOR: PHILIPP LANGE

AUTHOR(S): Lorenz Nierves[1,2] & Philipp Lange[1,2]

AFFILIATION(S): [1]Department of Pathology and Lab Medicine, University of British Columbia; [2]Michael Cuccione Childhood Cancer Research Program, B.C. Children's Hospital Research Institute

BACKGROUND/OBJECTIVES:
Presently, the 5-year survival rate for Acute Lymphoblastic Leukemia (ALL) in children is approaching 90%. But current treatment options are harsh and indiscriminate, leading to secondary cancers, late effects, or acute side effects. There is, therefore, a need to seek out more specific therapeutic targets for paediatric ALL. Proteins in the cell surface are prime therapeutic targets due to their accessibility. Paediatric ALL, however, has a relatively lower mutational burden; thus making it more challenging to select targets that are more specific to cancer cells. To overcome this challenge, we are interested in exploring the cell surface proteoforms that resulted from the presence of a unique microenvironment and deregulated proteases that are exclusive to cancer cells. We hypothesize that the unique microenvironment and proteases present in the bone marrow (BM) of ALL patients lead to new and cancer-specific proteolytic terminus for cell surface proteins.
Objectives: To optimize a proteomic workflow that will allow for the isolation and analysis of cell surface termini from limited amounts of clinical samples.

METHODS:
To enrich for cell surface proteins and termini, we began by incubating cells with biotin to label accessible amine (—NH2) groups present in the N-terminus and lysine side chains of cell surface proteins. After, cells were lysed, and proteins were isolated and digested with trypsin. Peptides resulting from trypsin digestion were then incubated with anti-biotin antibodies conjugated to agarose beads. Biotinylated peptides were positively selected for Tandem Liquid Chromatography Mass Spectrometry (LC-MS/MS) analysis.

RESULTS:
There are a few challenges associated with selection of proteins localized in the cell's plasma membrane due to the unique architecture of the organelle. Preliminary experiments have shown that these challenges can be overcome. We have optimized biotin labeling, cell lysis, and LC-MS/MS gradient for biotinylated peptide detection. Thus far, we have demonstrated that we can enrich for 500-800 biotinylated proteins, with about 50% annotated as "plasma membrane", "cell surface", or "extracellular". We are currently using this workflow to evaluate the link between neo-termini generation and specific protease activity through in vitro incubation of cultured cells with recombinant proteases.

CONCLUSIONS:
Optimizing a proteomic workflow to isolate and analyze proteins and termini localized on the cell's plasma membrane is the first step in demonstrating that there is cancer-specific proteolytic cleavage. The resulting net-termini are unlikely to be present in healthy cells and can therefore serve as highly specific targets for the development of new therapeutics.

TAYLOR POBRAN

TDP-43 ENRICHMENT USING A HIGH AFFINITY RNA APTAMER

SUPERVISOR: MARI DEMARCO

AUTHOR(S): Taylor Pobran[1], Junyan Shi[1] and Mari L. DeMarco[1,2]

AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada; [2]Department of Pathology and Laboratory Medicine, St. Paul's Hospital, Providence Health Care, Vancouver, Canada

BACKGROUND/OBJECTIVES:
Transactive response DNA binding protein 43 kDa (TDP-43) is a 414 amino acid heterogeneous nuclear ribonucleoprotein that is widely expressed in human tissues. In frontotemporal degeneration and amyotrophic lateral sclerosis, TDP-43 has been identified as the major component of the defining pathological inclusions. As such, there is great interest in detecting, characterizing and quantifying TDP-43 and its disease-related post-translational modifications to investigate pathogenesis. Detailed analysis of TDP-43 in human tissues and biofluids is hindered by sample complexity and the relatively low abundance of TDP-43. While immunoenrichment is a strategy commonly used for improving detection, use of antibodies can be associated with significant start-up costs, high ongoing reagent costs, and concerns regarding analyte specificity and antibody stability. Taking an alternate approach, we developed an RNA aptamer enrichment workflow for TDP-43 followed by detection using liquid chromatography tandem mass spectrometry (LC-MS/MS).

METHODS:
Specimens analyzed included recombinant full length human TDP-43 (recTDP-43), HeLa cell lysate, and human cerebrospinal fluid (CSF), plasma, and intravenous immunoglobulin G. Sodium dodecyl sulfate – polyacrylamide gel electrophoresis followed by western blot analysis for TDP-43 was used to investigate (1) cross-reactivity of detection antibodies, (2) specificity of immunoprecipitation antibodies, and (3) interference from immunoprecipitation substrates. Aptamer enrichment was performed using a synthesized RNA polynucleotide, with a 3' biotin–tetraethyleneglycol spacer arm modification, having previously documented high binding affinity for TDP-43 (KD = 5.3 nM). RecTDP-43 in buffer and endogenous TDP-43 from HeLa cell lysate, were subjected to aptamer enrichment, trypsin digestion and analysis by LC-MS/MS.

RESULTS:
Immunodetection of TDP-43 direct from biofluids demonstrated cross-reactivity with endogenous IgG and albumin. Immunoenriched fractions from recTDP-43 in buffer and HeLa cell lysate demonstrated nonspecific binding on western-blot, consistent with the presence of streptavidin polymers. By western-blot analysis, the RNA aptamer enrichment protocol successfully enriched TDP-43 from HeLa cell lysate. Tryptic digestion of TDP-43 yielded 6 proteotypic peptides, where 3 transitions per peptide were used for a multiple reaction monitoring method. LC-MS/MS analysis of the aptamer-enriched fraction yielded a lower limit of detection for TDP-43 (as determined by a signal-to-noise ratio of at least 3 and corresponding transition peak area ratios within 15 % of the standard) in the low ng/mL range from both buffer with recombinant TDP-43 and endogenous TDP-43 in HeLa cell lysate.

CONCLUSIONS:
By exploiting the RNA-binding function of TDP-43, we developed an aptamer-based enrichment method for TDP-43 coupled to selective detection by LC-MS/MS.

KATLYN RICHARDSON

INVESTIGATING THE ROLE OF GRANZYME K IN PSORIASIS

SUPERVISOR: **DAVID GRANVILLE**

AUTHOR(S): Katlyn C. Richardson[1,3], Christopher T. Turner[1,3], Sho Hiroyasu[1,3], Matthew R. Zeglinski[1,3], Richard I. Crawford[1,2], Angela Burleigh[1,2], David J. Granville[1,3,4]

AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia, BC, Canada; [2]Department of Dermatology and Skin Science, University of British Columbia, BC, Canada; [3]International Collaboration On Repair Discoveries (ICORD) Centre, Vancouver Coastal Health Research Institute, BC, Canada; [4]British Columbia Professional Firefighters' Burn and Wound Healing Group, Vancouver Coastal Health Research Institute, BC, Canada

BACKGROUND/OBJECTIVES:
Psoriasis is a common skin disease characterized by skin inflammation and increased epidermal proliferation forming thick, scaly plaques. Current therapies are not completely effective and present with a number of side effects. Thus, to allow for the development of new therapeutic options, a deeper understanding of the pathological mechanisms associated with psoriasis are necessary. Granzyme K (GzmK) is a serine protease recently elucidated as a mediator of cutaneous inflammation. GzmK is upregulated in sepsis, allergic asthma and burns. The pathological role of GzmK in psoriasis remains unknown. In the present study, we **hypothesize** *that GzmK levels are increased and contribute to the onset and progression of psoriasis through the augmentation of inflammation and/or epidermal proliferation*. The objective of our study is to characterize GzmK expression and function in psoriasis pathogenesis using a combination of *human tissues*, an *animal model* and cell *culture experiments*.

METHODS:
GzmK expression was evaluated histologically in tissue from psoriasis patients and compared to healthy skin controls. The role of GzmK was investigated in a murine model of psoriasis, comparing GzmK knockout to wild-type mice. Psoriasis severity was assessed macroscopically for onset and severity of erythema and plaque formation. Psoriatic tissue was examined histologically for epidermal thickness GzmK expression, collagen organization (Masson's Trichrome, picosirius red, Collage I/III), inflammation (markers of T-cells, macrophages, NK cells), angiogenesis (CD31) and fibrosis (alpha-SMA). To elucidate a mechanistic role, we are currently culturing keratinocytes with GzmK for assessment of epidermal proliferation, cytokine expression and the GzmK degradome.

RESULTS:
GzmK positive cells were markedly elevated in lesional skin from psoriasis patients compared to healthy control skin. Lymphocytes and dendritic cells were identified as the predominant cell type responsible for GzmK expression. Preliminary data suggest that GzmK knockout psoriasis mice have reduced erythema and plaque formation compared to wild-type mice.

