PATHOLOGY
laboratory medicine
FACULTY OF MEDICINE
UNIVERSITY OF BRITISH COLUMBIA

Pathology Day

Abstract Book 2018

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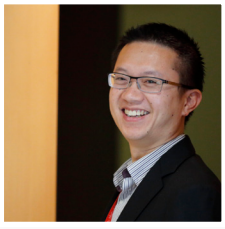
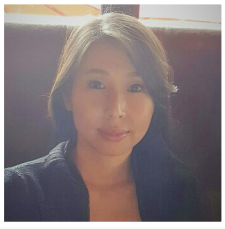
Acknowledgements

THE PATHOLOGY DAY COMMITTEE


Mike Allard


Corree Laule


Tony Ng


Sophia Wong


Daniel Owen


Brennan Wadsworth


Lise Matzke

WE HOPE YOU ENJOY PATHOLOGY DAY 2018.

Pathology Day is a team effort and we would like to extend our thanks to everyone who contributed to the 2018 edition.

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

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

This year these include:

Reza Alaghehbandan
Mike Allard
Vilte Barakauskas
Waren Baticados
Amanda Bradley
Jonathan Bush
Emily Button
Ellen Cai
Nevio Cimolai
Helene Cote
Andy De Souza
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Jeff Terry
Emma Todorovic
Peter van den Elzen
Susanne Vercauteren
Brennan Wadsworth
Yemin Wang
Sophia Wong
Aleksandra Stefanovic
Jennifer Xenakis
Wei Xiong
Damian Yap

Finally, sincere thanks to the staff who kindly assisted with technical and administrative support throughout the day and our photographers: Debbie Bertanjoli, Heather Cheadle, Helen Dyck, Sandra Izzard, Dan Kim, Julie Ho, Daniel Owen, Jenny Tai, Brennan Wadsworth, Joanne Wouterse, Jennifer Xenakis and Phuong Yung.

Message

Pathology Day

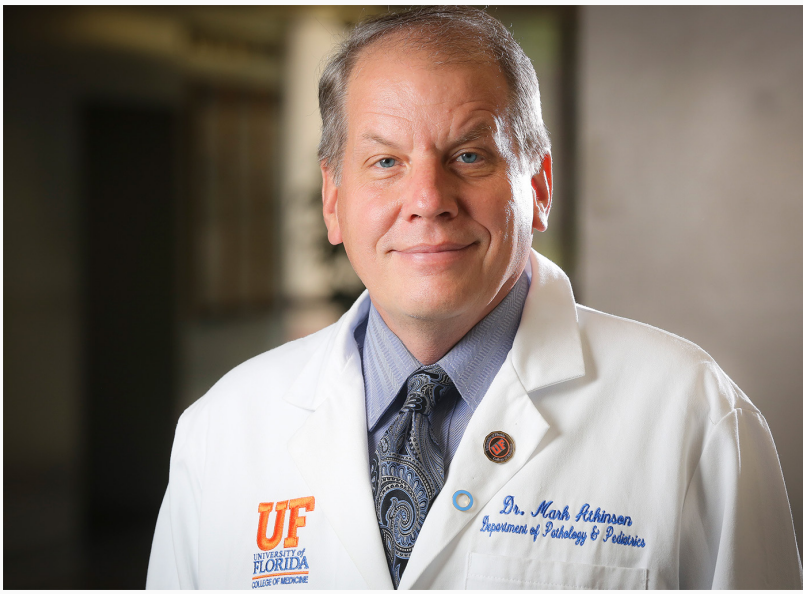
Pathology Day is a critically significant event in the departmental calendar as it serves as a time to showcase the broad spectrum of scholarly activities performed by our trainees and, by extension, our faculty. This gathering also provides a perfect venue to recognize outstanding contributions by members of the department. Pathology Day serves another very important function as well. It is an opportunity to get together to socialize and learn more about one another as well as gain an appreciation and understanding for the breadth of scholarly activities that take place in our geographically dispersed department. As for last year, a few members of our department will present a brief overview of their work as a way to facilitate this

We are very fortunate to have two outstanding individuals participate in the program this year, highlighting academic excellence and continuing in the tradition of having leaders in their disciplines speak at Pathology Day. Dr. Morris Pudek will give the James Hogg Lecture, while Dr. Mark A. Atkinson (Professor and American Diabetes Association, Eminent Scholar Chair at The University of Florida College of Medicine) is our Keynote Speaker.

I wish to extend my sincere thanks and gratitude to members of the committee responsible for organizing the event, including Dr. Corree Laule, Dr. Tony Ng, Dr. Sophia Wong, Dr. Daniel Owen, 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. MICHAEL ALLARD
DEPARTMENT HEAD



Keynote Speaker

MARK ATKINSON, PhD

Professor at The University of Florida
American Diabetes Association, Eminent Scholar Chair

"LESSONS FROM THE HUMAN PANCREAS - RE-WRITING THE TEXTBOOKS ON HOW AND WHY TYPE 1 DIABETES DEVELOPS"

4:25 pm - 5:25 pm
Cordula and Gunter Paetzold Health Education Centre, 1st floor, JPN, VGH

Bio: http://file.pathology.ubc.ca/Conference/MarkAtkinson.pdf

Oral presentations and guest speakers will be at the Cordula and Gunter Paetzold Health Education Centre, 1st floor & Taylor Fidler Jim Pattison Pavillion North 1411, Vancouver General Hospital

CONFERENCE OUTLINE

TIME	PAETZOLD AUDITORIUM & ATRIUM AT VGH
7:45am – 8:00am	Breakfast
8:00am – 8:10am	Opening remarks – Dr. Mike Allard, Department Head

ORAL PRESENTATIONS	LECTURE THEATRE (GRAD STUDENTS)	MULTIPURPOSE ROOM (RESIDENTS)	JPPN 1411 TAYLOR FIDLER (RESIDENTS)
8:10am – 8:25am	Tim Xue	Emilija Todorovic (AP)	Daniel Owen (AP)
8:25am – 8:40am	Yasir Mohamud	Ellen Cai (AP)	Hamish Nicolson (HP)
8:40am – 8:55am	Frank Lee	Jennifer Pors (AP)	Krista Marcon (HP)

9:00am – 9:20am

PDF GUEST SPEAKER [PAETZOLD LECTURE THEATRE] DR. SERENA SINGH

Dr. Singh is a postdoctoral fellow in Dr. Mari DeMarco's lab at St. Paul's Hospital. Her research focus is developing new diagnostics for Lewy body dementia using mass spectrometry. Previously, she worked on designing blood substitutes as a PhD student in Dr. Ronald Kluger's lab at the University of Toronto.

TITLE: **DEVELOPING SAMPLE PREPARATION WORKFLOWS AND MULTIPLE REACTION MONITORING PARAMETERS IN THE DESIGN OF A MASS SPECTROMETRY METHOD FOR DETECTION OF ALPHA-SYNUCLEIN IN CEREBROSPINAL FLUID**

9:25am – 9:45am

GUEST SPEAKER [PAETZOLD LECTURE THEATRE] DR. SUZANNE VERCAUTEREN

Dr. Vercauteren is a hematological pathologist at BC Children's Hospital, who obtained her MD and PhD at the University of Utrecht, The Netherlands. She did her residency in hematological pathology at the University of British Columbia. She is the Director of BC Children's Hospital BioBank as well as the Chair of the Childhood Cancer and Blood Research BioBank at BC Children's Hospital. She participated in a "Permission to Contact" study with Dr. Peter Watson and is interested in increasing engagement and participation of pediatric patients in research studies.

TITLE: **FUELING RESEARCH BY SAVING BODY BITS: BC CHILDREN'S HOSPITAL BIOBANK**

9:45am – 10:00am

BREAK (ATRIUM) 

10:00am – 10:30am

POSTER POWER PITCH SESSION [PAETZOLD LECTURE THEATRE]

10:35am – 11:35am

JAMES HOGG LECTURE [PAETZOLD LECTURE THEATRE] DR. MORRIS PUDEK

Dr. Pudek trained in Clinical Chemistry at VGH, following his PhD in Biochemistry from UBC, and obtained his certification from the CSCC in 1979. Morris served on the first board of directors of the Canadian Academy of Clinical Biochemistry and was the first recipient of the CACB award for outstanding service to the profession of Clinical Biochemistry. At the present time he is the Medical Leader for Clinical Chemistry for Vancouver Coastal Health. In addition he is a Clinical Professor in Pathology at UBC. Morris is the author or co-author of approximately 100 scientific publications. He has received many teaching awards from residents, science students and medical students over the years.

TITLE: **THE ADVENTURES OF A CLINICAL BIOCHEMIST: MY EDUCATION, EDUCATING AND TOXICOLOGY STORIES**

Poster session and awards reception will be at ICORD, Blusson Spinal Cord Centre, 818 West 10th Ave

CONFERENCE OUTLINE

ORAL PRESENTATIONS	LECTURE THEATRE (GRAD STUDENTS)	MULTIPURPOSE ROOM (RESIDENTS)	JPPN 1411 TAYLOR FIDLER (RESIDENTS)
11:40am – 11:55am	Zhouchunyang Xia	Adrian Levine (NP)	Lisa Li (MM)
11:55am – 12:10pm	Jennifer Ji	Catherine Cheng (MB)	Eric Eckbo (MM)
12:10pm – 12:25pm	Ling Li	Kyra Berg (AP)	Victor Yuen (MM)
12:30pm – 2:30pm	POSTER SESSION & LUNCH [ICORD]		
2:45pm – 3:05pm	GUEST LECTURE [PAETZOLD LECTURE THEATRE] DR. ANDREW MINCHINTON Andrew Minchinton is Head of Radiation Biology at BC Cancer He received his PhD from UCL in London and was a staff scientist in Cancer Biology at Stanford University before accepting a faculty position at the BC Cancer Agency. His main interests are the tumour microenvironment, defining its important in cancer therapy and how it can be exploited for therapeutic gain. He recently received major drug discovery funding from the Wellcome Trust in the UK to develop inhibitors of DNA damage repair and is currently engaged in lead optimization of 3 novel classes of anticancer agents. TITLE: RADIATION ONCOLOGY AND HYPOXIA - A NEVER ENDING STORY!		

ORAL PRESENTATIONS	LECTURE THEATRE (GRAD STUDENTS)	MULTIPURPOSE ROOM (RESIDENTS)	JPPN 1411 TAYLOR FIDLER (RESIDENTS)
3:10pm – 3:25pm	Lisa Decotret	Nissreen Mohammad (AP)	Basile Tessier-Cloutier (AP)
3:25pm – 3:40pm	Brennan Wadsworth	Deidre Ongaro (AP)	Maryam Al Bakri (HP)
3:40pm – 3:55pm	Abhinav Ajaykumar	Kenrry Chiu (AP)	Alisa Abozina (AP)
3:55pm – 4:10pm	Maryam Ghaedi	Jessica Saunders (AP)	
4:10pm – 4:25pm	BREAK (ATRIUM) 		
4:25pm – 5:25pm	KEYNOTE SPEAKER [PAETZOLD LECTURE THEATRE] DR. MARK ATKINSON Dr. Atkinson is currently the American Diabetes Association Eminent Scholar for Diabetes Research and the Jeffrey Keene Family Professor at The University of Florida. He also is the Director of the Diabetes Institute at UF. The author of over 400 publications, Dr. Atkinson is beginning his 34th year of investigation into the field of type 1 diabetes. TITLE: LESSONS FROM THE HUMAN PANCREAS - RE-WRITING THE TEXTBOOKS ON HOW AND WHY TYPE 1 DIABETES DEVELOPS		
5:40pm – 6:00pm	COCKTAILS & CANAPES (ICORD) 		
6:00pm – 8:30pm	AWARDS (ICORD)		

TABLE OF CONTENTS

#	NAME	ORAL PRESENTATIONS
12	LISA LI RESIDENT	LISA LI[1], PIERA CALISSI[2], EDITH BLONDEL-HILL[1,3], BING WANG[1,3], AMANDA WILMER[1,3] Improved vancomycin utilization with rapidly available GeneXpert® MRSA/SA BC PCR and microbiologist case review
13	JENNIFER JI GRADUATE STUDENT	JENNIFER (XIAO YE) JI[1,2], DAWN COCHRANE[2], BASILE TESSIER-CLOUTIER[1], LIEN HOANG[1], YEMIN WANG[2], YI KAN WANG[2], ANGELA CHEUNG[3], CHRISTINE CHOW[3], SHANE COLBORNE[2], CHRISTOPHER HUGHES[2], GREGG MORIN[2], DAVID HUNTSMAN[1,2] Argininosuccinate synthase deficient ovarian cancers are sensitive to arginine deprivation
14	CATHERINE CHENG RESIDENT	CATHERINE CHENG[1], ANDRE MATTMAN[1,2], SOPHIA WONG[1,3], ANGELA FUNG[1,2] Anti-psychotic medications risperidone and 9-hydroxyrisperidone interfere with point-of-care fentanyl urine drug screens
15	ERIC ECKBO RESIDENT	ERIC ECKBO[1,2], VINCENT TANG[1], DIANE ROSCOE[2,3], MARTHE CHARLES[3], COLLEEN FLETCHER[3], CHARLENE PORTER[3], MARC ROMNEY[2,4], SYLVIE CHAMPAGNE[2,4], JENNIFER BILAWKA[4], BING WANG[5], PAMELA KIBSEY[6], MIGUEL IMPERIAL[2,7], ROMINA REYES[2,7], MICHAEL KELLY[2,7], SHAZIA MASUD[2,8], DALE PURYCH[8], BENJAMIN MACK[2,8], AMANDEEP MINHAS[8], KULVINDER MANNAN[8], SUK DHALIWAL[9], PETER TILLEY[2,9], LINDA HOANG[1,2] Assessment of identification methods for *Candida auris* in microbiology laboratories in British Columbia
16	LING LI GRADUATE STUDENT	LING LI[1,4], KAREN JUNG[2,4], PASCAL LECLAIR[2,4], HEIDI VIEIRA[3], CHRISTOPHER MAXWELL[2,4], CHINTEN JAMES LIM[2,4] Functional characterization of the integrin α6 isoforms in breast cancer tumorigenic properties
17	KYRA BERG RESIDENT	KYRA BERG[1], JENNIFER TELFORD[3], LAURA GENTILE[4], AND DAVID SCHAEFFER[1,2] Malignant colorectal polyps - completeness of pathology reports and its direct impact on patient management: initial outcomes from a colon cancer screening program
18	VICTOR YUEN RESIDENT	VICTOR YUEN Citizen science approach to tick-borne disease surveillance in Manitoba
19	LISA DECOTRET GRADUATE STUDENT	LISA DECOTRET[1,4], KEVIN BENNEWITH[1,4], AND CATHERINE PALLEN[2,3,4] Protein Tyrosine Phosphatase Alpha (PTPα) positively regulates invadopodia-mediated cancer cell motility
20	NISSREEN MOHAMMAD RESIDENT	NISSREEN MOHAMMAD[1], AMY LUM[2], JONAH LIN[2], DEREK WONG[2], JULIE HO[3], CHENG-HAN LEE[1,4], TONY NG[1,3], STEPHEN YIP[1,3] Characterization of IDH1/IDH2 mutation and D-2-Hydroxyglutarate oncometabolite level in dedifferentiated chondrosarcoma
21	BASILE TESSIER-CLOUTIER RESIDENT	BASILE TESSIER-CLOUTIER[1,2], YEMIN WANG[1], SHANE COLBORNE[3], JOHNATHAN ZHANG[1], DAWN COCHRANE[1], ANTHONY KARNEZIS[1,2], GREGG MORIN[3], DAVID HUNTSMAN[1] PBRM1 expression is lost in small cell carcinoma of the ovary hypercalcemic type
22	BRENNAN WADSWORTH GRADUATE STUDENT	BRENNAN J WADSWORTH[1,2], KEVIN L BENNEWITH[1,2] Optimization of solid tumour perfusion can improve radiation therapy response by enhancing oxygen delivery
23	DEIDRE ONGARO RESIDENT	DEIDRE ONGARO & JEFFERSON TERRY Reproducibility of grading in chronic histiocytic intervillositis of unknown etiology