CONCLUSIONS:
GzmK, an important mediator of cutaneous inflammation, is elevated in human psoriatic tissue and may contribute to psoriasis lesional development. Overall, this work will provide significant insights into disease mechanisms as well as rationale for developing drugs for the improved treatment of psoriasis.

MARIE-SOLEIL SMITH

PRELIMINARY EVIDENCE THAT DOLUTEGRAVIR REDUCES CELL PROLIFERATION, INCREASES MITOCHONDRIAL DNA CONTENT, AND INDUCES DIFFERENTIATION IN A HUMAN EMBRYONIC STEM CELL LINE

SUPERVISOR: HÉLÈNE CÔTÉ

AUTHOR(S): Marie-Soleil Smith[1,2], Abhinav Ajay Kumar[1,2], Anthony Y.Y. Hsieh[1,2], Izabella Gadawska[1,2], Hélène C.F. Côte[1,2]

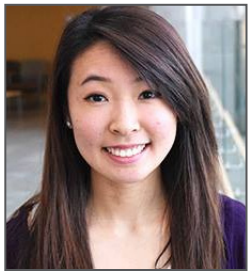
AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver; [2]Centre for Blood Research, University of British Columbia, Vancouver

BACKGROUND/OBJECTIVES:
Approximately 80% of women living with HIV worldwide now receive combination antiretroviral therapy (cART) during pregnancy, reducing their vertical transmission rates to <2%. Most antiretrovirals (ARVs) can cross the placenta, but the safety of many ARVs, such as the newer integrase inhibitors (InSTIs) dolutegravir (DTG) and raltegravir (RAL), have not been characterized in the context of pregnancy. Many ARVs are known to affect mitochondria, causing altered mitochondrial DNA (mtDNA) levels and/or mtDNA mutations, and can lead to mitochondrial dysfunction. Mitochondrial alterations at the initial stages of embryonic development could be catastrophic, as all cell types are derived from human embryonic stem cells (hESCs), which contain relatively few copies of mtDNA, creating a bottleneck. Our objective was to characterize and compare the effects of InSTI containing cART regimens to the current first line cART for pregnant women containing efavirenz (EFV), a non-nucleoside reverse-transcriptase inhibitor (NNRTI), on cultured hESCs, with respect to cellular and mitochondrial toxicities.

METHODS:
CA1S, a subset of the CA1 hES cell line, was used as a cell culture model as it maintains the properties of hESCs while being adapted for cell culture screening. For the preliminary experiments, CA1S cells were cultured in the presence of pharmacological concentrations of DTG-, RAL-, or EFV- containing cART on a tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) drug backbone. TDF+FTC, DTG, or RAL alone were also assessed. After 3.5 days, cells were harvested, their mtDNA content measured by qPCR, and their mitochondrial characteristics investigated by flow cytometry. Specifically, cells were stained with markers for mitochondrial mass, intermembrane potential, ROS, cell viability, and apoptosis. Pluripotency markers, specifically SSEA-3 and TRA-1-60, were also assessed in the same flow panel.

RESULTS:
Early experiments show that cells exposed to TDF+FTC+DTG and DTG alone demonstrated reduced proliferation compared to cells treated with all other drug treatments or with the 0.1% DMSO drug diluent control. Additionally, mtDNA content was elevated in TDF+FTC+DTG and DTG alone compared to the corresponding 0.1% DMSO control. Aligned with the elevated mtDNA content, an upward trend was noted in mitochondrial mass and ROS among the DTG-containing conditions. Pluripotency assessments suggest that DTG-containing conditions promote spontaneous differentiation of hESCs. TDF+FTC+RAL and RAL alone appear to elicit protection against apoptosis.

CONCLUSIONS:
These preliminary data suggest that treatment with DTG induces higher levels of mitochondrial and cellular toxicities than both the first-line cART therapy during pregnancy as well as the other InSTI, RAL. It is imperative to determine the most pregnancy-safe cART regimens, as most women now conceive on cART and DTG is rapidly becoming one of the most commonly used ARVs. This project will provide crucial information that could inform treatment guidelines, particularly for women of childbearing age, such that their regimens are safer during early embryo development.



LILY TAKEUCHI

DEVELOPMENT OF A CARDIAC-TARGETED MACROMOLECULAR IRON CHELATION SYSTEM FOR THE TREATMENT OF TRANSFUSIONAL IRON OVERLOAD

SUPERVISOR: JAYACHANDRAN N. KIZHAKKEDATHU

AUTHOR(S): Lily E. Takeuchi, Srinivas Abbina, Usama Abbasi, Jayachandran N. Kizhakkedathu

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BACKGROUND/OBJECTIVES:
Disorders of hemoglobin, such as sickle cell anemia and thalassemia, are an increasing global health concern requiring chronic transfusion as a life sustaining treatment. Unfortunately, a consequence of chronic transfusion is excess iron burden leading to transfusion-associated iron overload. With iron overload, excess iron is available to mediate oxidative damage of cells, and this iron accumulates in vital organs, namely, the liver and the heart. To date, no methods are available to remove iron from specific organs to prevent organ toxicity; consequently, iron overload remains associated with significant morbidity and mortality. In this work, we developed a macromolecular chelator with enhanced specificity toward heart as an effective system to treat cardiac iron overload. Here, we study the cellular and organ targeting ability, biodistribution, and biocompatibility of our novel cardiac-targeted macrochelator (CTMC).

METHODS:
Fluorescently-tagged CTMC and non-targeted controls were incubated in a co-culture model of iPS cardiomyocytes and BJ human fibroblasts. *In vitro* cellular uptake was measured by flow cytometry and laser scanning confocal microscopy methods. Next, *in vivo* organ targeting ability was determined by injecting ^{3}H-labelled CTMC in Balb/c mice. Qualitative distribution of CTMC was assessed through histological examination of heart tissue collected from mice injected with fluorescently-tagged CTMC. Finally, biocompatibility of the system was assessed by MTT cell viability assay.

RESULTS:
In vitro cellular uptake studies demonstrated a 2.5 ± 0.5-fold uptake of CTMC in as limited as 100 nM doses by iPS cardiomyocytes. *In vivo* radiolabeling study of CTMC demonstrated successful organ-targeting ability with 22 ± 4% injected dose/gram of cardiac tissue retained up to 8 hours post-injection, as compared to 7 ± 1% from non-targeted controls. Finally, biocompatibility testing showed that CTMC incubated cells experience 30% higher cell viability compared to cells incubated with a small molecule chelator.

CONCLUSIONS:
Here, we successfully demonstrate a biocompatible, cardiac-specific targeting approach to chelation therapy. As cardiac failure remains a leading cause of death in iron overload patients the development of chelators targeting heart will be a paradigm shift in the treatment of iron overload.

SIMON TESKEY

TARGETING THE NRF2 PATHWAY TO ENHANCE THE THERAPEUTIC EFFICACY OF TREATMENTS FOR ADVANCED PROSTATE CANCER

SUPERVISOR: MARIANNE SADAR

AUTHOR(S): Simon J.L. Teskey[1,2], Nasrin R. Mawji[2], Marianne D. Sadar[1,2]

AFFILIATION(S): [1]Department of Pathology & Laboratory Medicine, Faculty of Medicine, UBC, Vancouver, Canada; 2Michael Smith Genome Sciences Centre, BC Cancer Agency, 675 West 10th Ave, Vancouver, BC Canada

BACKGROUND/OBJECTIVES:
Prostate cancer is the second leading cause of male-related cancer deaths in the Western world. Primary treatments are often successful, yet 20-30% of patients will have recurrence after radical prostatectomy or radiation therapy. Most prostate cancers are dependent on the androgen receptor (AR) for growth and survival. Full length AR is a ligand activated transcription factor that mediates the effects of androgen. The standard care for advanced prostate cancer is androgen deprivation therapy (ADT) or castration. ADT induces disease regression but is not curative. Most disease will progress to the lethal form known as castration-resistant prostate cancer (CRPC). Additional treatment with chemotherapeutics or 2nd generation anti-androgens may provide a brief window of efficacy before the inevitable emergence of therapeutic resistance. ADT induces oxidative stress in prostate cancer cells and inflammation in prostate tumor tissues, and oxidative stress has been implicated in the conversion of androgen-dependent disease into treatment-refractory CRPC. With the objective to gain a clearer understanding of this progression, we explored strategies to maximize the impact of both current protocols and emerging treatments still in development by co-targeting the redox environment in tandem with AR directed agents. The NRF2 protein is the master regulator of the intracellular redox milieu and a sensitive rheostat for monitoring homeostatic disturbances. It functions as a transcription factor for a diverse set of protective and pro-survival genes including critical antioxidant proteins and phase II detoxification enzymes. Activated NRF2 contributes to the primary cellular defense mechanisms against xenobiotic, metabolic, and oxidative stress. Thus, we hypothesized that activation of the NRF2 pathway influences the efficacy of prostate cancer treatments and could be strategically modulated to enhance therapeutic outcomes.

METHODS:
In vitro experiments were performed in androgen-sensitive (LNCaP) and CRPC (LN-95) human prostate cancer cells. AR associated gene expression was measured using luciferase reporter assays for AR transcriptional activity following transfection to induce transient siRNA- mediated knockdown or cDNA over-expression of NRF2. Treatments assessed alone or in combination include the AR-N terminal domain inhibitor Ralaniten, the antiandrogen Enzalutamide, the NRF2 inhibitor trigonelline, and the redox inhibitor N-acetylcysteine in conditions both with and without the synthetic androgen R1881. AR induced protein expression and altered redox signaling were assayed by Western Blot.

RESULTS:
Transient siRNA- mediated knockdown or cDNA over-expression of NRF2 had a profound effect on AR signaling. Preliminary findings shows that combination AR/NRF2 treatment yielded enhanced inhibition of AR activity, compared to monotherapy.

CONCLUSIONS:
Activation of the NRF2 pathway influences the efficacy of prostate cancer treatments. This knowledge can be exploited to enhance patient outcomes and demonstrates the complexity of cellular mechanisms driving the response to targeted therapeutics.

BRENNAN WADSWORTH

THE LIFETIME OF PERFUSION-LIMITED HYPOXIC TUMOUR CELLS IN SOLID TUMOUR XENOGRAFTS

SUPERVISOR: KEVIN L. BENNEWITH

AUTHOR(S): Brennan J Wadsworth[1,2], Kevin L Bennewith[1,2]

AFFILIATION(S): [1]BC Cancer, Integrative Oncology, Radiation Biology Unit; [2]University of British Columbia, Pathology and Laboratory Medicine

BACKGROUND/OBJECTIVES:
Solid tumours often develop dysfunctional vasculature which can produce regions of diffusion-limited hypoxia (DLH) where cells too distant from blood vessels to receive sufficient oxygen for proliferation, as well as perfusion-limited hypoxia (PLH) where blood flow and therefore oxygen delivery is inconsistent and cells can experience hypoxia-reoxygenation cycles. Hypoxia is associated with resistance to general chemo- and radiation-therapy, while oxygen cycling is shown to increase expression of pro-metastatic genes, although little is understood about the in vivo response of tumour cells to the stress of hypoxia-reoxygenation exposure or how long PLH cells survive in a solid tumour. Aim: The model and rate for loss of PLH is unstudied and the purpose of this study was to quantify the rate of PLH cell loss relative to DLH.

METHODS/RESULTS:
We quantified the longevity of hypoxic tumour cells by administering exogenous markers of hypoxia, 'pimonidazole' and 'EF5', which are reduced and bound to protein in hypoxic cells. We administered pimonidazole between 96 and 24 hours before tumour harvest followed by EF5 3 hours before harvest and compared the relative abundance of pimonidazole and EF5 positive tumour cells. We compared two human tumour cell lines grown as subcutaneous xenografts in immunodeficient mice; 'SiHa' cervical carcinoma tumours demonstrated more rapid hypoxic cell loss than 'WiDr' tumours, with half lives between 50-60hr and 70-80hr respectively. We utilized the perfusion modifying drug pentoxifylline to reoxygenate PLH, indicated by reduced EF5, increased fraction of perfused blood vessels, and increased mismatch between endogenous hypoxia markers and EF5. Pentoxifylline was only effective in WiDr tumours, agreeing with past research that SiHa tumours do not exhibit rapid oxygen cycling. In longevity experiment, when pentoxifylline is administered prior to pimonidazole and EF5 we isolate DLH cells and found no difference in the rate of hypoxic cell loss in SiHa tumours between control and pentoxifylline treated mice, but observed slowed cell loss for pentoxifylline-treated WiDr tumours beginning at 48h hours, indicating a delay in loss of PLH cells compared to DLH. In vitro we observe that rapid oxygen cycling but not chronic hypoxia to induce significant DNA damage indicated by 53BP1 nuclear foci with a modest reduction in cell survival. In vivo we observe greater 53BP1 staining in WiDr tumours, and an enrichment of 53BP1 in hypoxic regions.

CONCLUSIONS:
Overall, these data suggest that PLH cells experience weakly cytotoxic DNA damage while cycling, and that loss of the majority of PLH cells follows DLH sequentially.

SAMUEL SHAO HUAN WENG

SENSITIVE IDENTIFICATION OF PROTEOLYTIC PROTEOFORMS IN LIMITED SAMPLES

SUPERVISOR: PHILIPP LANGE

AUTHOR(S): Samuel S. H. Weng[1,2,5], Fatih Demir[3,5], Enes K. Ergin[1,2], Sabrina Dirnberger[3], Anuli Uzozie[1,2], Domenic Tuscher[3], Lorenz Nierves[1,2], Janice Tsui[1,2,] Pitter F. Husegen[3,4], Philipp F. Lange[1,2]

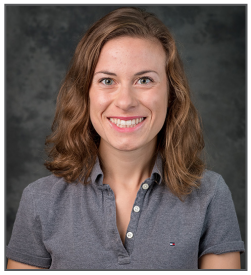
AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada; [2]Michael Cuccione Childhood Cancer Research Program, BC Children's Hospital Research Institute, Vancouver, Canada; [3]Central Institute for Engineering, Electronics and Analytics, ZEA-3, Forschungszentrum Jülich, Germany; [4]Medical Faculty and University Hospital, University of Cologne, Cologne, Germany; [5]These authors contributed equally: Samuel S. H. Weng and Fatih Demir

BACKGROUND/OBJECTIVES:
Protein N termini define different proteoforms arising from limited proteolytic processing, alternative translation initiation and co- or post-translational N-terminal modification. Abnormal proteolytic processing, catalyzed and driven by deregulated proteases, influences cellular regulation and signaling in most malignancies. Therefore, proteases are considered promising drug targets and proteolytic proteoforms may be used as clinical biomarkers. Currently, protein termini are commonly used to identify proteolytic proteoforms and monitor proteolytic activities by following the occurrence and loss of protein termini. However, most N termini enrichment techniques require relatively large amounts of starting material in the range of several hundred micrograms to milligrams proteins which is not feasible for clinical biopsies, particularly from pediatric patients, for comprehensive N terminome analysis. Hence, there is a necessity to improve the sample handling strategy to enable N terminome analysis of limited clinical samples. We hypothesize that utilizing carboxylate-modified magnetic beads along with hydrophobic tagging of internal peptides will reduce sample losses while increasing sensitivity of N termini identification.

METHODS:
To develop and optimize a High-efficiency Undecanal-based N Termini EnRichment (HUNTER) method, HeLa cells were used as a starting material. After cell lysis, protein N-terminus and lysine residues were derivatized by reductive dimethylation. Carboxylate-modified magnetic beads were then introduced to capture proteins and assist sample clean-up. Then, the purified proteins were digested with trypsin. This trypsin digestion generates new peptides with unprotected amine groups at the N-terminus, which are amenable to react with undecanal. Due to the increase in hydrophobicity of undecanal-labeled internal peptides, proteins' N-terminus can be negatively selected with reversed phase chromatography. Particularly, these N termini-enriched samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

RESULTS:
We have identified ~1,000 N termini from 10,000 HeLa cells (2µg of protein lysate) in one hour of LC-MS/MS analysis time or >5,000 N termini from 200µg of protein lysate using high-pH pre-fractionation. HUNTER is easily automated on liquid handling systems increasing data reproducibility. We show the application of HUNTER to sorted human immune cells, subcellular compartments, and plasma from pediatric cancer patients to identify distinct N-terminal profiles.

CONCLUSIONS:
A negative enrichment protocol to isolate protein N-termini from as low as 2µg proteins has been developed. The proposed method could be used in studying rare or precious clinical samples and potentially aid in discovering information related to proteolytic pathways.