#	NAME	ORAL PRESENTATIONS
24	MARYAM AL BAKRI RESIDENT	MARYAM AL BAKRI[1], SUZANNE VERCAUTEREN[1,2], NICHOLAS AU[1,2], ANGELA TSANG[2], MYKOLA MAYDAN[2] Assessment of platelet function using a multicolor flow cytometry
25	ABHINAV AJAYKUMAR GRADUATE STUDENT	ABHINAV AJAYKUMAR[1,2], MAYANNE ZHU[1], HUGO SOUDEYNS[3,4], FATIMA KAKKAR[5], JASON BROPHY[6], ARI BITNUN[7], ARIANE ALIMENTI[8,9], DEBORAH MONEY[9,10,11], HÉLÈNE CÔTÉ[1,2,10], & THE CIHR TEAM IN CELLULAR AGING AND HIV COMORBIDITIES IN WOMEN AND CHILDREN (CARMA) HIV-exposed uninfected (HEU) blood mtDNA content remains elevated from birth into early life (0-3y)
26	KENRRY CHIU RESIDENT	KENRRY CHIU[1,2], STEVE E. KALLOGER[1], CHRISTINE CHOW[1], SHOUKAT DEDHAR[3], DANIEL RENOUF[4], DAVID SCHAEFFER[1,2] Increased expression of carbonic anhydrase IX is associated with improved prognosis in resected pancreatic ductal adenocarcinoma
27	ALISA ABOZINA RESIDENT	ALISA ABOZINA[1], LAURA GENTILE[2], JENNIFER TELFORD[2,3], DAVID SCHAEFFER[1,4] Everything but adenomata: 4 years' experience of the British Columbia Colon Screening Program
28	MARYAM GHAEDI GRADUATE STUDENT	MARYAM GHAEDI[1,2], ZI YI SHEN[2], LISA WEI[3], ITZIAR MARTINEZ-GONZALEZ[1,2], ALIREZA HERAVI-MOUSSAVI[3], MARCO MARRA[3,4] & FUMIO TAKEI[1,2] Single cell RNA sequencing of lung lymphocytes in RORα lineage tracer mice reveals heterogeneity of lung ILC2s
29	JESSICA SAUNDERS RESIDENT	JESSICA SAUNDERS[1], CHRIS CONKLIN[2], CHENG-HAN LEE[2] Immunohistochemical characterization of locally aggressive sarcomatoid/rhabdoid renal cell carcinoma

#	NAME	POSTER PRESENTATIONS
30	ARDALAN AKBARI UNDERGRADUATE STUDENT	ARDALAN AKBARI[1], LAWRENCE LEE[2] Immunoglobulin G4-Related Disease (IgG4-RD) of the Ileocecal Region: A Case Report Resembling Malignancy
31	GURPREET AULAKH UNDERGRADUATE STUDENT	GURPREET AULAKH[1,2], JESSICA TUENGEL[2,3], DONGJUN ZHENG[2,3], SANYA RANCHAL[1,2,3], CETAREH MOHSENZADEH-GREEN[1,4], DAVID VERMIJLEN[5], PETER VAN DEN ELZEN[1,2,3], SOREN GANTT[1,2,3] Gamma-delta T lymphocyte responses to primary EBV & CMV infection in Ugandan infants
32	TIMOTHY AU-YEUNG UNDERGRADUATE STUDENT	TIMOTHY AU-YEUNG[1], SOPHIA WONG[2] Using machine learning to predict aspartate aminotransferase (AST) test results
33	MARIA - ELIZABETH BAEVA UNDERGRADUATE STUDENT	MARIA-ELIZABETH BAEVA[1], PIERRE BECQUART[1], JACQUELINE QUANDT[1] Characterizing neuroprotective roles for aryl-hydrocarbon receptor nuclear translocator expression and neuronal pas 4 domain protein in models of multiple sclerosis
34	HEIDI BRITTON UNDERGRADUATE STUDENT	HEIDI BRITTON, LEO HUANG, KATHRYN SHUM, JESSICA MCALPINE, BLAKE GILKS Application of the Proactive Molecular Risk Classifier of Endometrial Cancer (ProMisE) in Young Women

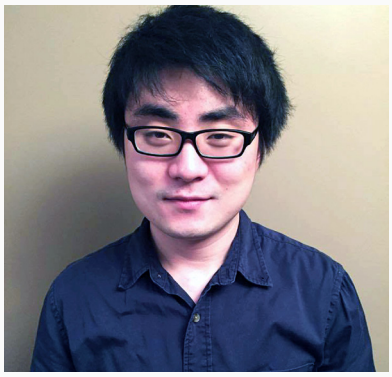
#	NAME	POSTER PRESENTATIONS
35	HONOR CHEUNG UNDERGRADUATE STUDENT	COLE VONDER HAAR[1,2] , KRIS MARTENS[1,2] , KURT MCINNES[3], ASMA BASHIR[2] , WAI HANG CHENG[2], HONOR CHEUNG[2], SOPHIE STUKAS[2], CARLOS BARRON[2], TESSA LADNER[2], KASSI WELCH[2], PETER CRIPTON[3], CATHARINE WINSTANLEY[2], AND CHERYL WELLINGTON[2] Repetitive closed-head impact model of engineered rotational acceleration injury in rats increases impulsive choice, alters the dopaminergic mesolimbic pathway and generates white matter tau pathology
36	BRIAN CHO UNDERGRADUATE STUDENT	BRIAN CHO[1], GIAN LUCA NEGRI[1], SIMRAN SIDHU[1], JORDAN CRAN[1], GABRIEL LEPRIVIER[2], MARC REMKE[2,3,4], JOHN M MARIS[5], ALBERTO DELAIDELLI[1,6], & POUL H SORENSEN[1,6] Targeting mRNA translation in MYCN amplified pediatric neuroblastoma
37	EVAN GIBBARD UNDERGRADUATE STUDENT	EVAN GIBBARD[1], DAWN COCHRANE[1], BASILE TESSIER-CLOUTIER[2], SHANE COLBORNE[3], ANGELA CHENG[1], JENNIFER PORS[5], GREGG MORIN[3], JESSICA MCALPINE[4], DAVID HUNTSMAN[1,2,4,5], LIEN HOANG[5] Identification of novel biomarkers for mesonephric-derived carcinomas
38	LEO HUANG UNDERGRADUATE STUDENT	LEO HUANG[1,3], BRESHELL RUSS[1,4], LEILYNAZ MALEKAFZALI[1,3], QIANLI MA[1,2], ZHE (JAY) YIN[1,2], CLARA JOHNSON[1,3], DAMIAN YAP[1,2], STEVE MCKINNEY[1,2], SAMUEL APARICIO[1,2] Understanding the role of PPP2R2A in ER-positive breast cancer cells
39	JAKE JOHNSTON UNDERGRADUATE STUDENT	JAKE JOHNSTON[1], PIERRE BECQUART[1], JACQUELINE QUANDT[1] Aryl hydrocarbon receptor nuclear translocator 2 expression characterizes the remyelinating and repair potential of oligodendrocyte differentiation in a model of multiple sclerosis
40	DANIEL KIM UNDERGRADUATE STUDENT	DANIEL KIM[1], VIJAY GADKAR[2], PETER TILLEY[1,2] Validation of novel lab-developed loop-mediated isothermal nucleic acid amplification (LAMP) assay for herpes simplex and varicella zoster viruses for clinical use in infected children
41	KEIR MARTYN UNDERGRADUATE STUDENT	CAMERON ORAM[1,2], KEIR MARTYN[1,2], SOHRAB MANESH[1,4], GREG DUNCAN[1,3], JIE LIU[1], WOLFRAM TETZLAFF[1,3,5] & DAVID GRANVILLE[1,2] Attenuation of granzyme b reduces tissue damage and improves functional recovery following spinal cord injury
42	ZI YI SHEN UNDERGRADUATE STUDENT	ZI YI SHEN[1], MARYAM GHAEDI[1,2], GRACE POON[1,2], ITZIAR MARTINEZ-GONZALEZ[1,2] & FUMIO TAKEI[1,2] Lipopolysaccharide inhibits innate lymphoid cells type 2 (ILC2)-mediated allergic lung inflammation
43	JORDAN STEWART UNDERGRADUATE STUDENT	JORDAN STEWART[1], DAILIN LI[2,3], MORRIS PUDEK[2,3], SOPHIA WONG[2,3] Utility of differential oligoclonal banding pattern in the diagnosis of multiple sclerosis
44	GUNJAN KUMAR GRADUATE STUDENT	GUNJAN KUMAR[1,2,4], ELIE RITCH[2,4], DAVIDE TORTORA[2,3,4], BEIBEI ZHAI[2,4], ALEXANDER WYATT[2,4], MADS DAUGAARD[1,2,3,4], PETER BLACK[1,2,4] Genomic screen of cisplatin resistance genes in muscle-invasive bladder cancer using genome-wide CRISPR knockout screen
45	ASMA BASHIR GRADUATE STUDENT	ASMA BASHIR[1], SOPHIE STUKAS[1], KURT MCINNES[2], ZELALEM ABEBE[2], IGOR TATARNIKOV[3], WAI HANG CHENG[1], J. CARLOS BARRON[1], GUILAINE BOYCE[1], ANNA WILKINSON[1], ANDREW YUNG[4], PIOTR KOZLOWSKI[4], MATTHEW FARRER[3], PETER CRIPTON[2], CHERYL WELLINGTON[1] Moderate-severe traumatic brain injury elicits injury-dependent neuropathological, biochemical, and electrophysiological changes in a biofidelic murine model of impact-acceleration head injury

#	NAME	POSTER PRESENTATIONS
46	EMILY BUTTON GRADUATE STUDENT	EMILY B BUTTON[1,2], JEROME ROBERT[1,2], MEGAN GILMOUR[1,2], JIANJIA FAN[1,2], HARLEEN CHEEMA[1,2], EMMA MARTIN[1,2] & CHERYL WELLINGTON[1,2] Methodological considerations to prepare high-density lipoprotein particles with optimal function in cerebrovascular health and Alzheimer's disease
47	RACHEL CEDERBERG GRADUATE STUDENT	RACHEL CEDERBERG[1,2], ALVINA SO[3], ELIZABETH FRANKS[1], JENNA COLLIER[1,2], BRENNAN WADSWORTH[1,2], MICHAEL HUGHES[4], KELLY MCNAGNY[4], KEVIN BENNEWITH[1,2,5] Deciphering the role of eosinophils and B cells in lung cancer and breast cancer lung metastasis
48	WAI HANG CHENG GRADUATE STUDENT	WAI HANG CHENG[1], KRIS MARTENS[1], SOPHIE STUKAS[1], EMILY BUTTON[1], ANNA WILKINSON[1], ASMA BASHIR[1], CARLOS BARRON[1], PETER CRIPTON[2], CHERYL WELLINGTON[1] CHIMERA repetitive mild traumatic brain injury induces long-term pathological and PTSD-like behaviour in APP/PS1 mice
49	TANYA DE SILVA GRADUATE STUDENT	TANYA DE SILVA[1] & WILLIAM LOCKWOOD[2] Characterization of the novel candidate tumor suppressor gene SHPRH in lung cancer development
50	NATALIE FIRMINO GRADUATE STUDENT	NATALIE FIRMINO[1], KEVIN BENNEWITH[1,2] Hypoxia develops in human and murine lymph nodes during B cell-driven immune responses to tumor antigens
51	LAUREN FORGRAVE GRADUATE STUDENT	LAUREN FORGRAVE[1], IAN MACKENZIE[1,2] & MARI DEMARCO[1,3] TDP-43 proteinopathies
52	KENDALL GREENING GRADUATE STUDENT	KENDALL GREENING[1,2], EVAN GIBBARD[2], DAWN COCHRANE[2], ANTHONY KARNEZIS[2], DAVID HUNTSMAN[1,2] Immunohistochemical characterization of normal gynecological tissue: implications for the pathogenesis and prevention of gynecologic cancers
53	ANTHONY HSIEH GRADUATE STUDENT	ANTHONY HSIEH[1,2], BEHEROZE SATTHA[1], NICOLE BERNARD[3], CECILE TREMBLAY[4], HÉLÈNE CÔTÉ[1,2], FOR THE CIHR TEAM ON CELLULAR AGING AND HIV COMORBIDITIES IN WOMEN AND CHILDREN (CARMA) Shorter cell subset telomeres in HIV slow progressors than in HIV non-slow progressor women
54	EMEL ISLAMZADA GRADUATE STUDENT	EMEL ISLAMZADA[1], KERRYN MATTHEWS[2], QUAN QUO[2], ALINE SANTOSO[2], MARK SCOTT[1,3,6], AND HONGSHEN MA[1,2,3,4,5] Microfluidic isolation of drug-resistant plasmodium falciparum parasites to enable rapid target identification of antimalarial drug candidates
55	FARHIA KABEER GRADUATE STUDENT	FARHIA KABEER[1,2], HOSSEIN FARAHANI[1,8], DANIEL LEI[1,8], EMMA LAKS[1,4], JAZMINE BRIMHALL[1,4], JUSTINA BIELE[1,4], JECY WANG[1,4], KIERAN CAMPBELL[1,8], CIARA O'FLANAGAN[1,4], DAMIAN YAP[1,4], PETER EIREW[1], SOHRAB SHAH[1,3,5,6,7,8] & SAM APARICIO[1,2,3,4,5,6] Exploring predictive biomarkers of resistance in breast cancer pre-clinical models at single cell genomics and transcriptomic level