ANNA-CATHARINA WILHELM

THE EFFECT OF GRANZYME B-CLEAVED THROMBOSPONDIN 2 ON ENDOTHELIAL CELL PHENOTYPE AND FUNCTION

SUPERVISOR: DAVID GRANVILLE

AUTHOR(S): Anna-Catharina Wilhelm[1,2], Matt Zeglinski[1,2], David Granville[1,2]

AFFILIATION(S): [1]Pathology and Laboratory Medicine, UBC; [2]International Collaboration On Repair Discoveries (ICORD)

BACKGROUND/OBJECTIVES:
Angiogenesis produces new blood vessels by growth, migration, and differentiation of endothelial cells. Thrombospondin-2 (TSP2) is a glycoprotein, secreted by fibroblasts and smooth muscle cells, demonstrated to be a potent inhibitor of angiogenesis in several models. Granzyme B (GzmB) is a serine protease known for its intracellular role in cytotoxic lymphocyte-mediated apoptosis; additionally, evidence demonstrates that extracellular GzmB has the ability to cleave a variety of important extracellular proteins. Extracellular GzmB targets several angiogenic factors including plasminogen, plasmin, and von Willebrand factor. Although both TSP2 and GzmB have been shown to play a role in angiogenesis, it is unknown whether GzmB targets TSP2 and what effect GzmB-cleaved TSP2 may have on new vessel formation. We **hypothesize** that TSP2 inhibits activation of human umbilical vein endothelial cells (HUVECs) and that cleavage of TSP2 by GzmB will negate this inhibitory effect *In vitro*.

METHODS:
TSP2 was cleaved with GzmB (100nM) for 2 hrs and fragmentation was visualized by Coomassie blue. For the inhibition condition, serpinA3N (SA3N) (500nM) and GzmB (100nM) were preincubated for 30 min at room temperature. Cleavage reactions were stopped by the addition of SDS-PAGE loading buffer. Dose-response cleavage was tested using TSP2 incubated with GzmB (1, 10, and 100 nM) at 37 □C for 2 hrs. Time-dependence of TSP2 cleavage using 1nM GzmB was stopped at 0.5, 2, 4, 8, and 18 hours. SA3N dose-response inhibition of 1nM GzmB was tested using 10, 100, and 500 nM SA3N. Viability: Confluent monolayers were exposed to full length TSP2 or GzmB (1, 10, 100 nM) cleaved TSP2 fragments for 24 hours. HUVEC viability was determined using MTT. **Adhesion**: TSP2 coated culture plates were exposed to increasing concentrations of GzmB prior to seeding HUVECs. HUVECs were left to adhere for 24 hrs and non-adherent cells were washed away. Adherent cells were identified by crystal violet.

RESULTS:
Predictive analysis of human TSP2 identified 5 potential cleavage sites for GzmB, most located towards the C-terminal end of TSP2. Cleavage experiments identified two fragments at 115 kDa and 90 kDa. The GzmB dosed-response cleavage assay demonstrated that full length TSP2 was cleaved by 1 nM GzmB, while 10 nM GzmB resulted in a pair of cleavage fragments of 115 and 90 kDa, and 100 nM GzmB resulted in fragmentation around 90 kDa. The time-course cleavage assay demonstrated that the 115 kDa fragment lightened as the 90 kDa band intensified over time. SA3N dosed assay showed complete inhibition of GzmB cleavage of TSP2 with 500 nM SA3N. We detected no major difference in viability between treatment groups of HUVECs treated with GzmB-cleaved TSP2; however, we saw decreased adhesion of cells treated with TSP2 cleaved by increasing GzmB concentrations.

CONCLUSIONS:
We were able to successfully identify human recombinant TSP2 as a substrate of GzmB. With TSP2 fragments obtained from GzmB cleavage, as seen in the cleavage assays, we observed altered cell adhesion but not viability; ongoing work will aim to further test the function of these fragments on HUVEC behavior.

DAPHNE WU

IDENTIFICATION OF BIOMARKERS TO PREDICT PLATELET TRANSFUSION OUTCOME

SUPERVISOR: **DANA DEVINE**

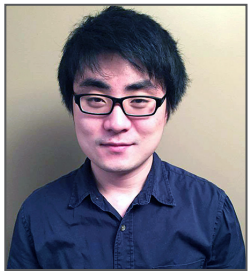
AUTHOR(S): Daphne Wu[1,2], Dana Devin[1,2]

AFFILIATION(S): [1]Department of Pathology & Laboratory Medicine, UBC; [2]Center for Blood Research, UBC

BACKGROUND/OBJECTIVES:
Platelet concentrates deteriorate throughout storage affecting product quality. Although the degree of deterioration varies from donor to donor, there is currently no marker than can identify poor storing donors. Previous research in our lab used proteomic techniques to compare platelets transfused to recipients who subsequently had a good response to the transfusion and those whose transfusion did not work as a way to distinguish good quality platelets from poor quality platelets. Several proteins were identified to be potential indicators of transfusion efficacy. The goal of this study is to validate these proteins as storage biomarkers. Initially, we will compare expression levels of these proteins in apheresis platelets at multiple time points throughout the storage to explore the effect of platelet storage on protein levels. We will then compare expression across multiple donors to understand the range of levels in the platelet donor population. Markers that behave in a predictable manner throughout storage, and which show clear discrimination between good and poor storing platelet donors will make strong candidates. We anticipate being able to access pedigreed samples from a clinical trial of platelet transfusion outcome conducted by colleagues in the UK in order to challenge the ability of candidate biomarkers to predict transfusion outcomes.

METHODS:
The specific candidate biomarkers under study are TMP4, catalase myosin 14 and myosin IIA. To determine protein behaviour throughout the storage period, whole apheresis units are obtained from the Blood4Research program of Canadian Blood Services, stored at room temperature with agitation, and sampled on days 2, 6, and 8 of storage. Each sample is assessed for platelet count by Sysmex hematology analyzer and pH, glucose and lactate by blood gas analyzer (Instrumentation Laboratories). Using flow cytometry, the levels of platelet activation and apoptosis are assessed in each sample. Platelet lysates are prepared and analyzed via SDS-PAGE and western blots using antibodies to catalase, TMP4, myosin 14 and myosin IIA (Abcam).

RESULTS/CONCLUSIONS:
These studies are just starting, and I am currently gaining experience and preliminary results with the techniques. If we are successful in identifying biomarkers that can be used to identify donors whose platelets that do not store well, we can consider new strategies of donor management within blood collection organizations as well as different inventory management strategies in hospital blood banks.



TIM XUE

ENTEROVIRAL INFECTION IN MOUSE BRAIN LEADS TO AMYOTROPHIC LATERAL SCLEROSIS-RELATED PROTEIN PATHOLOGIES AND MOTOR DYSFUNCTION

SUPERVISOR: **HONGLIN LUO**

AUTHOR(S): Yuan Chao Xue[1,2], Gabriel Fung[1,2], Yasir Mohamud[1,2], Eric Deng[1,2], Huitao Liu[2,3], Jingchun Zhang[1,2], Ralph Feuer[4], Neil Cashman[5], Honglin Luo[1,2]

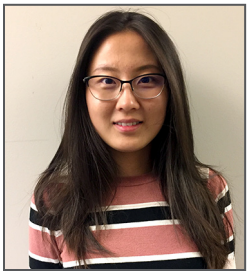
AFFILIATION(S): [1]Centre for Heart Lung Innovation, St. Paul's Hospital; [2]Department of Pathology and Laboratory Medicine, University of British Columbia; [3]Department of Experimental Medicine, University of British Columbia; [4]Department of Biology, San Diego State University; [5]Centre for Brain Health, University of British Columbia

BACKGROUND/OBJECTIVES:
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that targets the motor neurons in the brain and spinal cord, which control the movements of the body. Currently, there are ~3,000 Canadians living with ALS, and the lifetime risk of developing ALS is 1 in 1,000. Currently, without any effective therapies, the destruction of motor neurons leads to paralysis, and eventually death. It has been suspected that enteroviruses (EVs), a family of positive, single-stranded RNA viruses including poliovirus, coxsackievirus, EV-A71, and EV-D68 and the major human pathogens for CNS and heart infection, can induce ALS pathogenesis due to the viruses' ability to target motor neurons. We and others have found that in vitro EV infections, via EV-encoded proteases, can result in protein aggregation, RNA-processing defects, and disruption of nucleocytoplasmic trafficking and autophagy. Of particular interest was the virus-induced cytoplasmic aggregation and cleavage of TDP-43, one of the hallmarks of ALS. **We hypothesis that enterovirus is a putative causal/risk factor for the development of ALS.**

METHODS AND RESULTS:
To test our hypothesis, we intracranially infected neonatal BALB/C mice with either GFP-expressing coxsackievirus B3 (107 pfu/ml) or DMEM (mock infection), and brain samples were collected at days 2, 5, 10, 30 and 90 post-infection and analyzed by immunohistochemical staining. We showed brain lesions and sustained inflammation (GFAP-reactive astrocytes, Iba1-microglia, pSTAT3-astrogliosis, and ChAT-NK maturation) in multiple regions of the brain (hippocampus, cerebral cortex, striatum, olfactory bulb and putamen) in parallel with virus detection through GFP and dsRNA as early as 2 days post-infection. Most notably, ALS-like pathologies, i.e., TDP-43 mislocalization and/or aggregation, were clearly present within virus-infected areas. Moreover, co-localization of TDP-43 with other known ALS-relevant proteins such as p62 and ubiquitin has also been strongly detected within the infected regions, similarly observed in ALS patient tissues.