#	NAME	POSTER PRESENTATIONS
56	KEVIN KUCHINSKI GRADUATE STUDENT	KEVIN KUCHINSKI[1], JUN DUAN[1], WILLIAM HSIAO[1,2], NATALIE PRYSTAJECKY[1,2] Designing hybridization probes for target enrichment and deep sequencing of highly diverse viral genomes
57	HAKWOO LEE GRADUATE STUDENT	HAKWOO LEE[1], FARHIA KABEER[1,2], CIARA O'FLANAGAN[2,3], SAMUEL APARICIO[1,2,3] Decoding the functional molecular profiles of tumour clones in breast cancer metastasis using patient-derived xenograft model
58	YU (MICHAEL) LI GRADUATE STUDENT	MICHAEL YU LI[1,2], SHANNON HEALY[2], FONG CHUN CHAN[2] & CHRISTIAN STEIDL[1,2] Determining the functional role of repressor element-1 silencing transcription factor co-repressor 1 (RCOR1) in diffuse large B cell lymphoma
59	BRYAN LIN GRADUATE STUDENT	BRYAN LIN[1,2,] MICHAEL SUTHERLAND[1,2,3], JAMES MORRISSEY[4] & EDWARD PRYZDIAL[1,2,3] Function and prevalence of host-derived coagulation factors on the virus surface.
60	ANAM LIU GRADUATE STUDENT	ANAM LIU[1,2], JENNIFER BAKER[1], XINHE LIU[1], ALASTAIR KYLE[1], JUDIT BANATH[1], TAIXIANG WANG[1], ANDREW MINCHINTON[1] Evaluating normal tissue toxicity resulting from inhibitors of radiation-induced-damage-repair in murine models
61	JENNIFER LUU GRADUATE STUDENT	JENNIFER LUU[1,2], ELIZABETH FRANKS[2], KEVIN BENNEWITH[1,2], WILLIAM LOCKWOOD[1,2] Uncovering immune changes that promote immune evasion in early lung cancer
62	ERIN MARSHALL GRADUATE STUDENT	ERIN MARSHALL[1,2], GREG STEWART[1,2], ADAM SAGE[1,2], WAN LAM[1], CAROLYN BROWN[3] Analysis of XIST as a sex-specific miRNA sponge in non-small cell lung cancer
63	BRENDA MINATEL GRADUATE STUDENT	BRENDA MINATEL[1], VICTOR MARTINEZ[1], TOMAS TOKAR[2], ADAM SAGE[1], ERIN MARSHALL[1], KEVIN NG[1], DAIANA BECKER-SANTOS[1], PATRICIA REIS[3], WENDY ROBINSON[4], IGOR JURISICA[2], WAN LAM[1] Characterizing the landscape of oncofetal small non-coding RNAs in the liver
64	ANGELA MO GRADUATE STUDENT	ANGELA MO[1,2], SERGIO MARTINEZ-HOYER[1,2], ALY KARSAN[1,2] Investigating the effects of TP53 mutations on lenalidomide resistance in del(5q) myelodysplastic syndrome patients
65	HAISLE MOON GRADUATE STUDENT	HAISLE MOON[1,2], PETER RAHFELD[3], IREN CONSTANTINESCU[1], STEPHEN WITHERS[3], & JAYACHANDRAN KIZHAKKEDATHU[1,2,3] Towards universal blood - enzymatic conversion of blood group A antigens
66	QUYEN (AMY) NGUYEN GRADUATE STUDENT	AMY NGUYEN[1], J. GRACE VAN DER GUGTEN3, ALICE FOK[2], GING-YUEK ROBIN HSIUNG[2] & MARI DEMARCO[1,3] Routine clinical use of mass spectrometry for quantification of amyloid-beta 1-40 and 1-42: pre-Analytical and analytical considerations
67	LORENZ NIERVES GRADUATE STUDENT	LORENZ NIERVES[1,2], & PHILIPP LANGE[1,2] Optimizing a proteomic workflow for the isolation of cell surface termini

#	NAME	POSTER PRESENTATIONS
68	JESSICA PILSWORTH GRADUATE STUDENT	JESSICA PILSWORTH[1,2], DAWN COCHRANE[2], BAHAR MOUSSAVI[2], SAMANTHA NEILSON[2], ANNIINA FÄRKKILÄ[3,4], HUGO HORLINGS[5], SATOSHI YANAGIDA[6], JANINE SENZ[2], YI KAN WANG[2], DANIEL LAI[2], ALI BASHASHATI[2], JACQUELINE KEUL[7], ADELE WONG[8], SARA BRUCKER[7], FLORIN-ANDREI TARAN[7], BERNHARD KRÄMER[7], ANNETTE STAEBLER[9], ESTHER OLIVA[8], SOHRAB SHAH[2,10], STEFAN KOMMOSS[7], FRIEDRICH KOMMOSS[11], C. BLAKE GILKS[5], DAVID HUNTSMAN[2,5] Genomic characterization of adult-type granulosa cell tumours of the ovary: Are there features of importance beyond FOXL2?
69	SONJA SOO GRADUATE STUDENT	SONJA SOO[1,3], JIANJIA FAN[3], WENCHEN ZHAO[2,3], JEROME ROBERT[3], CHERYL WELLINGTON[3] Increasing APOE in primary human brain pericytes does not modify migration in a scratch-wound assay
70	LILY TAKEUCHI GRADUATE STUDENT	LILY E. TAKEUCHI[1], SRINIVAS ABBINA[1,2], PRASHANT KUMAR[1,2], USAMA ABBASI[1], JAYACHANDRAN N. KIZHAKKEDATHU[1,2] Use of a cardiac-targeting approach enhances uptake of polymer therapeutics in cardiomyocytes
71	TSEDAY Z TEGEGN GRADUATE STUDENT	TSEDAY Z. TEGEGN[1,2], MICHAEL SUTHERLAND[1,2], ED PRYZDIAL[1,2] Dengue virus induced protein synthesis in platelets
72	SREEPARNA VAPPALA GRADUATE STUDENT	SREEPARNA VAPPALA[1,2], MANU THOMAS KALATHOTTUKAREN[1,2], CHANEL LA[1,3], SRINIVAS ABBINA[1,2], EDWARD CONWAY[1,4], CHARLES HAYNES[1,5], JAYACHANDRAN KIZHAKKEDATHU[1,2] Antithrombotics without bleeding side effects: inhibition of prothrombotic polyanions with polymeric inhibitors
73	CHRIS WANG GRADUATE STUDENT	CHRIS KEDONG[1,2], NADER AL NAKOUZI[1,3], DESMOND HUI[1], HTOO ZARNI OO[1,3], JAMIE RICH[4], JOHN S. BABCOCK[4], ALI SALANTI[5], MADS DAUGAARD[1,2] Internalization mechanism of malaria parasite protein in cancer cells
74	SHAO HUAN (SAMUEL) WENG GRADUATE STUDENT	SHAO HUAN WENG[1,2], ERIN KIM[1,2], PHILIPP LANGE[1,2] An improved microscale N termini enrichment technique with activated magnetic beads and hydrophobic tagging
75	ANNA - CATHARINA WILHELM GRADUATE STUDENT	ANNA-CATHARINA WILHELM[1,2], STEPHANIE SANTACRUZ[1,2], CHRISTOPHER TURNER[1,2], PAUL HIEBERT[1], THEO KLEIN[3], CHRISTOPHER OVERALL[3], DAVID GRANVILLE[1,2] Granzyme B cleaves tenascin-c: role in chronic wound healing
76	DEREK WONG GRADUATE STUDENT	DEREK WONG[1,2], KOHL LOUNSBURY[3], AMY LUM[2], VERONIQUE LEBLANC[4,5], JUNGEON SONG[4], SUSANNA CHAN[4], SUGANTHI CHITTARANJAN[4], MARCO MARRA[4,5], & STEPHEN YIP[1,2] Exploring the functional relationship between capicua (CIC) and ataxin-1-like (ATXN1L) in glioma
77	GUANGZE ZHAO GRADUATE STUDENT	GUANGZE ZHAO[1,2], YE QIU[1,2], HUIFANG ZHANG[1,2], XIN YE[1,2], DECHENG YANG[1,2] Coxsackievirus b3-induced reduction of intercalated disc proteins in viral pathogenesis
78	WENCHEN ZHAO GRADUATE STUDENT	WENCHEN ZHAO[1], CHERYL KOH[2], JIANJIA FAN[1], ANDREA ZUHL[3], IVA KULIC[1], JEROME ROBERT[1], OLA ENGKVIST[4], JOHAN MEULLER[5], QI WANG[6], RYAN HICKS[7], MARCELLO MARESCA[7], CARINA RAYNOSCHEK[7], SAMANTHA BARICHIEVY[3], MICHAEL W. WOOD[6], CAMERON PARRO[1], HSIEN-YA CHOU[1], NICHOLAS J. BRANDON[6], CHERYL L. WELLINGTON[1] Axl receptor tyrosine kinase regulates the expression of apolipoprotein E in human astrocytes

#	NAME	POSTER PRESENTATIONS
79	HANQI (WAYNE) ZHAO GRADUATE STUDENT	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], & DANA DEVINE[1,2,3] Evaluation and Optimization of Cold Stored Platelets in Transfusion
80	ADAM ZIADA GRADUATE STUDENT	ADAM SHERIF ZIADA[1, 2], MENG YING LU[1, 2], JAREK IGNAS-MENZIES[3], SARA SABERI[1,2], ANTHONY HSIEH[1,2], BEHEROZE SATTHA[1,2], STEVE KALLOGER[1], HÉLÈNE CÔTÉ[1,2,4], & THE CIHR TEAM GRANT ON CELLULAR AGING AND HIV COMORBIDITIES IN WOMEN AND CHILDREN (CARMA) Smoking and high HIV viremia damage mitochondrial DNA, and may accelerate mtDNA aging
81	JEROME ROBERT PDF	JEROME ROBERT[1,2], EMILY BUTTON[1,2], MEGAN GILMOUR[1,2], EMMA MARTIN[1,2], WENCHEN ZHAO[1,2], CHERYL WELLINGTON[1,2*] High-density lipoproteins reduce amyloid-beta-deposition in a novel in vitro model of the human brain vasculature
82	JUNYAN SHI PDF	JUNYAN SHI[1], YU ZI ZHENG[1], MARI DEMARCO[1,2] Targeting production of a fast-forming proteotypic peptide for rapid quantification of apolipoprotein A1 in plasma by LC-MS/MS
83	SERENA SINGH PDF [ORAL PRESENTATION]	SERENA SINGH[1] & MARI DEMARCO[1,2] Developing sample preparation workflows and multiple reaction monitoring parameters in the design of a mass spectrometry method for detection of alpha-synuclein in cerebrospinal fluid
84	ELLEN CAI RESIDENT	ELLEN CAI[1,4], PAULA TELLEZ[1,2], ROBYN CAIRNS[3,4], NEIL CHADHA[2,4], ANNA LEE[1,4] Large 7 cm parathyroid cyst in a 14 year old boy: case report and review of literature
85	VIRGINIA YOUNG MLT (TECHNOLOGIST)	BRENDA SHKURATOFF[1], VIRGINIA YOUNG[1], ABBAS SYED[1], NICOLE WATSON[1], ELIZABETH BROWNE[1], LAURA BOOK[1], MARY-JANE MARGACH[1], PETER TILLEY[2], GHADA AL-RAWAHI[2] Evaluation of a two-step algorithm for the diagnosis of C. difficile infection in children: is it different from adults?
86	PIERRE BECQUART RESEARCH ASSISTANT	PIERRE BECQUART[1], MARIA ELIZABETH BAEVA[1], JACQUELINE QUANDT[1] Aryl hydrocarbon receptor nuclear translocator-2 and Neuronal PAS Domain Protein 4 expression in cortical neurons is influenced by inflammatory mediators in vitro and in models of multiple sclerosis



ENTEROVIRAL INFECTION LEADS TO MISFOLDED PROTEIN AND NEURODEGENERATION

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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. Even though 5% of all ALS cases have been associated with inherited genetic mutations that have been categorized as familial ALS, the majority of all ALS cases (95%) is sporadic, and occurs in the absence of any ALS-related family history. Enterovirus (EV), a family of RNA viruses, including poliovirus and coxsackievirus, is suspected to influence ALS development due to the viruses' ability to target motor neurons. Our lab has recently made the discovery that EV infection can disrupt normal cell function through the action of a viral proteinase 3C, which causes a critical RNA regulating protein, TDP-43 to undergo abnormal structural changes and mislocalized from the nucleus to the cytoplasm. Of particular interest was our finding that the same abnormal changes in this protein are also the hallmark of ALS. Based on these findings, we hypothesize that EV infection is a causative and/or risk factor in the development of sporadic ALS.

METHOD(S)
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 H&E and immunohistochemical staining of different markers. Based on our data, we were able to show brain lesions and sustained inflammation (Iba1-microglia), pSTAT3-astrogliosis and GFAP-reactive astrocytes) 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, we were able to demonstrate clear ALS-like pathologies such as TDP-43 mislocalization and/or aggregation, without the presence of any fused in sarcoma (FUS) protein pathologies within the areas of infection. Moreover, co-localization of TDP-43 with other proteins such as p62 and ubiquitin has also been strongly detected within the infected regions, similarly observed in ALS patient samples.

CONCLUSION(S)
Our results reveal that enterovirus infection, such as coxsackievirus, is able to cause ALS-like pathologies in vivo in the form of TDP-43 protein pathologies and co-localization with p62 and ubiquitin. Furthermore, these findings may lead to the development of anti-viral drugs as an effective therapeutic option for sporadic ALS patients.

SCLEROSING ODONTOGENIC CARCINOMA WITH LOCAL RECURRENCE: CASE REPORT AND REVIEW OF LITERATURA

BACKGROUND/OBJECTIVES
Sclerosing odontogenic carcinomas (SOCs) are newly added to the latest World Health Organization (WHO) classification of head and neck tumors (4th Edition) as a distinct entity. With only 10 case reports in the literature to date it is a perplexing neoplasm with locally aggressive behavior, although there have been no reports of loco-regional recurrence following complete excision to date. Here we report a case of a 62-year-old male with sclerosing odontogenic carcinoma involving the left maxilla. Following extensive surgery with close surgical margins the patient had a recurrence of the tumor documented on imaging involving the skull base and underwent a course of radiotherapy with clinical remission. This case report is the first to our knowledge of a tumor recurrence following clean macroscopic margins and highlights the difficulties of assessing margin status in these tumors on hematoxylin & eosin-stained sections. We will review the clinical, radiographic and histologic features of SOCs in literature to date and discuss the differential diagnoses. This rare tumour presents a challenge when diagnosed on a small biopsy. More studies of this rare tumor would be needed to further knowledge of their behavior and to standardize management.

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MOLECULAR CHARACTERIZATION OF *ERBB2*-AMPLIFIED COLORECTAL CANCER IDENTIFIES POTENTIAL MECHANISMS OF RESISTANCE TO TARGETED THERAPIES: A REPORT OF TWO INSTRUCTIVE CASES

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BACKGROUND/OBJECTIVES

ERBB2 amplification has been identified in approximately 5% of KRAS wild-type colorectal cancers. A recent clinical trial showed response to HER2-directed therapy in a subset of *ERBB2*-amplified metastatic colorectal cancers resistant to chemotherapy and EGFR-directed therapy. With the aim of better understanding mechanisms of resistance to HER2-directed and EGFR-directed therapies, we report the complete molecular characterization of two cases of *ERBB2*-amplified colorectal cancer.

METHOD(S)

PCR-free whole genome sequencing was used to identify mutations, copy number alterations, structural variations and losses of heterozygosity. *ERBB2* copy number was also measured by fluorescence in situ hybridization. Single stranded mRNA sequencing was used for gene expression profiling. Immunohistochemistry and protein mass spectrometry were used to quantify HER2 protein expression.

RESULT(S)

The cases showed *ERBB2* copy number of 86 and 92, respectively. Both cases were immunohistochemically positive for HER2 according to colorectal cancer-specific scoring criteria. Fluorescence in situ hybridization and protein mass spectrometry corroborated significantly elevated *ERBB2* copy number and abundance of HER2 protein. Both cases were microsatellite stable and without mutation of RAS pathway genes. Additional findings included altered expression of *PTEN*, *MET* and *MUC1* and mutation of *PIK3CA*. The potential effects of the molecular alterations on sensitivity to EGFR and HER2-directed therapies were discussed.

CONCLUSION(S)

Identification of *ERBB2* amplification in colorectal cancer is necessary to select patents who may respond to HER2-directed therapy. An improved understanding of the molecular characteristics of *ERBB2*-amplified colorectal cancers and their potential mechanisms of resistance will be useful for future research into targeted therapies and may eventually inform therapeutic decision making.

ENTEROVIRUS SUBVERTS AUTOPHAGY THROUGH CLEAVAGE OF FUSION ADAPTOR PROTEINS AND SELECTIVE AUTOPHAGY RECEPTORS

BACKGROUND/OBJECTIVES
Autophagy, an evolutionarily-conserved intracellular degradation pathway, can target misfolded proteins, damaged organelles, and invading pathogens for lysosomal clearance. Despite traditionally being considered an anti-viral pathway, we and others have shown that enterovirus can hijack the cellular autophagic machinery to disrupt its degradative capacity and facilitate viral propagation. However, the exact mechanism by which enterovirus inhibit autophagic flux is unclear. We **hypothesize** that enterovirus disrupts autophagy-flux through viral-proteinase mediated cleavage of key autophagy-regulators.

METHOD(S)/ RESULT(S)
To delineate the possible mechanism involved, we focused on proteins previously reported to be involved in autophagosome fusion. Notably, we found that the autophagosomal SNARE protein SNAP29 and the tethering protein PLEKHM1, two critical proteins known to regulate autophagosome-lysosome fusion, were cleaved upon CVB3 infection. Further *in vivo* (in cells transfected with protease constructs) and in vitro (using recombinant proteases) cleavage assays demonstrated that CVB3-encoded proteinase $3C^{pro}$, not 2^{Apro} or caspases, is responsible for these cleavages. We identified the cleavage sites on SNAP29 (Q161) and PLEKHM1 (Q668), respectively, leading to impaired SNARE complex formation. Moreover, we showed that gene-silencing of SNAP29/PLEKHM1 inhibited autophagic flux, resulting in a significant increase in viral growth, likely due to enhanced accumulation of autophagosomes that provide sites for viral RNA replication and assembly. Finally, we also identified the xenophagic receptor, NDP52, as a bona fide substrate of viral proteinase $3C^{pro}$. The cleavage of NDP52 takes place at Q139, separating the N-terminal LC3-interacting region from the C-terminal ubiquitin-binding domain. The functional significance of NDP52 cleavage is currently under investigation

CONCLUSION(S)
We identified a novel underlying mechanism by which enterovirus, through viral encoded proteinases, subverts the host autophagic pathway to promote viral propagation. Our findings in this study provide strong evidence of a potential therapeutic benefit by targeting the autophagy-virus interface.

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A COMPARISON OF CK19, AFP AND GLYPICAN-3 IN DISTINGUISHING YOLK SAC TUMORS FROM OTHER GERM CELL TUMORS IN THE OVARY

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BACKGROUND/OBJECTIVES
Ovarian yolk sac tumors (YST) can exhibit a wide variety of morphologic patterns. Variant morphology can be particularly difficult to distinguish from other germ cell tumors. Although immunohistochemical (IHC) stains for alpha-fetoprotein (AFP) and glypican-3 have traditionally been used to diagnose YST, staining for AFP is often focal and glypican-3 is known to be less specific. It was recently reported that CK19 is a more sensitive marker than AFP in testicular YST. To date, the diagnostic utility of CK19 in ovarian YST has not yet been investigated.

METHOD(S)
We examined a total of 34 ovarian germ cell tumors (9 pure YST, 5 mixed germ cell tumors with a significant YST component, 8 dysgerminoma, 11 immature teratoma and 1 gonadoblastoma). The expression of CK19, AFP, glypican-3, and SALL-4 were assessed on tissue microarrays and/or whole sections. Staining patterns were characterized by an intensity score (0 = no staining, 1 = weak, 2 = moderate, 3 = strong), proportion score in 10% increments and overall H-score (intensity score multiplied by proportion score).

RESULT(S)
CK19, in comparison to AFP and Glypican-3, demonstrated the most conspicuous staining, with the highest overall proportion and H-score. However, it had a lower sensitivity than AFP and glypican-3 (86% compared to 100% and 100% respectively). Specificity was highest for AFP (100%), intermediate for CK19 (85%) and lowest for glypican-3 (75%). No dysgerminomas nor gonadoblastoma displayed any staining for CK19, AFP and glypican-3. Immature neuroepithelium exhibited focal weak staining for CK19 and glypican-3 but not AFP. SALL-4 was a good overall marker for ovarian germ cell tumors.

CONCLUSION(S)
1. CK19 has a stronger staining than AFP and Glypican-3, but has lower sensitivity than AFP and Glypican-3, and lower specificity than AFP.
2. CK19 and Glypican-3 also stained immature neuroepithelium.
3. Given that CK19 is widely available, it can be used as an alternative marker for differentiating YST from other ovarian germ cell tumors.
4. SALL4 was a sensitive marker for ovarian germ cell neoplasms.

QUALITY ASSURANCE REVIEW TO EVALUATE THE USE AND EFFICACY OF CRYOPRECIPITATE TO TREAT HYPOFIBRINOGENEMIA IN NEONATES UNDER 1 YEAR OF AGE OVER A TWO YEAR PERIOD AT BRITISH COLUMBIA CHILDREN'S HOSPITAL

BACKGROUND/OBJECTIVES
Fibrinogen replacement plays an important role in the management of bleeding in trauma, obstetrical hemorrhage and post cardiac surgery. Products available to treat acquired hypofibrinogenemia include frozen plasma (FP), cryoprecipitate and fibrinogen concentrate (FC). In Canada, cryoprecipitate remains the primary product used for the treatment of acquired hypofibrinogenemia in many Canadian centers, including British Columbia Children's Hospital (BCCH). Most current guidelines support the transfusion of cryoprecipitate if fibrinogen is <0.8-1.0 g/L and is associated with clinical bleeding or an anticipated surgical procedure. We performed a quality assurance review to evaluate the use and efficacy of cryoprecipitate to treat hypofibrinogenemia in neonates under 1 year of age over a two year period at BCCH.

METHOD(S)
This was a retrospective review of all neonates less than 1 year of age treated with cryoprecipitate for hypofibrinogenemia over a 2 year period. The electronic patient record was used to identify all patients and their corresponding diagnoses, laboratory parameters (fibrinogen, aPTT, INR and TT pre- and post-transfusion), cryoprecipitate and plasma transfusions.

RESULT(S)
Forty-three patients, 24 (56%) male and 19 (44%) female, and a total of 85 cryoprecipitate transfusion episodes were identified during the 2015/2016 and 2016/2017 fiscal years at BCCH. Patient weight ranged from 0.6 kg to 9.0 kg. Weight based cryoprecipitate dosing was variable, ranging from 2 to 20mls/kg, with an average of 6mls/kg. The estimated amount of fibrinogen transfused per transfusion event ranged from 0.4 to 1.3 g, with an average of 0.6 g. The primary indication for cryoprecipitate transfusion was coagulopathy (56%), followed by surgical bleeding (24%), malignancy (5%) and other causes (2%). The commonest clinical scenario was prematurity associated with sepsis related coagulopathy followed by congenital cardiac disease requiring surgery. Pre-transfusion fibrinogen levels were measured in 81 transfusion episodes and ranged from 0.7 (lowest reportable detection limit) to 3.0 g/L, with an average level of 0.9 g/L. Where post-transfusion fibrinogen levels were available, there was no consistent predictable response to cryoprecipitate.

CONCLUSION(S)
In neonates under 1 year of age at BCCH, cryoprecipitate transfusion is administered to treat acquired hypofibrinogenemia in patients with coagulopathy and requiring surgery when fibrinogen levels are less than 1.0 g/L with no consistent predictable response to post transfusion fibrinogen levels. This likely reflects clinical scenario with ongoing coagulopathy or bleeding and time delay between product administration and subsequent fibrinogen testing. This information informs laboratory transfusion medicine practice at BCCH to better understand fibrinogen replacement product usage in neonates less than 1 year of age. This data may be used to make product comparisons between cryoprecipitate and fibrinogen concentrate for fibrinogen replacement in neonates less than one year of age.

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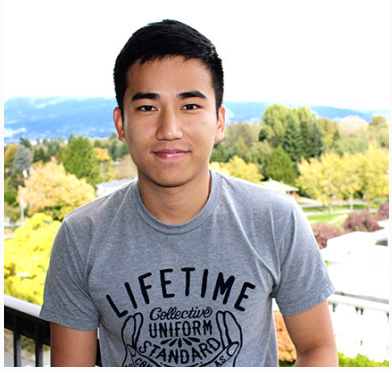

PLASMIN CONVERTS CLOTTING FACTOR VA FROM A PROCOAGULANT TO FIBRINOLYTIC COFACTOR

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BACKGROUND/OBJECTIVES
Cardiovascular disease is the leading cause of death worldwide. The major contributor is blood clots (i.e. thrombi) that block normal blood flow. Thrombi are comprised largely of fibrin, which provides structural support to the clot. Current clot-dissolving (i.e. thrombolytic) drugs were designed based on the protease tissue plasminogen activator (tPA), which activates plasminogen (Pg) into plasmin, the enzyme ultimately responsible for cleaving and dissolving fibrin. However, tPA is an active enzyme that is given at high doses to overcome its rapid clearance and can work systemically to cause life-threatening bleeding. Treating thrombosis may be improved by instead accelerating intrinsic tPA using a non-enzymatic therapy localized to the clot itself. We have discovered that plasmin cleaves and converts the essential coagulation cofactor, factor Va (FVa), into a fibrinolysis cofactor capable of accelerating tPA-mediated Pg activation. Complex plasmin cleavages dissociate the FVa heavy chain (FVaH) from the light chain (FVaL) into fragments derived from either FVaH or FVaL. Our goal is to determine which FVa-derived fragment(s) generated following plasmin cleavage can accelerate tPA activity.

METHOD(S)
To correlate the fragmentation of FVa following plasmin cleavage to accelerated tPA cofactor activity, a chromogenic assay was developed to measure Pg activation to plasmin. Either intact or plasmin-cleaved FVa was incubated with tPA/Pg and samples were taken over time to measure plasmin generation through chromogenic substrate cleavage. FVaH-derived fragments were separated from FVaL-derived fragments by density-dependent centrifugation on sucrose-containing aPL. Fragmentation of FVa-derived samples was concomitantly analyzed by polyacrylamide gel electrophoresis and Western blot to confirm chain dissociation and correlate fragmentation to tPA cofactor activity. Additionally, autoradiography was used to assess direct binding of FVa fragments to 125I-radiolabelled Pg.

RESULT(S)
Cleavage of FVa by plasmin prior to incubation with tPA and Pg significantly accelerated Pg activation, demonstrating that proteolytic modulation by plasmin confers tPA cofactor activity. This enhancement was observed only in the presence of anionic phospholipid (aPL). Interestingly, when separated, both free FVaH-derived and aPL-binding FVaL-derived fragments both enhanced plasmin generation. However, only a ~50 kDa FVaH-derived fragment was shown to directly bind to 125I-radiolabelled Pg, suggesting that the FVaL-derived fragment enhances tPA cofactor activity through a yet unknown mechanism.

CONCLUSION(S)
The logical extension of our work is in thrombotic disease such as stroke and heart attack where thrombolysis is required to restore blood flow. Since FVaL has an intrinsic high affinity-binding site for anionic phospholipid present exclusively at site of the clot, generating a recombinant fibrinolytic fragment of FVaL may localize plasmin generation to the site of thrombosis. Due to side effects associated with exogenous tPA use, a non-enzymatic cofactor-based approach to dissolving clots by accelerating intrinsic tPA may be an effective alternative.

A COMPARISON OF GATA3, TTF1, CALRETININ AND CD10 IN IDENTIFYING MESONEPHRIC CARCINOMAS OF THE GYNECOLOGIC TRACT

BACKGROUND/OBJECTIVES
Mesonephric carcinomas are very often under-recognized due to their varied morphologic patterns, which can be mistaken for other types of gynecologic carcinomas. They are aggressive tumors, as 40% of patients will develop locoregional recurrences or metastases. Recently, it has been reported that GATA3 and TTF1 are useful markers for mesonephric carcinomas, but these findings have not been validated in a large series. It is also unclear how GATA3 and TTF1 compare to the traditional markers used for mesonephric carcinomas, CD10 and calretinin.

METHOD(S)
Using tissue microarrays and/or whole sections, we assessed the immunohistochemical expression of GATA3, TTF1, PAX8, calretinin and CD10 in a series of 12 mesonephric carcinomas, 600 uterine carcinomas, and 94 cervical adenocarcinomas. Any intensity of staining (nuclear for GATA3, TTF1 and PAX8, nuclear/cytoplasmic for calretinin and cytoplasmic/membranous for CD10) in >1% of cells was considered positive.