A similar experimental protocol as above was implemented in both ALS and wild-type control mouse models (SOD1-G85R and C57BL/6, respectively) to investigate the effects of subclinical viral infection mimicking chronic viral infection (500 pfu/mouse) in the development of ALS. The experiment so far showcased significant motor dysfunction in the infected mice starting 28 weeks after the initial infection using both hanging test and footprint analysis. Upon the near future, changes in immune responses and ALS-related protein pathologies will be further investigated after the completion of 60-weeks post-infection scheduled endpoint.

CONCLUSIONS:
Our results reveal that in vivo EV infection, such as coxsackievirus, can cause ALS-like pathologies, especially in the case of TDP-43 abnormality, in virus-infected mouse brains. More tailored experimentation that looks into the exacerbation of viral infection in the development of ALS in ALS and WT mouse model is currently undergoing, which hints the appearance of motor dysfunctions in infected mice.



JOYCE ZHANG

MODELLING INITIATION EVENTS OF SEROUS OVARIAN CANCERS WITH ORGANOID CULTURES AND SINGLE CELL SEQUENCING

SUPERVISOR: DAVID HUNTSMAN

AUTHOR(S): Joyce Zhang[1,2], Dawn Cochrane[1], Kieran Campbell[1], James Hopkins[1], Genny Trigo[1], Germain Ho[1], Winnie Yang[1], Maya DeGrood[1], Sorab Shah[1,2], David Huntsman[1,2]

AFFILIATION(S): [1]Department of Molecular Oncology, BC Cancer Research Centre; [2]Department of Pathology and Laboratory Medicine, University of British Columbia

BACKGROUND/OBJECTIVES:
Ovarian cancer is the fifth most common cause of cancer mortality in women. While high-grade serous carcinoma (HGSC) is the most malignant form of ovarian cancer, accounting for 70% of cases, low grade serous carcinoma (LGSC) is much less common, accounting for 5% of all epithelial ovarian cancer cases. The two subtypes are both thought to arise from epithelial cells at the distal fimbriated end of Fallopian tube; however, they significantly differ in their mutational background as well as clinical prognosis. HGSC is characterized by genome instability and multiple copy-number gains and losses, commonly with co-occurring loss of function mutations in TP53 and BRCA1/2. LGSC has instead more stable genomes, rarely harbours TP53 mutations but has typical gain of function events affecting BRAF, KRAS and NRAS. Interestingly, LGSC NRAS mutations are found with concomitant EIF1AX mutations.

While studies of primary ex vivo cultures of normal Fallopian tubes and mouse models have improved our understanding of serous cancers, there is a need to validate and complement those findings in novel, human-model systems. An organoid is an in vitro 3D multicellular unit that resembles a tissue or organ of the body, better recapitulating cellular states compared to traditional monolayer cell lines.

Objective: to investigate how mutations drive the early stages of HGSC and LGSC tumorigenesis, with the organoid system and single-cell sequencing.

METHODS:
Mutations are introduced into primary cells of fimbriated end of Fallopian tube tissues from individuals undergoing surgical procedures for non-cancerous reasons. To model HGSC, epithelial cells are subjected to knockout of TP53 and BRCA1 with CRISPR/Cas9 system. Each vector contains a different fluorescence marker that enables sorting of the organoids for sequencing analysis. Similarly, to model LGSC, activating mutations of Nras(Q61R) and eIF1a (G8E), are introduced along with different fluorescent markers. Organoids are cultured from transduced primary cells and genomic perturbations are resolved with single-cell sequencing.

RESULTS:
Histological analysis of HGSC-modelling organoids showed cells with aneuploidy, pleomorphic nuclei, and mitotic figures. A pilot experiment of single cell RNA-sequencing (scRNA-seq) was conducted, and consistent with literature, mutants are enriched with secretory cell type, with several potential targets we plan to validate with immunohistochemistry. LGSC-modeling organoids show intense staining of nuclei, with a few cells show multiple nucleoli and mitotic figures. Pilot scRNA-seq experiment has shown upregulation of genes involved in mRNA translation such as ribosomal subunits, translation initiation and elongation factors.

CONCLUSIONS:
Organoid culture and single-cell sequencing represent a powerful duo in studying the initiation events in serous carcinomas of the ovary. Our work is crucial for developing early detection and prevention strategies as well as more informed and targeted treatment options. We plan to seek new treatment options targeting the perturbed pathways as informed by scRNA-seq analysis.

GUANGZE ZHAO

COXSACKIEVIRUS B3-INDUCED CLEAVAGE OF DESMOSOME PROTEINS ENHANCES DEGRADATION OF GAMMA-CATENIN AND BENEFITS WNT/BETA-CATENIN SIGNALING PATHWAY

SUPERVISOR: DECHENG YANG

AUTHOR(S): Guangze Zhao[1,2], Huifang M. Zhang[1,2], Ye Qiu[1,2], and Decheng Yang[1,2]

AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]The Centre for Heart Lung Innovation, St. Paul's Hospital, Vancouver, CanadaUniversity of British Columbia

BACKGROUND/OBJECTIVES:
Viral myocarditis is major heart disease in children and young adolescents, the late phase of this disease may lead to heart failure and sudden death. Coxsackievirus B3 (CVB3) is the primary pathogen for myocarditis. However, the mechanism by which CVB3 destroys cardiomyocytes is still unclear. Recent progress in understanding the structure of Intercalated Disc (ICD) helps to elucidate the relationship between CVB3 pathogenesis and myocarditis. ICDs are substantial connections maintaining cardiac structure and mediating signal communication among cardiomyocytes. Deficiency in ICD components, such as desmosome proteins, leads to heart dysfunction. Gamma-catenin is a component of ICD directly binding to desmocollin2 and desmoglein2 in desmosomes, thus gamma-catenin not only plays an essential role in the stability of cardiac muscle but also participates in signal transduction pathways.

METHODS AND RESULTS:
We found that CVB3 infection downregulated gamma-catenin at the protein level but not at mRNA level in mouse HL-1 cardiomyocytes. We further found that the reduction of gamma-catenin is due to the ubiquitin-proteasome-mediated pathway of protein degradation since proteasome inhibitor MG132 could rescue the protein decrease. In addition, we also found that desmocollin2 and desmoglein2 were cleaved by viral protease 2A and virus-activated cellular caspase, respectively. These cleavages led to the release of their interacting protein gamma-catenin from desmosome into the cytosol, resulting in rapid degradation. Since gamma-catenin shares high sequence homology with beta-catenin in binding the TCF/LEF transcription factor, we further studied the effect of gamma-catenin degradation on Wnt/beta-catenin signaling. Luciferase assay after co-transfecting HeLa cells with the reporter and gamma-catenin plasmid showed that gamma-catenin inhibited Wnt/beta-catenin signal. This finding was substantiated by q-PCR to show the downregulation of Wnt signal target genes such as c-Myc, cyclin D1, and MMP9.

CONCLUSIONS:
Taken together, our results indicate for the first time that CVB3 infection kills the cardiomyocytes by damaging the desmosome structure and altering the Wnt/beta-catenin singling pathway.