RESULT(S)
GATA3 had a sensitivity of 100% and specificity of 94% in distinguishing mesonephric carcinomas from other gynecologic adenocarcinomas. GATA3 staining decreased significantly in the more solid and poorly differentiated areas of tumor. However, GATA3 expression was also seen in 18% of carcinosarcomas (in both the carcinomatous and/or sarcomatous components). TTF1 had comparable specificity (99%) to GATA3 but a much lower sensitivity (58%). TTF1 expression was only seen in the uterine mesonephric carcinomas and not in any of the cervical tumors. Four of 12 (33%) mesonephric carcinomas showed an inverse staining pattern between GATA3 and TTF1, a finding not previously reported. All mesonephric carcinomas were PAX8 positive. Three uterine and 1 cervical adenocarcinoma were PAX8 negative and GATA3 positive, mimicking the profile of breast carcinomas. CD10 had comparable sensitivity (92%) and specificity (88%) to GATA3, while calretinin had inferior performance (sensitivity 50%, specificity 64%).

CONCLUSION(S)
GATA3 demonstrated the best sensitivity and specificity for mesonephric carcinomas compared to TTF1, CD10 and calretinin. GATA3 however, also stained a subset of carcinosarcomas and thus cannot be used to distinguish mesonephric carcinosarcomas from uterine carcinosarcomas. In small biopsies where mesonephric carcinoma is being considered but GATA3 is negative, TTF1 should be performed due to their inverse staining pattern.

FUTURE DIRECTIONS
We are currently using in-depth proteomics profiling to identify more robust markers for mesonephric carcinoma and have identified several candidate proteins. Antibodies to these proteins are currently being tested. We are also in the process of performing targeted sequencing in our series mesonephric carcinomas, which may help uncover potential molecular-based therapeutic options for these aggressive tumors.

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IMPACT OF PRE-PRINTED ORDER IMPLEMENTATION FOR PROTHROMBIN COMPLEX CONCENTRATES AT VANCOUVER GENERAL HOSPITAL: A QUALITY ASSURANCE AUDIT

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BACKGROUND/OBJECTIVES
Prothrombin Complex Concentrates (PCCs) are plasma-derived vitamin K dependent coagulation factor concentrates used for urgent reversal of warfarin. Following their approval by Health Canada in 2008, all usage of PCCs at Vancouver General Hospital required approval by a hematopathologist (HP) before release, with the sole exception being intracranial hemorrhage (ICH) due to its high acuity. In March 2014, after 6 years of local experience with PCCs, it was decided that a pre-printed order (PPO) could replace the HP approval process to simplify and expedite PCC usage in other urgent warfarin reversal scenarios, including urgent surgery and major bleeding. The goals of the PPO were to standardize PCC dosing, increase appropriate use of vitamin K, decrease turn-around-time (TAT), and decrease non-value-added calls to HPs. The purpose of this quality assurance study was to review PCC usage pre- and post-PPO implementation to determine the impact of the PPO in achieving its intended goals.

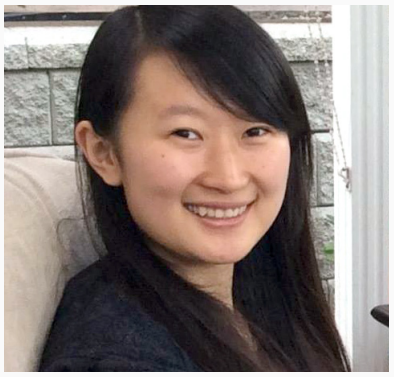
METHOD(S)
A retrospective chart review of PCC usage was performed for two years pre- and post-PPO implementation (March 2012-2014 and March 2014-2016, respectively). Relevant demographics and medical information, including PCC indication and dose, use of vitamin K, and adverse events (thrombosis and mortality) were recorded. Appropriateness of PCC dosage was adjudicated using the Canadian National Advisory Committee (NAC) PCC guidelines. Appropriateness of PCC indication was adjudicated based on chart review. Bleeding episodes were verified by review of clinical notes, endoscopic examination reports, and imaging reports when applicable. Surgical start times were documented from the anesthetic record, with urgent surgery defined as <6 hours from PCC order.

RESULT(S)
Baseline characteristics of patients receiving PCC pre-implementation (n=207) and post-implementation (n=202) were similar, including sex, age, warfarin use, and INR levels. The proportion of patients receiving appropriate PCC doses within 500 IU of NAC guidelines was significantly higher in the post-PPO period (67.2% vs. 79.0%, p=0.015). The appropriate administration of at least 5mg of intravenous vitamin K was more frequent following PPO introduction (70.5% vs. 75.2%, p=0.015). No significant differences were observed in TAT, additional doses of PCC, red cell transfusion, INR correction, thrombosis, or mortality.

After the introduction of the PPO, call volume to HPs for PCC approval was significantly lower (67.1% vs. 16.3%, p <0.001). The PPO was used to release PCC for inappropriate indications 23.2% of the time: 15 cases for patients on warfarin for "urgent surgery" when surgery was >6 hours from the PCC request; and 27 cases for patients not on warfarin without a documented HP consultation.

CONCLUSION(S)
Many of the goals of the PPO were achieved, with an increase in appropriate PCC dosing and vitamin K usage. The PCC ordering process was streamlined, preventing unnecessary delays in PCC administration. Although the PPO was used inappropriately in approximately a quarter of cases, these instances can guide refinements to the PPO to improve appropriate PCC utilization.

L1 RETROTRANSPOSON-MEDIATED DNA TRANSDUCTIONS IN ENDOMETRIOID AND CLEAR CELL OVARIAN CANCER



BACKGROUND/OBJECTIVES
Endometrioid ovarian cancer (ENOC) and clear cell ovarian cancer (CCOC) are distinct subtypes of epithelial ovarian cancer with a common precursor lesion, endometriosis (ectopic growth of uterine lining). How do two distinct cancers arise from the same precursor lesion is unknown, and effective biomarkers of early cancer development and recurrence are lacking. We performed whole genome sequencing (WGS) on 29 ENOC and 35 CCOC cases, and we observed recurring retrotransposition events originating from an active LINE-1 (L1) retrotransposon in the *TTC28* gene in 34% and 31% of the cohorts respectively. L1 retrotransposons are mobile repetitive genetic elements littered across our genome. They encode a set of protein machineries that helps them to "copy-and-paste" their own sequences into random genomic places. L1s are also capable of taking unique downstream DNA sequences in a process called 3' transduction. All these processes may fuel genomic instability, as such they are epigenetically silenced in normal tissues, but are found to become re-activated in epithelial cancers in association with global hypomethylation. As the gradual loss of L1 methylation has been found from normal endometrium to contiguous endometriosis and to ovarian tumors, we **hypothesize** that *TTC28*-L1 transductions occur early in ENOC and CCOC tumorigenesis and could be used as markers of tumor development and evolution.

METHOD(S)
To compare the clonal relationship between *TTC28*-L1 transductions and somatic mutations, we used PCR and next-generation sequencing assays on FFPE tumor tissues of different tumor samplings for 4 ENOC and 3 CCOC cases. To identify novel *TTC28*-L1 events in tumor and FFPE tissues, we developed a DNA-probe based target capture sequencing method and validated the assay on WGS cases with known retrotranspositions. L1 protein expression were assessed via immunohistochemical (IHC) staining of ovarian tissue microarray (TMA) and correlated with survival.

RESULT(S)
TTC28 L1 retrotransposition events were present at all five tumor sites in 75% (6/8) of cases, while some SNV/frameshift mutations were either absent or were present at varying allelic frequencies. Target capture sequencing had identified retrotransposition events corresponding to the events identified in WGS for the four cases tested, as well as in FFPE without WGS. L1 expression were found in 57% of ENOC and 63% of CCOC and correlated with poor disease specific survival in ENOC (five-year cut off: hazard ratio=4.08, p=0.007).

CONCLUSION(S)
Our results suggest that *TTC28*-L1 events occur early in ovarian cancer development and may reflect the pre-malignant transformation of ENOC and CCOC. The use of L1 protein IHC and our target capture assay could be explored as a potential method to track such development.

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A DEEP LEARNING APPROACH FOR CARCINOMA IDENTIFICATION AND CLASSIFICATION ON TISSUE MICROARRAYS

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BACKGROUND/OBJECTIVES

"Deep Learning" describes a set of machine learning algorithms that have, over the past decade, underpinned significant breakthroughs in the analysis of natural data forms, such as images, text, and speech. In particular, an application of deep learning known as Convolutional Neural Networks (CNNs) has become the standard approach for a range of computer vision tasks including image recognition, classification, and segmentation. CNNs are now being applied to tasks in numerous medical specialties, in many cases obtaining accuracy rates comparable to board certified specialists. In this project we employed CNNs on two tasks using tissue microarray (TMA) cores: (1) the classification of breast, pancreatic, and colorectal cancers and (2) the identification of colorectal cancer (CRC) versus normal colonic tissue.

METHOD(S)

Digitized TMAs of breast, colorectal, and pancreatic ductal adenocarcinomas, and of normal colon, supplied data to train and test the CNNs. The TMAs were de-arrayed using QuPath, evaluated for tissue quality of each core by a pathology resident, and exported as image files of individual cores, which were then stain normalized against a reference image. The CNN classifier was trained through transfer learning: Google's Inception-V3 architecture—pre-trained on the Imagenet database—was fine-tuned with images from our own dataset. The model was built in Python using the Keras API with a Tensorflow backend, and training was run on a multi-GPU server hosted at the Genome Sciences Center. Approximately 75% of the core images were allocated to the training set, which is used to tune the parameters of the model, with the remaining 25% split evenly between validation and test sets. While the validation set was evaluated at the end of each training epoch, in order to tune the model's hyperparameters and determine the optimal point to terminate training, the test set was only evaluated on the final model. The tumor type classification was performed on whole core images, while the CRC identification task classified each core by splitting the larger images into smaller tiles, and aggregating their classification to generate a core level prediction.

RESULT(S)

When distinguishing between the three types of adenocarcinoma, our CNN achieved 94% accuracy on the validation set and 89% accuracy on the test set. On the identification of colorectal cancer, the CNN achieved 91% validation set accuracy and 95% test set accuracy on individual tiles, and performed slightly better on whole cores, with 92% validation set accuracy and 96% test set accuracy.

CONCLUSION(S)

Deep learning is an effective approach to the computational analysis of digitized histopathology slides. As with most medical applications of machine learning, the greatest barrier to achieving an accurate, generalizable model is the challenge of obtaining a large, quality set of training images annotated by physicians. In the future we plan to significantly upscale our slide digitization efforts and establish a user-friendly interface for slide annotation, in order to generate a robust pipeline of deep learning training data.

IMPROVED VANCOMYCIN UTILIZATION WITH RAPIDLY AVAILABLE GENEXPERT® MRSA/SA BC PCR AND MICROBIOLOGIST CASE REVIEW

BACKGROUND

Staphylococcus aureus bloodstream infection is life-threatening and requires timely antibiotic therapy. The first-line empiric treatment is vancomycin, which covers methicillin-resistant *S. aureus* (MRSA). In the case of eventually proven methicillin-sensitive *S. aureus* (MSSA) bloodstream infection, delay in switching vancomycin to beta lactam therapy may be detrimental to patient outcomes due to clinical inferiority of vancomycin as compared to cloxacillin or cefazolin for MSSA. In October 2016, the GeneXpert® MRSA/SA BC assay, a PCR-based method to detect MRSA from blood cultures, was implemented at Kelowna General Hospital (KGH) laboratory. The medical microbiologist began using the results to perform real-time antimicrobial stewardship. In most cases, a negative PCR result allows rapid reporting of MSSA which leads to rapid de-escalation or avoidance of vancomycin all together. ***Objective:*** To assess the impact of a rapidly available MRSA PCR on the amount and duration of vancomycin use in MSSA bloodstream infections.

METHOD(S)

The study included MSSA bloodstream infections from KGH, a tertiary referral centre, and 4 community hospitals served by the KGH laboratory. After implementation, KGH microbiology technologists performed MRSA PCR on blood cultures with at least 3 bottles positive for gram positive cocci in clusters, or at the microbiologist's request. Results were given to the microbiologist immediately, who phoned the most responsible physician to de-escalate and/or streamline antibiotics. A retrospective review was performed, comparing cases pre- and post-implementation of the MRSA PCR. All episodes of MSSA bloodstream infection between January 1, 2013 to September 30, 2016 (pre-implementation group) and November 1, 2016 to January 31, 2018 (post-implementation group), were identified using the laboratory information system. These cases were matched with measures of vancomycin usage, defined daily doses (DDD) and days of therapy (DOT), obtained from the Meditech® Pharmacy System, using Insight® data extraction software, by selecting for vancomycin prescriptions ordered within 3 days of the collection date of each positive blood culture. For patients with ≥5 DOTs, electronic medical records were reviewed to look for study exclusion criteria, which were contraindications to beta lactam antibiotics, polymicrobial infections with organisms requiring vancomycin, transfer from another hospital with known infection, or vancomycin convenience dosing in hemodialysis. Mean vancomycin DDDs and DOTs between groups were compared, as was the proportion of patients receiving at least one dose of vancomycin. Categorical variables were analyzed using the chi-square test, while continuous variables were compared using the t-test.

RESULT(S)

In the pre-implementation group, 392 episodes of MSSA bacteremia were identified, with 29 excluded, leaving 363 for analysis. In the post-implementation group, 103 episodes were identified, with 6 excluded, leaving 97 for analysis. Significantly more patients received at least one dose of vancomycin in the pre-implementation group (70.5%) compared to the post-implementation group (54.6%) ($P<0.01$). The mean DDD was 1.5 in the post-implementation group, which was less than the pre-implementation group at 2.8 ($P<0.01$). The mean DOT also decreased, with the post-implementation group receiving less vancomycin (1.6 days) than the pre-implementation group (2.5 days) ($P<0.01$).

CONCLUSION(S)

Rapidly available MRSA PCR for *S. aureus* bloodstream infection coupled with antimicrobial stewardship performed by the medical microbiologists led to a significant decrease in vancomycin use.

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ARGININOSUCCINATE SYNTHASE DEFICIENT OVARIAN CANCERS ARE SENSITIVE TO ARGININE DEPRIVATION

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BACKGROUND/OBJECTIVES
In 2017, over 22,440 women were diagnosed with epithelial ovarian carcinoma (EOC) in the North America. EOC is divided into subtypes based on histology and prognosis. Each subtype is its distinct disease with unique clinical and molecular features. Despite these differences, the gold-standard treatment for all subtypes is still platinum-based chemotherapy, to which many are resistant to. In this study, we focus on targeting aberrant metabolism in three less common EOC subtypes: clear cell ovarian cancer (CCOC), endometrioid ovarian cancer (ENOC), and small cell hypercalcemic ovarian cancer (SCHCOC). The metabolic enzyme of focus is Argininosuccinate synthase (ASS1), it is a crucial enzyme in the cellular synthesis of arginine. Previous studies have shown that a deficiency in ASS1 makes cancer cells dependent on extracellular arginine for survival, therefore sensitive to arginine deprivation. Since most normal cells express ASS1, extracellular arginine deprivation therapy has little side effects. Therefore, targeting the arginine synthesis pathway in cancers without ASS1 expression has been studied in sarcoma, methothelioma, and hepatocellular carcinoma. Here, we explore the expression of this protein and its therapeutic implications in the less common EOC subtypes.

METHOD(S)
Using a mass spectrometry-based study, we characterized the global proteome of 17 formalin-fixed, paraffin-embedded patient CCOC tumors. The CCOC cases separated into 2 distinct subgroups based on unsupervised hierarchical clustering. We identified the top 250 most differentially expressed proteins between these 2 groups using Protein Expression Control Analysis (PECA) with a false discovery rate of <0.05. The identified targets were then subjected to pathway analysis through KEGG. Targets were validated using immunohistochemistry (IHC) on archival patient tissues using tissue microarrays on a Vantana Ultra machine, in addition to western blot validation using cell lines. Arginine deprivation studies were carried out for representative cell lines, an Incucyte machine was used to characterize the growth in response to arginine deprivation.

RESULT(S)
Of the 250 differentially expressed proteins between the 2 groups, 56 were metabolism-related, including ASS1. Using IHC, we observed varied ASS1 expression in ovarian cancer subtypes. While the most common subtype of EOC, high grade serous ovarian cancer, had the most expression of ASS1, 30% of all CCOC had lower expression of this protein, and almost all ENOC had low to no ASS1 protein expression. Strikingly, almost all SCHCOC were completely null for ASS1 expression. On western blot, representative cell lines also reflect this variability in ASS1 expression, and we show that cell lines deficient in ASS1 are sensitive to extracellular arginine deprivation regardless of EOC subtype.

CONCLUSION(S)
ASS1 has a varied expression in less common EOC subtypes, therefore could present as a therapeutic target through arginine deprivation.

ANTI-PSYCHOTIC MEDICATIONS RISPERIDONE AND 9-HYDROXYRISPERIDONE INTERFERE WITH POINT-OF-CARE FENTANYL URINE DRUG SCREENS

BACKGROUND/OBJECTIVES
The increasing number of deaths secondary to fentanyl abuse underscores the importance of accurate urine drug screens (UDSs). Recently there were several false positive cases in British Columbia and Yukon, wherein a positive point-of-care (POC) fentanyl UDS was subsequently negative on confirmatory LC-MS/MS. Case reviews and literature search suggest the commonly prescribed anti-psychotic medications, risperidone and 9-hydroxyrisperidone, may interfere with POC fentanyl UDSs. Here we evaluated the extent of risperidone and 9-hydroxyrisperidone interference on various fentanyl UDSs.

METHOD(S)
Blank urine specimens collected from healthy volunteers were spiked with risperidone and 9-hydroxyrisperidone (Sigma-Aldrich) at various concentrations. The spiked urines were analyzed by Alere SureStep, BTNX Rapid Response, American Screening Corp. OneScreen, and Immunalysis homogeneous enzyme immunoassay (HEIA) on Siemens ADVIA1800.

RESULT(S)
The SureStep single fentanyl strip was the POC UDS most showing cross-reactivity when urine concentrations of risperidone and 9-hydroxyrisperidone were greater than or equal to 1000 µg/L. The SureStep and Rapid Response multidrug panels showed cross-reactivity in the range of 2500-4000 µg/L. OneScreen and Immunalysis HEIA showed no cross-reactivity up to 5000 and 15000 µg/L of risperidone and 9-hydroxyrisperidone, respectively.

CONCLUSION(S)
POC fentanyl UDSs are susceptible to interference from risperidone and 9-hydroxyrisperidone at various concentrations. The SureStep single fentanyl strip cross-reacts with urine anti-psychotic concentrations of 1000 µg/L, which is at the upper range for a patient on a high-dose regimen of risperidone or 9-hydroxyrisperidone. Thus, this degree of interference may result in clinically misleading false positive fentanyl UDS results in patients that could misdirect care in patients who are taking these antipsychotics.

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ASSESSMENT OF IDENTIFICATION METHODS FOR *CANDIDA AURIS* IN MICROBIOLOGY LABORATORIES IN BRITISH COLUMBIA

Supervisor:

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BACKGROUND/OBJECTIVES

Candida auris is an emerging, potentially multidrug-resistant organism linked to healthcare outbreaks. In September 2017, the first *C. auris* isolate was identified in a clinical specimen in British Columbia. We sought to assess the abilities of frontline laboratories in B.C. to accurately and reliably identify *C. auris* isolates, and investigate factors affecting successful identification.

METHOD(S)

A panel of 20 yeast isolates, including 10 *C. auris* isolates, were forwarded to twelve participating laboratories. Laboratories were instructed to work-up the samples as per their local protocol for yeast identification from sterile sites. Ten laboratories received the full panel; two received an abbreviated panel. After results were returned, a questionnaire was distributed in order to elucidate factors associated with successful identification of the unknown samples.

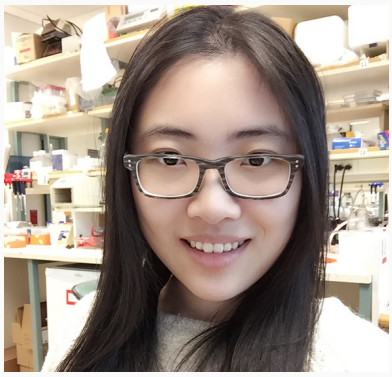
RESULT(S)

Three laboratories utilized the Vitek®-MS MALDI-TOF. While use of the standard IVD database failed to correctly identify *C. auris*, the SARAMIS RUO database accurately identified all isolates. Seven laboratories utilized the Bruker Biotyper® MALDI-TOF, correctly identifying *C. auris* with ≥90% accuracy at each laboratory. Correct identification required updating of the system to the 6903/7311 library. Two laboratories utilized the Vitek®-2 phenotypic system exclusively, with other laboratories using this system as a secondary identification system. Vitek®-2 did not reliably identify *C. auris*; the most common misidentification was *Candida haemulonii*.

CONCLUSION(S)

C. auris can reliably be identified by Vitek®-MS using the SARAMIS database. Correct identification on the Bruker Biotyper® requires updating to the 6903 library or later, and tube formic acid extraction improved scores compared to spot extraction. The Vitek®-2 did not reliably identify *C. auris*. Seven of twelve laboratories were unable to identify *C. auris* isolates with 100% accuracy. This assessment may assist participating laboratories implement updates to ensure greater accuracy in *C. auris* identification.

FUNCTIONAL CHARACTERIZATION OF THE INTEGRIN A6 ISOFORMS IN BREAST CANCER TUMORIGENIC PROPERTIES



BACKGROUND/OBJECTIVES
Cancer stem cells (CSCs) represent a small population of cancer cells thought to be responsible for tumor progression, enhanced drug resistance, cancer relapse and metastasis. Integrin α6 (aka CD49f) is a usefull marker for over 30 different stem cell populations, including the CSCs of breast and other cancers. Yet, little is known regarding its contribution through integrin-mediated signaling. Integrins are cell surface adhesion receptors that serve to communicate environmental cues to inside of cells. Each integrin heterodimer is composed of an α- and a β-subunit, both having an extracellular domain for interaction with ligands and a cytoplasmic tail for transduction of intracellular signaling. Alternate splicing of integrin α6 mRNAs yields two isoforms, α6A and α6B, each possessing different protein sequences within the cytoplasmic tail possible to transmit differential signaling. We hypothesize that integrin α6 contributes to the tumorigenic properties of breast cancer cells via the functionally distinct α6A and α6B isoforms.

METHOD(S)
The triple negative subtype of breast cancer (TNBC) has worse prognosis, attributable to greater numbers of the CSCs. We use TNBC cell line MDA-MB-231 that have endogenous expression (α6WT), lack expression (α6null), or have isoform-specific expression (α6A or α6B) to evaluate their tumorigenic properties. CRISPR/Cas9 is used to knock-out expression of both isoforms, in which cells the specific α6 isoforms are re-introduced (sorted by FACS). Surface expression of various integrin subunits, e.g. β1 and β4 subunits that can dimerize with α6, are evaluated by flow cytometry. Tumor-initiating ability is quantified by tumorsphere formation assay. Cell invasiveness is evaluated in three aspects: adhesive ability by adhesion assay on substrates, motility by wound healing assay, and invasion by Transwell invasion assay through Matrigel, a reconstituted basement membrane.

RESULT(S)
While β1 production is unaffected by α6 expression, α6null cells lack surface β4 expression which is reconstituted by re-expression of α6A, but not α6B. Knocking out integrin α6 decreases the number of tumorspheres formed. Adhesion assays show α6null cells are defective in adhering to Matrigel, containing substrate laminin specific for integrin α6, which is re-established upon expression of either α6A or B isoforms. Integrin α6 does not affect adhesion to fibronectin, an ECM substrate for integrin β1. α6null cells are unable to invade through Matrigel which is restored by re-expression of α6A but not α6B.

CONCLUSION(S)
Integrin α6 contributes to TNBC cell tumorsphere formation, adhesion and invasion capacities in vitro. The α6A and B isoforms may have differential pairing with the β subunits, possibly facilitating signaling mediated by the β subunits. Future analysis on their contributions to tumorsphere formation and cell migration will assist understanding of unique attributions from specific isoforms. Evaluation on known signaling pathways (e.g. stem cell pathways) could provide insight into signaling mediated by the α subunit cytoplasmic tails.

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MALIGNANT COLORECTAL POLYPS - COMPLETENESS OF PATHOLOGY REPORTS AND ITS DIRECT IMPACT ON PATIENT MANAGEMENT: INITIAL OUTCOMES FROM A COLON CANCER SCREENING PROGRAM

Supervisor:
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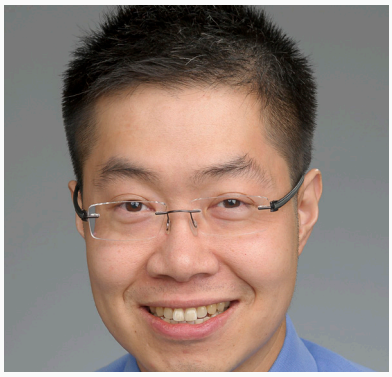
BACKGROUND/OBJECTIVES
With the widespread introduction of colon cancer (CRC) screening programs, there has already been evidence of a shift towards detection of CRC at an earlier stage, and pathologists can expect to see increasing numbers of malignant polyps/early pT1 CRC. Some patients with malignant polyps may be treated by polypectomy alone, but between 9-24% have lymph node metastases at the time of presentation. Lack of certain pathologic poor prognostic factors (high grade tumor differentiation, lymphovascular invasion, positive resection margin of 1mm or less, and high grade tumor budding) has repeatedly been shown to predict patients at low risk of residual malignancy and lymph node metastases. These criteria have now been included to stratify patients into those who do not require any further treatment after polypectomy in the National Comprehensive Cancer Network guidelines, as well as the Japanese guidelines. We assessed if these reporting standards are being met in a large colon cancer screening program, and the resulting effect on patient management.

METHOD(S)
289 malignant polyps were identified retrospectively from the colon cancer screening program database by ICD-O v.3 code and pathology reports were screened for appropriate classification, leaving 236 cases. Pathology reports were reviewed for reporting of tumor grade, lymphovascular invasion, tumor budding, and resection margin. Outcome data was collected from subsequent rebiopsy and endoscopic resection reports, surgical pathology reports, oncology consultations, imaging, and autopsy reports.

RESULT(S)
From the 26,399 patients that were enrolled in the screening program from 2011 to 2016, there have been 236 malignant polyps from 233 patients (0.9%). Complete reporting was seen in 40.7% of reports. Margin status was reported in 90.3%, grade in 83.9%, lymphovascular invasion in 80.5%, and tumor budding in 44.1%. Of these 233 patients, 144 patients had a subsequent surgery and only 32 had residual carcinoma identified. 20 patients (14%) had lymph node metastases identified. 63 patients had no poor prognostic factors on biopsy, and 26 (41%) of these patients went for surgery. Of those 26 patients, 21 had an incomplete report.

CONCLUSION(S)
Routine pathologic reporting in malignant polyps is incomplete, with only 40.7% complete reporting in this cohort of 236. This has a major impact on patient care, with 41% of patients with no identified poor prognostic factor on their pathology report going for subsequent surgery and the majority (80%) of those patients having an incomplete report.

CITIZEN SCIENCE APPROACH TO TICK-BORNE DISEASE SURVEILLANCE IN MANITOBA



BACKGROUND/OBJECTIVES
In Manitoba, the burden of tick-borne diseases (TBDs) is increasing as a result of the range expansion of *Ixodes scapularis* ticks, likely due to climate change. The incidence of Lyme disease in 2016 in Manitoba was 3.88 per 100,000 but is thought to be underestimated due to challenges in diagnosis and under-reporting by physicians. Surveillance using citizen science may enhance current tick and TBD surveillance to better estimate the true burden of TBDs and improve prevention efforts.

METHOD(S)
We propose a two-pronged approach that consists of passive tick surveillance to track the geographic distribution of tick species and estimate carriage rates of TBDs, and syndromic Lyme disease surveillance to capture clinical cases that may be missed under current reporting practices. A mobile phone app will be launched and promoted as a data collection tool. Respondents will be asked for demographic information as well as the place and date the tick was found, along with an uploaded image. Tick reports and photographs will be screened and respondents whose ticks were identified as I. scapularis will be asked to submit the ticks. Individuals to whom ticks have been attached will be advised about the symptoms of Lyme disease, and after four weeks they will be asked whether they were diagnosed and treated for early Lyme disease.

RESULT(S)
Tick counts will be calculated along with the prevalence of ticks harbouring tick-borne diseases. Exposed individuals who were clinically diagnosed or treated will be counted for calculation of the incidence of clinical cases of early Lyme disease. Cases that were diagnosed serologically or reported by physicians will be removed to avoid double counting. Surveillance results will be disseminated to support primary and secondary prevention efforts.

CONCLUSION(S)
The strength of this proposed surveillance system lies in its acceptability, sensitivity, and simplicity. We propose consultation with local stakeholders in British Columbia to consider a similar surveillance system here.