HANQI (WAYNE) ZHAO

CHARACTERIZATION OF COLD-STORED PLATELETS *IN VITRO*

SUPERVISOR: **DANA DEVINE**

AUTHOR(S): **Hanqi (Wayne) Zhao[1,2], Katherine Serrano[1,2,3], Peter Schubert[1,2,3], Elena Levin[2,3], Brankica Culibrk[2,3], Zhongming Chen[2,3], and Dana V.Devine[1,2,3]**

AFFILIATION(S): **[1]Department of Pathology and Laboratory Medicine; [2]Centre for Blood Research, University of British Columbia, Vancouver, British Columbia, Canada; [3]Centre for Innovation, Canadian Blood Services, Vancouver, British Columbia, Canada**

BACKGROUND/OBJECTIVES:
For every 4 platelet units collected, 1 unit will be thrown away due to expiration. Platelet unit wastage costs our health care system some 7.2 million dollars in 2016. Currently, platelet concentrates are stored at room temperature (22°C) (RPs) under constant agitation for only seven days. Cold stored platelets (CPs) have been proposed as a potential storage method to extend storage life by preserving platelet hemostatic function. However, refrigeration can drastically reduce the number of platelets during storage by inducing aggregate formation. The effect of refrigeration on CPs is also not well established for buffy coat derived platelets. Furthermore, most CPs are stored at 4°C without agitation. This can lead to platelet settling in close proximity and facilitating aggregation. We hypothesize that cold storage will better preserve the function of platelets compared to room temperature storage. In addition, agitating CPs during storage may alleviate the decrease in platelet count during cold storage.

METHODS:
Platelet concentrates were produced using standard buffy coat method by Canadian Blood Services. In order to remove the effect of donor variability, platelets were pooled together and split into three standard storage bags. Platelet concentrates were either stored at 22°C with constant agitation, 4°C without agitation or 4°C with constant agitation. Sample from each bag was taken on day 1,2,4,7,9,11 and 14 of storage. The *in vitro* characteristics were assessed by hematology analyzer, blood gas analyzer and flow cytometry. The hemostatic capability of platelets was assessed by aggregometry.

RESULTS:
Throughout 14 days of storage, RPs maintained a relatively constant platelet count whereas CPs, both agitated and non-agitated, had significantly lower platelet counts. Agitated CPs seem to have the lowest count out of three storage conditions. Storing at 4°C significantly slowed the metabolism of platelets as shown by the relatively constant pH, decreased consumption of glucose and production of lactate in CPs compared to RPs. No significant difference was observed between agitated and non-agitated CPs in this case. Refrigeration also pre-activated platelets as shown by platelet degranulation marker (CD62P). Platelets stored overnight at 4°C showed similar CD62P expression compared to RPs stored up to 5 days. CPs showed improved responses to physiological agonists (ADP, collagen with epinephrine) as assessed by platelet aggregometer. From storage day 7 and onwards, RPs did not have any aggregation response when stimulated with ADP whereas CPs maintained response until day 14. There was no significant difference between agitated and non-agitated CPs in agonist response.

CONCLUSIONS:
CPs have reduced metabolism and maintained acceptable pH. CPs also have better response to agonists compared to RPs. However, agitating CPs did not improve platelet count during storage. Overall, CPs have better hemostatic potential than RPs *in vitro*.

ADAM ZIADA

PROPOSAL TO INVESTIGATE THE EFFECTS OF INTERRUPTIONS IN HIV THERAPY ON MITOCHONDRIAL AGING

SUPERVISOR: HÉLÈNE CÔTÉ

AUTHOR(S): Adam S. Ziada[1,2], Nancy Yang[1,2], Beheroze Sattha[1,2], Hélène C.F. Côté[1,2,3], and The CIHR Team Grant on Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; [2]Centre for Blood Research, University of British Columbia, Vancouver, BC, Canada; [3]Women's Health Research Institute, Vancouver, BC, Canada

BACKGROUND/OBJECTIVES:
HIV+ individuals experience accelerated aging, with a reported decrease in lifespan of up to 10 years, as well as earlier onset and higher prevalence of age-related comorbidities, including cardiovascular disease, non-AIDS-defining cancers, neurocognitive decline, and osteoporosis. It is unclear if this is due to HIV infection, off target effects of HIV antiretroviral therapy, or both. Many antiretroviral drugs are known to negatively affect mitochondrial DNA (mtDNA). Current theories of aging describe both de novo mtDNA mutations, as well as the clonal expansion of pre-existing mutations, as potential mechanisms for the increased prevalence of mtDNA mutations seen with aging. HIV+ individuals may age faster in part because they experience an accelerated accumulation of mtDNA mutations, either somatic (*de novo*) mutations, or higher frequency mtDNA variants (heteroplasmy) that likely result from the clonal expansion of mutations.

My preliminary work suggests that uncontrolled HIV replication, as would occur during interruption(s) in HIV therapy, may be associated with higher blood mtDNA somatic point mutation burden. A previous clinical trial that examined the effects of scheduled HIV treatment interruptions clearly demonstrated the negative clinical impact of therapy interruptions whereby those in the interruption arm had increased risk of cardiovascular disease, renal disease, liver disease and mortality. These have also been associated with aging and/or mitochondrial dysfunction. Given that HIV therapy can alter mtDNA content, it is plausible that stopping HIV therapy, hence removing drug pressure on the mitochondria, promotes the clonal amplification of mtDNA mutations.

Hypothesis: Interruptions in HIV antiretroviral therapy will be associated with an increase in the frequency of blood somatic mtDNA substitutions and/or the occurrence of mtDNA heteroplasmy.

Objective: To measure and compare mtDNA somatic point mutations and heteroplasmy among HIV-negative controls, HIV+ individuals with no antiretroviral therapy interruptions, and HIV+ individuals who have had one or more interruptions.

METHODS:
I will measure blood mtDNA somatic mutation frequency and heteroplasmy via Illumina next generation sequencing in HIV-negative (n=72) and HIV+ (n=219) individuals, ranging between 1 and 75 years of age, and already enrolled in the CARMA cohort. Among HIV+ individuals, 53 have never experienced an interruption in HIV therapy, while 166 have had between 1 and 17 therapy interruptions lasting >28 days. In addition to number of interruptions, both general (e.g. age, smoking status) and HIV specific (e.g. HIV viral load, CD4+ cell count) factors will be investigated as possible predictors of mtDNA somatic mutation frequency and heteroplasmy via multivariable linear regression modeling.

SIGNIFICANCE:
While HIV antiretroviral therapy has allowed HIV+ individuals to control their viral burden and prevent disease progression to AIDS, therapy interruptions remain common for a variety of reason. My study will inform health care providers and people living with HIV about the potential effects associated with therapy interruptions.

JOHN AIRD

MISMATCH REPAIR PROTEIN IMMUNOHISTOCHEMISTRY IN CASES OF RECURRENT GYNECOLOGIC AND GASTROINTESTINAL MALIGNANCIES: IS THERE A NEED FOR REPEAT STAINING?

SUPERVISOR: DAVID SCHAEFFER

AUTHOR(S): John Aird[1,2], Mike Steel[1], Robert Wolber[2,3], Lien Hoang[1,2], David Schaeffer[1,2]

AFFILIATION(S): [1]Division of Anatomic Pathology, Vancouver General Hospital, Vancouver, BC, Canada; [2]The University of British Columbia, Vancouver, BC, Canada; [3]Department of Laboratory Medicine and Pathology, Lions Gate Hospital, North Vancouver, BC, CanadaServices, Vancouver, British Columbia, Canada

BACKGROUND/OBJECTIVES:
The use of immune checkpoint inhibitors for the treatment of recurrent/progressive malignancies which fail to respond to prior cytotoxic therapy has been endorsed in a variety of tumor types. As a result, there have been increased requests to re-test metastatic/recurrent tumors for mismatch repair (MMR) proteins, despite testing having already been done on the original tumor. It remains unclear however, whether there is any benefit in re-testing patients. The aim of our study was to compare MMR staining patterns in primary and metastatic/recurrent tumors and document any changes in MMR staining pattern. We also assessed the MMR profiles of synchrononus and metachronous colorectal adenocarcinomas.

METHODS:
Using the pathology laboratory database, we identified patients who had MMR immunohistochemistry (IHC) performed on their primary tumor and who had a recurrence confirmed by tissue biopsy. We performed MMR IHC on the recurrence if it had not been done at the time of diagnosis. A tissue microarray was constructed for IHC testing consisting of duplicate 0.6mm cores stained for PMS2, MLH1, MSH2 and MSH6. We also identified patients who had a synchronous or metachronous colorectal adenocarcinoma. All synchronous and metachronous colorectal adenocarcinomas had MMR IHC performed on both tumors at the time of diagnosis.

RESULTS:
3/144 (2%) tumors showed a difference in MMR profile in a recurrence although mitigating factors were recognised in all 3 cases. 2/14 (14%) metachronous tumors showed a difference in MMR profile. 7/27 (26%) synchronous tumors showed a difference in MMR profile.

CONCLUSIONS:
There is no benefit to retesting tumor recurrences for MMR if the original tumor has been tested. MMR should be performed on both tumors in cases of synchronous and metachronous colorectal adenocarcinoma. Pathologists should be cautious reporting MMR IHC in tissue that has been previously radiated and if there is no internal control.