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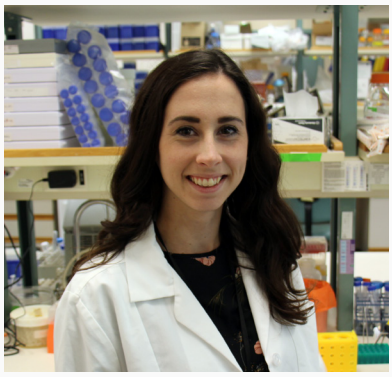

PROTEIN TYROSINE PHOSPHATASE ALPHA (PTPA) POSITIVELY REGULATES INVADOPODIA-MEDIATED CANCER CELL MOTILITY

Supervisor:
Dr. Kevin Bennewith and Dr. Catherine Pallen

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BACKGROUND/OBJECTIVES
Normal cell migration is a highly conserved process that plays a critical role in many physiological processes such as wound healing. Aberrant cell migration is implicated in cancer metastasis, which accounts for 90% of cancer -related mortality. In normal cells, the dynamic process of cellular migration by which cells move along the extracellular matrix (ECM) is mediated by the formation of focal adhesions. In highly invasive tumour cells, the ability to invade is mediated by similar integrin-mediated structures called invadopodia. These are dynamic Src-regulated, actin-based protrusions of the plasma membrane that mediate ECM degradation. Protein tyrosine phosphatase alpha (PTPα), a widely expressed transmembrane protein, acts in normal cells to promote focal adhesion formation and cell migration. However, little is known about the role of PTPα in cancer cell motility. *Therefore, we hypothesize that PTPα regulates invadopodia formation and function to promote the invasive motility of malignant cells.*

METHOD(S)
Small interfering-RNA (siRNA) was used to deplete PTPα in MDA-MB-231 cells and a siRNA-resistant PTPα-expressing plasmid was used to rescue PTPα. Transwell migration and invasion assays were used to investigate the role of PTPα in tumour cell motility. An invadopodia-mediated ECM-degradation assay was used to determine the degradation ability of control vs. PTPα-depleted cells. Short-hairpin RNA (shRNA) was then used to stably knockdown PTPα in MDA-MB-231 cells. The shRNA control and knockdown cells were stained for invadopodial markers to determine the role of PTPα in invadopodia formation and if PTPα localizes to invadopodia. Statistical analysis was performed using a Student's t-test for a two-group comparison or a one-way ANOVA for the analysis of three or more groups.

RESULT(S)
PTPα knockdown showed reduced migration and invasion through the Transwell membrane 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 compared to the control and this defect was rescued by restoring PTPα expression. Interestingly, control and PTPα-depleted cells formed equivalent numbers of invadopodia, and PTPα co-localized with cortactin, actin, and MT1-MMP to punctate invadopodia-like structures. Together, these findings indicate that PTPα, present within mature invadopodia, positively regulates invadopodia-mediated ECM degradation while not affecting invadopodia formation. Furthermore, preliminary data show that PTPα depletion decreased MMP9 expression and activity as well as cell surface expression of MT1-MMP outlining a possible reason for the dysfunctional invadopodia.

CONCLUSION(S)
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.

CHARACTERIZATION OF *IDH1/IDH2* MUTATION AND D-2-HYDROXYGLUTARATE ONCOMETABOLITE LEVEL IN DEDIFFERENTIATED CHONDROSARCOMA

BACKGROUND/OBJECTIVES
Dedifferentiated chondrosarcoma (DDCHS) is an aggressive type of chondrosarcomas, and results from high-grade transformation of a low-grade chondrosarcoma. Isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* mutations have been recently described in low-grade cartilaginous neoplasms. These mutations lead to increased D-2-hydroxyglutarate (2HG) oncometabolite production, promoting tumorigenesis through CpG island and histone hypermethylation. Our aim is to determine the frequency of *IDH1/2* mutations in DDCHS and correlate it with 2HG levels.

METHOD(S)
We examined a series of 21 resection specimens of primary DDCHS using Sanger sequencing and qPCR genotyping to interrogate for *IDH1/2* mutations, and using a fluorimetric assay to evaluate the level of 2HG in formalin-fixed paraffin-embedded (FFPE) tumor and matched normal tissue samples.

RESULT(S)
Seventy-six percent of DDCHS (16/21) harbored either *IDH1* or *IDH2* mutations, with R132L (3/16), R132H (2/16), R132S (3/16), R132C (1/16) and R132G (1/16) in IDH1, and R172S (4/16) and R172M (2/16) in *IDH2*. *IDH1/2*-mutated DDCHS showed elevated 2HG in the tumor relative to matched normal tissue, with 2HG level being highest in tumors with IDH2 R172S mutation, whereas DDCHS with wild-type *IDH1/2* showed no elevation in 2HG level. There were no consistent histologic differences between *IDH1/2*-mutated tumors and wild-type tumors. The mean age of patients with *IDH1/2*-mutated DDCHS was 65.7 years. Amongst these 16 mutation-positive patients, follow-up data was available for 14 patients, with an average follow-up period of 22 months. 11 of 14 patients (78%) died from their disease, most within a year of diagnosis, while 2 patients were alive with recurrent disease.

CONCLUSION(S)
Our study confirms the frequent presence of *IDH1/2* mutations in DDCHS, which is seen in about three-quarter of the cases. A broad range of *IDH1/2* mutation variants is seen, indicating that a sequencing-based approach to mutation analysis is required for DDCHS if it is to be used as a diagnostic marker. As seen in other *IDH1/2*-mutated tumor types (i.e. glioma and acute myeloid leukemia), *IDH1/2*-mutated DDCHS also shows elevated 2HG level, indicating that it may depend at least in part on the oncometabolite activity for its oncogenesis.

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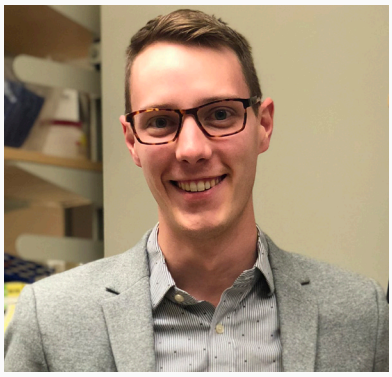

PBRM1 EXPRESSION IS LOST IN SMALL CELL CARCINOMA OF THE OVARY HYPERCALCEMIC TYPE

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BACKGROUND/OBJECTIVES

Small cell carcinoma of the ovary hypercalcemic type (SSCOHT), is a lethal malignancy affecting young women and children. Little is known about the origin of this tumor but dysfunctional SWI/SNF proteins SMARCA4 and SMARCA2 is well established as part of its pathophysiology. In an effort to further define the expression of SWI/SNF proteins in SCCOHT, we performed comprehensive proteomic analysis based on mass spectrometry.

METHOD(S)

Full proteome analysis of 16 cases of SCCOHT, and a mix of 12 tubo-ovarian high grade serous carcinomas and Sertoli-Leydig cell tumors with no known SWI/SNF alterations. The SP3-clinical proteomics protocol was performed on formalin fixed tissue and was run on an ThermoFisher Orbitrap Fusion. Analytic pipeline included batch effect correction (ComBat) and Protein Expression Control Analysis (PECA). Unsupervised cluster analysis was performed between tumor types in function of 28 known SWI/SNF complex proteins.

RESULT(S)

The proteomic analysis identified 7329 proteins, 1230 were significantly enriched in the SCCOHT cohort when compared to tubo-ovarian tumors with no known SWI/SNF alterations. SWI/SNF cluster analysis and PECA showed decreased expression of SMARCA4, SMARCA2 and PBRM1 in SCCOHT compared to other ovarian malignancies ($p<<0.0001$).

CONCLUSION(S)

This is the first report of PBRM1 loss in SCCOHT, an important gene involved in the stability of the SWI/SNF complex. PBRM1 has shown potential as an immunotherapy biomarker in RCC but further testing in SCCOHT is needed to evaluate its predictive role. Immunohistochemical validation is underway.

OPTIMIZATION OF SOLID TUMOUR PERFUSION CAN IMPROVE RADIATION THERAPY RESPONSE BY ENHANCING OXYGEN DELIVERY

BACKGROUND/OBJECTIVES
Aggressive solid tumours often contain significant regions with inadequate oxygen supply (ie hypoxia) due to high tumour cell metabolism and an immature blood vessel network. Extensive evidence demonstrates that hypoxia provides tumour cells with significant resistance to radiation therapy, which is reflected in clinical studies where hypoxia predicts poor outcome across a variety of cancers.

Interventions to reduce hypoxia at the time of radiation include drugs that aim to acutely enhance tumour perfusion. Nicotinamide and pentoxifylline are two drugs cited to provide acute perfusion enhancement to tumours, however, clinical trials combining these drugs with radiation therapy largely conclude non-significant benefits. In retrospect, the body of pre-clinical evidence suggests that many tumours are indeed not responsive to these drugs. We hypothesize that the lack of significant clinical benefit from nicotinamide and pentoxifylline is due to this heterogeneity in response to these drugs. The first aim of this study was to quantify differences in oxygen delivery and response to acute perfusion modifying drugs between responder and non-responder tumour models. The second aim of this study was to test if chronic pharmacological intervention with the intent of reducing intra-tumour pressure could convert non-responsive tumours into tumours that will respond to acute perfusion modifying drugs.

METHOD(S)
Male NSG mice were subcutaneously implanted with both WiDr human colorectal adenocarcinoma and SiHa human cervical squamous cell carcinoma xenografts for all experiments. Prior to endpoint measurements mice were administered either nicotinamide, pentoxifylline, or control. 2-^{18}F-fluoroethanol uptake was quantified using PET to measure perfusion to tumour and normal tissue. 2-Nitroimidazole uptake was detected using immunofluorescent microscopy and flow cytometry to measure hypoxia. For radiation experiments mice were subject to whole body 10 Gy x-irradiation and tumour cell survival was assessed using clonogenic assay. For experiments addressing the second aim of this study mice were administered 20mg/kg losartan or PBS for 14 days prior to endpoint. Losartan is an angiotensin receptor blocker cited to reduce compression of blood vessels in solid tumours.

RESULT(S)
Both pentoxifylline and nicotinamide alone reduced hypoxia in WiDr tumours but not SiHa, losartan treatment reduced hypoxia in both. Analysis of immunofluorescent microscopy revealed that central, higher pressure and more hypoxic, regions of WiDr tumours were responsive to pentoxifylline alone, while the combination of losartan and pentoxifylline was required to oxygenate central regions of SiHa tumours. Similarly, chronic losartan treatment alone induced a significant increase in radiation sensitivity for WiDr, while the combination of losartan followed by pentoxifylline was required to produce a significant increase in radiation sensitivity for SiHa tumours.

CONCLUSION(S)
Acute enhancement of solid tumour perfusion can improve tumour response to radiation and the response rate to acute perfusion modifying agents can be optimized by chronic losartan treatment.

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REPRODUCIBILITY OF GRADING IN CHRONIC HISTIOCYTIC INTERVILLOSITIS OF UNKNOWN ETIOLOGY

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BACKGROUND/OBJECTIVES
Chronic histiocytic intervillositis of unknown etiology (CIUE) is a rare inflammatory process associated with pregnancy loss and recurrence risk. CIUE is characterized by intervillous histiocytosis with variable associated fibrin and can be graded according to proportion of involved intervillous space. Definitive therapy is unclear but high grade CIUE may prompt steroid therapy in subsequent pregnancies in an attempt to delay or prevent recurrence. Accurate and reproducible grading is important to avoid unnecessary steroid-related side effects. We conducted a quality assurance study to assess diagnostic accuracy and reproducibility of CIUE grading at our institution.

METHOD(S)
Twenty cases of CIUE were identified at our institution between 2010-2016 including 15 first trimester and 5 second trimester pregnancy losses. No third trimester losses or live births were found. De-identified slides from all submitted placental tissue from each case were reviewed by 7 perinatal pathologists in 2 sequential rounds. In the first round, 20 slides (15 cases) were graded according to the Rota scheme: 0= negative, 1= <10%, 2= 10 to <50%, and 3= ≥50% of intervillous space occupied by histiocytes +/- associated fibrin. Accuracy and agreement for the initial 20 slides were reviewed as a group after which the remaining 11 slides (5 cases) were assessed. The majority Rota grade for each slide was considered to be correct for measuring accuracy and percent agreement. Interobserver variability was assessed by Fleiss' kappa. Research ethics board approval was obtained for reporting of study results.

RESULT(S)
For round 1, diagnostic accuracy was 94%, average percent agreement was 79% (range 43-100%), and Fleiss kappa value for interobserver variability was 0.54 (95% CI 0.48-0.60). After review, 2 cases were reclassified as negative (1 no CIUE and 1 acute neutrophilic intervillositis). Disagreement between Rota grades 0/1 and 1/2 was thought to be the greatest source of variability. In round 2, diagnostic accuracy was 83%, average percent agreement was 70% (range 43-100%), and Fleiss kappa value for interobserver variability was 0.36 (95% CI 0.28-0.43).

CONCLUSION(S)
CIUE was diagnosed primarily in trimesters 1 and 2 in our study population. Diagnostic accuracy was high and agreement on Rota grade was moderate. Group review did not improve accuracy and agreement, which may be due to a higher proportion of Rota grade 0/1/2 slides in round 2 (66% vs. 56% in round 1). Simplifying grading to a low grade/high grade scheme (<50% or ≥50%) might improve reproducibility.

ASSESSMENT OF PLATELET FUNCTION USING A MULTICOLOR FLOW CYTOMETRY

BACKGROUND/OBJECTIVES
Platelets play a pivotal role in hemostasis by preventing bleeding at a site of vascular injury. Therefore, assessment of platelet function is essential in patients with bleeding disorders. Flow cytometry has proven beneficial in investigation of inherited and acquired platelet disorders such as Glanzman thrombasthenia, Bernard-Soulier syndrome, and storage pool disease. In this study we describe the initial steps of development of a clinical platelet flow cytometry assay using normal healthy volunteers to assess the expression of platelet surface glycoproteins and activation-dependent platelet surface markers in response to ADP agonist.

METHOD(S)
Citrated whole blood samples from five healthy volunteers were obtained. All samples were analyzed using two platelet flow cytometry panels. The first panel measured the expression of the surface glycoproteins using the following antibodies: CD41, CD42a, CD42b, CD49b, CD61 and GPVI. The second panel measured the expression the activation dependent markers, CD62P and CD63, in response to stimulation by ADP. The median florescent intensity (MFI) and the normal ranges for each antibody were obtained.