JOHN AIRD

PROGNOSTIC ASSESSMENT OF IMMUNOGENICITY IN AMPULLARY CARCINOMA POINTS TOWARDS AN IMMUNOMODULATORY THERAPEUTIC OPTION

SUPERVISOR: DAVID SCHAEFFER

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AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; [2]Centre for Blood Research, University of British Columbia, Vancouver, BC, Canada; [3]Women's Health Research Institute, Vancouver, BC, Canada

BACKGROUND/OBJECTIVES:
The prognostication of ampullary carcinoma (AC) has largely relied on differentiation of the two main subtypes: pancreatobiliary (PB-Type) and intestinal (I-Type). Previous studies have shown that PB-Type has an inferior prognosis relative to I-Type. While much of the recent literature has focused on how best to categorize AC, little attention has been given to intrinsic characteristics such as immunogenicity which may have prognostic effects that transcend subtypes.

METHODS:
A fully clinically annotated duplicate 0.6mm core tissue microarray of the epithelial and stromal components of 97 pathologically confirmed AC was assessed for CD8 & CD3 T-cells. Absolute counts were conducted for each core and the maximum count between cores was taken as the score for the histological component. Absolute counts and localization of the respective T-cell phenotypes were compared across histological subtypes and subjected to univariable and multivariable prognostic assessments. Association with PD-L1 staining of the epithelial component was also evaluated.

RESULTS:
Parametric survival analysis for the quantitative assessment of infiltrating lymphocytes showed a statistically significant improvement in disease specific prognosis with increasing quantities of infiltrating T-cells. Univariable assessment of compartment specific T-cell infiltrates indicated that the presence of either CD3+ or CD8+ intraepithelial T-cells confers a superior prognosis relative to T-cells being confined to the stroma or completely absent. Multivariable analysis indicated that increased CD3+ and CD8+ T-cell counts confer improved survival adjusted for ampullary subtype. CD8+ T-cell counts confer a larger improvement on a per unit basis.

CONCLUSIONS:
This study suggests that immune infiltrate may be of more importance than ampullary subtype for prognostic assessment. The strongest prognostic associations were conferred by CD8+ intraepithelial T-Cells.

WAI HANG (TOM) CHENG

CHIMERA REPETITIVE MILD TRAUMATIC BRAIN INJURY INDUCES CHRONIC BEHAVIORAL AND NEUROPATHOLOGICAL PHENOTYPES IN WILD-TYPE AND APP/PS1 MICE

SUPERVISOR: CHERYL WELLINGTON

AUTHOR(S): Wai Hang Cheng[1], Kris M. Martens[1], Asma Bashir[1], Honor Cheung[1], Sophie Stukas[1], Ebrima Gibbs[2], Dhananjay R. Namjoshi[1], Emily B. Button[1], Anna Wilkinson[1], Carlos J. Barron[1], Neil R. Cashman[2], Peter A. Cripton[3], Cheryl L. Wellington[1]

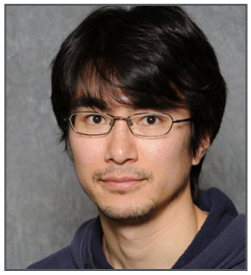
AFFILIATION(S): [1]Department of Pathology and Laboratory Medicine, UBC; [2]Department of Neurology, UBC; [3]Department of Mechanical Engineering, International Collaboration on Repair Discoveries, UBC

BACKGROUND/OBJECTIVES:
The annual incidence of traumatic brain injury (TBI) in the USA is over 2.5 million, with over 3 million people living with chronic sequelae. Compared to moderate-severe TBI, the long-term effects of mild TBI (mTBI) are less understood but important to address particularly for contact sport athletes and military personnel who have high mTBI exposure. The purpose of this study was to determine the behavioral and neuropathological phenotypes induced by the closed head impact model of engineered rotational acceleration (CHIMERA) model of mTBI in both wild-type (WT) and APP/PS1 mice up to 8 months post-injury.

METHODS:
Male WT and APP/PS1 littermates received sham or repetitive mTBI (rmTBI; 2x 0.5J impacts 24 hr apart) at 5.7 months of age. Animals were assessed for acute neurological deficits by loss of righting reflex and neurological severity score, and chronic (up to 8 months) behavioral changes using passive avoidance, Barnes maze, elevated plus maze and rotarod. Neuropathological assessments included white matter damage, grey matter inflammation, A-beta levels, amyloid, and aducanumab-binding activity.

RESULTS:
CHIMERA rmTBI used here produced no significant acute neurological or motor deficits in WT and APP/PS1 mice, but profoundly inhibited extinction of fear memory specifically in APP/PS1 mice over the 8-month assessment period. Spatial learning and memory were affected both by injury and genotype. Anxiety and risk-taking behavior were affected by injury but not genotype. CHIMERA rmTBI induced chronic white matter microgliosis, axonal injury and astrogliosis independent of genotype in the optic tract but not corpus callosum, and altered microgliosis in APP/PS1 amygdala and hippocampus. RmTBI did not alter long-term tau, A-beta or amyloid, but increased aducanumab-binding activity.

CONCLUSIONS:
RmTBI leads to chronic changes in behaviour and neuropathology. The presence of A-beta significantly modifies TBI-induced neuroinflammation and induces post-traumatic stress disorder (PTSD)-like changes.



SHO HIROYASU

GRANZYME B: A NOVEL TARGET FOR PEMPHIGOID DISEASES

SUPERVISOR: DAVID GRANVILLE

AUTHOR(S): Sho Hiroyasu[1,2,3], Valerio Russo[1,2,3], Matthew R. Zeglinski[1,2,3], Hongyan Zhao[1,2,3], Yoan Machado[4,5], Anika Kasprick[6], Chiharu Tateishi[7], Wataru Nishie[8], Angela Burleigh[9], Peter A. Lennox[10], Nancy Van Laeken[10], Nick J. Carr[10], Richard I. Crawford[2,9], Hiroshi Shimizu[8], Daisuke Tsuruta[7], Christopher M. Overall[4,5], Ralf J. Ludwig[6], and David J. Granville[1,2,3]

AFFILIATION(S): [1]International Collaboration On Repair Discoveries (ICORD) Research Centre, Vancouver, BC, Canada; [2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; [3]BC Professional Firefighters' Burn and Wound Healing Research Laboratory, Vancouver, BC, Canada; [4]Centre for Blood Research, University of British Columbia, Vancouver, BC, Canada; [5]Department of Oral Biological and Medical Sciences, Faculty of Dentistry, University of British Columbia, Vancouver, BC, Canada; [6]Lübeck Institute of Experimental Dermatology, University of Lübeck, Lübeck, Germany; [7]Department of Dermatology, Osaka City University Graduate School of Medicine, Osaka, Japan; [8]Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [9]Department of Dermatology and Skin Science, University of British Columbia, Vancouver, BC, Canada; [10]Department of Surgery, University of British Columbia, Vancouver, BC, Canada

BACKGROUND/OBJECTIVES:
Pemphigoid diseases are a subgroup of autoimmune skin blistering diseases characterized by widespread tense skin blisters. The standard treatment is systemic corticosteroid, which often causes fatal adverse effects. Proteases such as neutrophil elastase and MMP-9 play important roles in the pemphigoid diseases through the cleavage of hemidesmosomal proteins. However, to date, clinical trials targeting proteases have been limited, because application of these inhibitors often causes severe adverse effects. In this study, we investigated the role of the serine protease, granzyme B (GzmB), in pemphigoid disease pathology. GzmB expression in non-inflammatory skin is quite low, therefore the inhibition of its function in the skin may not induce intolerable adverse effects. We hypothesized that GzmB contributes to both the afferent and efferent phases of the diseases through the cleavage of hemidesmosomal proteins.

METHODS:
Skin biopsies and blister fluids were collected from patients with bullous pemphigoid (BP) or inflammatory epidermolysis bullosa acquisita (EBA). Tissue and blister fluids were assessed for GzmB using immunohistochemistry and enzyme-linked immune sorbent assay (ELISA), respectively. Hemidesmosomal proteins in the lesional tissues were also assessed by immunohistochemistry. GzmB-mediated hemidesmosomal protein cleavage and cell attachment assays were performed. To test its function in the efferent phase of the pemphigoid diseases, a passive transfer model of EBA on GzmB-deficient mice was analyzed with clinical score, histological blistering score, immunohistochemistry, and western blotting. Finally, to test the role of GzmB in the afferent phase, cleavage sites of collagen XVII by GzmB were analyzed using western blotting or mass spectrometry.

RESULTS:
Abundant GzmB and loss of hemidesmosomal proteins were identified on the pemphigoid-disease patient lesional samples. GzmB cleaved hemidesmosomal proteins (collagen XVII and alpha6beta4 integrin) to reduce cell attachment strength. GzmB-deficient mice with EBA exhibited a reduction in affected-body surface area, histological blisters, neutrophil infiltration, and hemidesmosomal-protein loss, compared to the wild-type mice with the disease. Cleavage analyses of collagen XVII suggested that GzmB cleavage occurs in the NC16a domain, which is the pathological-autoantibody recognition site.

CONCLUSIONS:
GzmB contributes to pemphigoid disease pathology through the cleavage of hemidesmosomal proteins. The results support the hypotheses that GzmB is a potential target for the treatment in human patients of pemphigoid diseases.

ANULI UZOZIE

INTEGRATED PROTEOME, PHOSPHO-PROTEOME AND N TERMINOME CHARACTERIZATION OF PEDIATRIC PATIENT AND MATCHED ENGRAFTED LEUKEMIA

SUPERVISOR: PHILIPP LANGE

AUTHOR(S): Anuli Uzozie[1,3], Janice Tsui[1], Christopher Maxwell[2,3], James Lim[2,3], Gregor Reid[2,3], Philipp Lange[1,3]

AFFILIATION(S): [1]Department of Pathology, University of British Columbia, Vancouver, BC, Canada; [2]Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada; [3]Michael Cuccione Childhood Cancer Research Program, BC Children's Hospital, Vancouver, BC, Canada

BACKGROUND/OBJECTIVES:
Patient leukemic cells implanted in mouse exhibit the genomic diversity of the primary clinical sample. Often referred to as patient derived xenograft (PDX), these models generate conducive environments to study cancer growth, monitor disease development, and evaluate treatment options for the original patient. The extent to which implanted outgrowths reflect protein expression and processing in primary samples is not clear. This study employed mass spectrometry-based proteomics methods to characterize the proteome, phospho-proteome and N terminome of patients and matched xenografts.

METHODS:
Bone marrow mononuclear cells collected from 14 patients diagnosed with B- and T- cell acute leukemia were implanted in immunodeficient mice to generate PDX models for each patient. Multi-proteomics was performed on a Q-Exactive HF mass spectrometer. Whole proteome of each sample was investigated with data independent acquisition method. Phosphorylated peptides enriched with Fe-NTA beads where analyzed by data dependent and data independent acquisition. Protein N termini was studied using our newly developed HUNTER method for sensitive negative selection of peptide N termini.

RESULTS:
Our robust study quantified 5484 protein groups, 2578 phosphorylated peptides, and 3075 N termini across samples. Strong similarity between primary and PDX samples was mostly maintained at the proteome level (R2>0.80). Disease-related processes and signaling network that were not well established in the PDX models were also captured, in particular, in cell secretion and immune response.

CONCLUSIONS:
This study will further characterize cancer-driven pathways that are retained or absent in patient PDX models, and explore the interplay between protein abundance, phosphorylation and proteolysis in childhood leukemia.

MATTHEW MASOUDI

TRANSLATING RESEARCH INTO PRACTICE: INVESTIGATING THE IMPACT OF ALZHEIMER'S DIAGNOSTICS IN CANADA (IMPACT-AD)

SUPERVISOR: MARI DEMARCO

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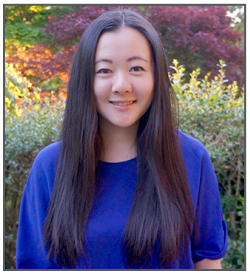
BACKGROUND/OBJECTIVES:
A current challenge in Alzheimer's disease research and patient care is the ability to confidently diagnose Alzheimer's disease *in vivo*, that is, to distinguish the early symptoms of Alzheimer's disease from other causes of mild cognitive impairment. Fortunately, we now know that the measure of amyloid-beta and tau proteins in cerebrospinal fluid can improve diagnostic accuracy. As a result, many countries have adopted testing for amyloid-beta and tau testing for Alzheimer's disease. Canada, however, has yet to act on current recommendations due to a lack of evidence of the utility and benefit of these biomarkers within our healthcare system. The goal of this study is to bridge this knowledge translation gap between diagnostic accuracy studies and clinical utilization of testing for amyloid-beta and tau within the Canadian healthcare system.

METHODS:
IMPACT-AD is an observational study that will develop a comprehensive understanding of how Alzheimer's disease biomarker testing impacts medical and personal decision-making, and healthcare costs. This study has partnered with the COMPASS-ND study, which is enrolling individuals with various types of cognitive disorders from 12 sites across Canada. Participants (n = 400) will undergo a thorough diagnostic workup by a dementia specialist, including a lumbar puncture for analysis of amyloid-beta and tau proteins in cerebrospinal fluid. Dementia specialists will be surveyed pre- and post-disclosure of biomarker results for data including: diagnosis, diagnostic confidence, and medical management plans. The biomarker results will also be shared with the participants and their study partners, who will also be surveyed pre- and post-biomarker results for information including, for example, understanding of the diagnosis, and long-term care and financial planning.

RESULTS:
IMPACT-AD will generate first-of-its-kind data on the diagnostic, economic and personal utility of Alzheimer's disease biomarkers as part of the diagnostic workflow for patients with cognitive impairment.

CONCLUSIONS:
IMPACT-AD was designed to inform positive change within the Canadian health care system to improve care and support for individuals living with Alzheimer's disease and their families.



VEENA LIN (POSTER WITHOUT PRESENTER, AL ROHET HOSSAIN WILL BRING IN THE POSTER)

DIFFERENTIAL EXPRESSION OF NUP98-ERBB4-PSEN1-NRG1 (NEPN) SIGNALING AXIS AS POTENTIAL BLOOD-BASED BIOMARKERS FOR VIRAL MYOCARDITIS

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BACKGROUND/OBJECTIVES:
Myocarditis, inflammation of the heart muscle, is a leading cause of sudden and unexpected heart failure in young adults. Viruses are the most common etiology in developed countries of myocarditis, which can manifest in a wide range of clinical presentations, ranging from asymptomatic to cold and flu-like symptoms to sudden heart failure. Chronic myocarditis results from persistent inflammation, leading to life-threatening arrhythmias and dilated cardiomyopathy, which may necessitate mechanical assist devices or cardiac transplantation. The current gold standard for diagnosis relies upon the antiquated Dallas Criteria, which requires a heart biopsy and has a high rate of false negatives, with correct diagnoses made in only 30% of patients. The combination of all of these factors make myocarditis exceedingly difficult to diagnose, hence why its prevalence continues to be underestimated. To address these challenges, we are examining expression of NUP98-ERBB4-PSEN1-NRG1 (NEPN) signaling axis proteins, all of which have previously been implicated in immune response and heart failure. Our goal is to identify novel biomarkers and their cleavage fragments that are highly specific to viral myocarditis and may be detected in the blood for non-invasive, more sensitive diagnosis of the disease.

METHODS:
HeLa cells and human induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CM) were infected with sham, UV-irradiated CVB3 or CVB3. Western blot analysis and confocal microscopy were performed to determine the expression and subcellular localization of NEPN signaling axis proteins in tissue culture. In addition, tissue and blood from murine models of viral myocarditis (A/J and C57BL/6 mice) were collected at 4 dpi (viremic phase), 5 to 8 dpi (acute phase), and 21 dpi (chronic phase) for Western blot analysis, IHC and IF.

RESULTS:
NUP98, ERBB4, PSEN1 and NRG1 all show changes in expression and subcellular localization throughout the infection time course. NUP98 is upregulated, cleaved and accumulates in the nucleus during infection. NRG1 is upregulated in both lysates and supernatant, although at differing protein sizes. ERBB4 co-localizes at the cell membrane with the protease PSEN1. ERBB4 cleavage fragments are secreted and detected in both tissue culture supernatant and mice plasma. In the plasma of the less susceptible C57BL/6 mice, an ERBB4 cleavage fragment increases throughout the acute and viremic phase. However, in the highly susceptible A/J mice, ERBB4 cleavage fragments were not detected in the plasma until 6 dpi (acute phase), where it highly upregulated, even more so than in C57BL/6 mice.

CONCLUSIONS:
Differential expression, subcellular localization and cleavage fragments of NEPN signaling axis proteins were detected during the pathogenesis of viral myocarditis, which may be useful for the development of a blood-based diagnostic for the disease.

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