RESULT(S)
Panel 1 showed good separation in most antibodies form their negative isotype controls. The MFI and the normal range for each antibody in panel 1 were 31.46 (27.61-46.21) for CD41, 42.61 (36.56-61.52) for CD61, 39.60 (32.52-44.00) for CD42b, 26.47 (22.24-28.66) for CD42a, 11.93(9.63-18.40) for CD49b and 6.79 (5.33-7.10) for GPVI. Panel 2 showed a good separation of CD62P level in response to ADP stimulation compared to pre-activation level, however the peaks are very broad in general, which led to highly variable X-median values. Analysis of CD63 showed minimal overlap with the isotype control except in one sample but overall there was a good separation between activated and non-activated levels.

CONCLUSION(S)
We described the results of initial steps of developing a multicolor platelet flow cytometry assay using normal controls. Increasing the number of agonists used and validating this assay using known patients with well-defined platelet bleeding disorder will be of interest. In conclusion, we are the first clinical laboratory in British Columbia to develop a multicolour platelet flow cytometry test. This test will be especially helpful in children as only a small amount of whole blood is required in comparison to platelet aggregometry.

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HIV-EXPOSED UNINFECTED (HEU) BLOOD MTDNA CONTENT REMAINS ELEVATED FROM BIRTH INTO EARLY LIFE (0-3Y)

Supervisor:
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Author(s):
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BACKGROUND/OBJECTIVES
More than 35 million people live with HIV globally, of which 48% are women and 10% are children. Maternal combination antiretroviral (ARV) therapy (cART) during pregnancy has been highly successful in preventing mother-to-child HIV transmission. However, in utero exposure to ARVs, many of which can cross the placenta, could have long term effects on HIV-exposed uninfected (HEU) infants. Some ARVs can cause mitochondrial toxicity and affect mitochondrial DNA (mtDNA) quantity and quality. Previous studies among HEU infants exposed to cART in utero have been inconsistent, with some reporting increased blood mtDNA levels at birth compared to HIV-unexposed uninfected (HUU) infants, while others report a decrease. Our objective was to compare HEU and HUU infant blood mtDNA content at birth and over the first 3 years of life, and investigate any relationship to cART exposure.

METHOD(S)
Peripheral blood mtDNA content was measured by monochrome multiplex qPCR in 324 HEU (0-3y, of whom 214 had ≥2 blood samples) and 308 HUU children (0-3y, each with a single blood sample) enrolled in the CARMA cohort study. A subset of these children was randomly age- and sex-matched 1:1 for a cross-sectional comparison of mtDNA content over the first 3y of life. Factors showing an association univariately ($p<0.1$) were considered in multivariable linear regression models.

RESULT(S)
mtDNA content at birth (0-3d) was obtained for 114 HEU and 86 HUU children. In a multivariable model of mtDNA content at birth (n=192) that included HEUs categorized according to type of in utero cART exposure (ref. HUU), gestational age (GA) at birth, and maternal smoking during pregnancy, lower GA was the only factor independently associated with higher mtDNA content ($p=0.003$). Among infants born at term (GA>37w, n=161), GA was no longer associated with birth mtDNA content, and only HEUs exposed in utero to Ritonavir-boosted PIs in combination with ABC+3TC ($p=0.009$) or AZT+3TC ($p=0.03$) had higher birth mtDNA content compared to HUUs. Additionally, infant AZT prophylaxis did not affect mtDNA content at 6w vs. birth. Lastly, among age and sex-matched children (n=214:214), HEU children had persistently higher mtDNA content than HUUs ($p<0.01$) throughout the first 3 years of life.

CONCLUSION(S)
HEUs and infants born preterm have higher mtDNA content at birth, an effect that persisted among HEUs up to age three. This may represent a long-term effect, possibly resulting from adaptive mitochondrial biogenesis in response to in utero stresses.

INCREASED EXPRESSION OF CARBONIC ANHYDRASE IX IS ASSOCIATED WITH IMPROVED PROGNOSIS IN RESECTED PANCREATIC DUCTAL ADENOCARCINOMA

BACKGROUND/OBJECTIVES
Carbonic anhydrase IX (CAIX), a transmembranous enzyme that is part of the hypoxia-inducible factor pathway, has been implicated as an adverse prognostic marker and oncogenic protein in various malignancies, including pancreatic ductal adenocarcinomas (PDACs). The aim of this study was to assess CAIX as a prognostic marker in our cohort of pancreatic ductal adenocarcinomas (PDACs).

METHOD(S)
A duplicate 0.6mm core tissue microarray containing tumor samples of PDACs from 257 patients was stained for CAIX with the M75 antibody. CAIX membranous expression was assessed by an H-score, obtained as the product of a semi-quantitative estimate of percentage of positive cells and staining intensity (0 = negative, 1 = weak, 2 = moderate, and 3 = strong). The maximum score was used for each case. The H-score cutpoint for high and low CAIX expression was selected to optimize the binarization of disease-specific survival (DSS). Comparisons with clinicopathologic variables were performed as well as univariate and multivariate analysis of DSS.

RESULT(S)
Eighty-one patients had tumors with high CAIX expression (H-score ≥240), while 176 patients had low expression of CAIX (H-score <240). High expression of CAIX was associated with less lymphovascular invasion (p = 0.01) and less perineural invasion (p = 0.01). High CAIX expression was associated with a higher median DSS of 1.88 y (95% confidence interval (CI), 1.52 – 2.42) compared to low CAIX expression (median DSS 1.33 y, 95% CI, 1.10-1.63, P = 0.0127). On multivariate analysis, CAIX expression was not an independent prognostic marker.

CONCLUSION(S)
PDACs with high expression of CAIX were less likely to be associated with lymphovascular invasion and perineural invasion. High expression of CAIX in PDACs was associated with improved DSS, but was not an independent prognostic marker. Our findings contrast with previous studies that reported CAIX expression as an adverse prognostic marker.

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EVERYTHING BUT ADENOMATA: 4 YEARS' EXPERIENCE OF THE BRITISH COLUMBIA COLON SCREENING PROGRAM

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BACKGROUND/OBJECTIVES

The British Columbia Colon Screening Program (BCCSP) was implemented in November 2013 with the goal of reducing colorectal cancer mortality via the early detection of colorectal cancer and removal of its precursor lesions. In addition to this, various lesions of epithelial and mesenchymal origin have been incidentally discovered. This study focuses on lesions detected beyond the conventional adenomata.

METHOD(S)

The pathology of specimens collected through the BCCSP from November 2013 to December 2017 was assessed using data collected by the British Columbia Cancer Agency (BCCA).

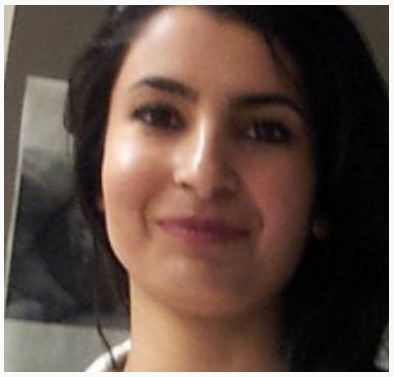
RESULT(S)

There were 88,541 colonoscopies performed in the period from November 2013 to December 2017. Most lesions (n = 55595, 51.6%) were tubular adenomata, followed by hyperplastic polyps (n = 21343, 19.8%), tubulovillous adenomas (n = 7100, 6.6%), sessile serrated adenomas (n = 5107, 4.7%), villous adenomas (n = 545, 0.5%), and traditional serrated adenomas (n = 271, 0.3%). Only 0.7% (n = 739) were adenocarcinomas. Eight percent (n = 8643) were normal, while 1.5% (n = 1658) showed only inflammation. The remainder (n = 6372, 5.9%) showed other pathology, including inflammatory polyps (n = 1020), mesenchymomas (n = 220), leiomyomas (n = 210), neuroendocrine tumours (n = 65), juvenile polyps (n = 56), Peutz-Jeghers polyps (n= 24), squamous cell carcinomas (n = 13), and one gastrointestinal stromal tumour.

CONCLUSION(S)

In addition to the early detection of colorectal cancer and its precursors, the BCCSP has detected a variety of incidental non-adenomatous pathologies which has allowed for adequate patient assessment and follow up.

SINGLE CELL RNA SEQUENCING OF LUNG LYMPHOCYTES IN RORA LINEAGE TRACER MICE REVEALS HETEROGENEITY OF LUNG ILC2S



BACKGROUND/OBJECTIVES
Group 2 innate lymphoid cells (ILC2s) are defined as lineage marker negative Thy1+CD127+ST2+CD25+ cells that express the transcription factors GATA3 and RORα in mouse lungs. ILC2s mediate diverse functions as they produce a variety of cytokines, growth factors and receptors/ligands including IL-5, IL-13, amphiregulin, IL-4, IL-2, IL-9, IL-10, GITR/L and ICOS/L. Our lab findings have indicated that ILC2s play an important role in allergic lung diseases including asthma. These cells are also involved in the development of a broad range of lung diseases, including COPD, lung fibrosis and tumors. It is unknown whether a single population of ILC2s produces all these cytokines and growth factors. The objective of this study is to fully characterize the heterogeneity of mouse lung ILC2s and dissect them into functionally distinct subsets, elucidate their developmental pathways and determine their roles in lung immunity.

METHOD(S)
To characterize the heterogeneity of mouse lung ILC2s, we crossed RORα-IRES-Cre mice with Rosa26-floxSTOPflox-YFP mice to generate RORα/YFP mice. Flow cytometric analyses of lymphocytes in RORα/YFP mice showed that ILC2s were marked by YFP. However, lung YFP+ ILC2s were heterogeneous with respect to the expression of the known ILC2 markers. To further analyze ILC2 heterogeneity, we sorted YFP- and YFP+ cells from adult and neonatal RORα/YFP mouse lungs and analyzed by single cell RNA sequencing (scRNAseq).

RESULT(S)
Our scRNAseq data analyses have confirmed that ILC2s heterogeneously express the known ILC2-associated genes. Also, our analyses have comprehensively revealed the genes that are specifically and/or differentially expressed in ILC2s compared to other lung immune cells.

CONCLUSION(S)
These results suggested that the lung ILC2 population is highly heterogeneous and can be divided into functional subsets, each expressing a distinct set of ILC2-associated genes. This study has also shown the expression of novel genes with potential immune regulatory/stimulatory functions on ILC2 subsets. ILC2s are recognized as possible therapeutic targets in many diseases and it is important to elucidate their complexity so that proper subsets are targeted in therapies.

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IMMUNOHISTOCHEMICAL CHARACTERIZATION OF LOCALLY AGGRESSIVE SARCOMATOID/RHABDOID RENAL CELL CARCINOMA

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BACKGROUND/OBJECTIVES
Sarcomatoid features in renal cell carcinoma (RCC) confers a poor prognosis and is of unknown pathogensis. In RCC, sarcomatoid features are observed in 5% of tumors. However, among individuals with clinical stage IV disease, it occurs in up to 15% of cases.
Rhabdoid cells have been recognized as one of the cellular components of sarcomatoid RCCs. Sarcomatoid RCCs with rhabdoid features are exceedingly rare and their significance remains unclear. The limited data available suggests that rhabdoid features portend a poor prognosis and are most likely associated with high-grade tumors of advanced stage. Our understanding of the genetic events associated with sarcomatoid and rhabdoid differentiation in renal tumors remains limited. To gain better insight into the tumor biology of these aggressive lesions, we performed immunohistochemical analysis on cases which presented with pathologic stage pT3 or higher and sarcomatoid differentation over a 17 year period at Vancouver General Hospital.

METHOD(S)
This study is a retrospective review of cases which presented to Vancouver General Hospital between the years 2001-2017, with locally aggressive (pathologic stage pT3 or higher) renal cell carcinoma that also showed sarcomatoid differentiation. 33 cases were identified which met inclusion criteria. 31 cases were included in the construction of a tissue microarray. A panel of immunohistochemical stains was run on the tissue microarray including MDM2, PAX8, p53, SMA, BAP1, BRM, BRG1, and INI1.

RESULT(S)
Of the 33 sampled cases, 27 contained a carcinoma component, which consisted of 17 clear cell, 3 chromophobe, 3 collecting duct, 2 papillary, and 2 mixed clear cell papillary. PAX8 expression was uncommon in carcinoma showing positive expression in 4 (17.4%) of cases, and showed retained expression in 2 rhabdoid cases. Strong diffuse nuclear p53 staining indicative of TP53 mutation was seen in the sarcomatoid or rhabdoid component of 5 of 32 cases (15.6%) while the corresponding carcinoma component showed wild-type p53 expression. MDM2 showed expression in 2 (8.3%) of sarcomatoid areas, one case from a pure sarcoma, both cases showing wild-type p53 expression. BRG1 and BRM showed complete loss of of nuclear expression in 1 and 2 rhabdoid cases, respectively, while the corresponding carcinoma showed retained expression. INI1 was retained in all cases.

CONCLUSION(S)
Locally aggressive RCC showing sarcomatoid or rhabdoid features frequently lack PAX8 expression in the carcinoma component. The acquisition of TP53 mutation and MDM2 overexpression may be associated in sarcomatoid transformation in a subset of sarcomatoid RCC. The loss of BAP1, BRM and BRG1 expression may represent a mechanism that results in rhabdoid histology. The findings of occasional MDM2 overexpression in sarcomatoid RCC also suggest that MDM2 overexpression is not specific for well differentiated/dedifferentiated liposarcoma in a retroperitonel location unless an underlying renal mass can be excluded radiologically.

IMMUNOGLOBULIN G4-RELATED DISEASE (IGG4-RD) OF THE ILEOCECAL REGION: A CASE REPORT RESEMBLING MALIGNANCY

BACKGROUND/OBJECTIVES
Immunoglobulin G4-related disease (IgG4-RD) is a chronic mass forming inflammatory disease characterized by fibroblastic proliferation and mixed inflammatory cell infiltration, predominantly lymphocytes and plasma cells. IgG4-RD can involve one or multiple organs, and the most commonly affected organs include the pancreas, salivary glands, and the orbit. The diagnosis of IgG4-RD rests on the combination of characteristic histopathological features and increased IgG4 plasma cells. Key histopathologic findings include a dense lymphoplasmacytic infiltrate, a storiform pattern of fibrosis, and obliterative phlebitis, and at least two of these features are necessary for the diagnosis of IgG4-RD. An initial therapeutic response to glucocorticoid therapy is often a hallmark of IgG4-RD.

METHOD(S)
We present a case of a 66-year-old male presenting with an obstructing ileocecal mass. The patient also had biopsy-proven pulmonary sarcoidosis. He underwent an open right hemi-colectomy for suspected malignancy. The mass was sectioned and the tissue was used for microscopic examination, elastin and immunohistochemical staining.

RESULT(S)
Sections from the mass showed lymphoplasmacytic infiltrates, lymphoid follicles, and storiform fibrosis in the subserosa extending into the submucosa. Elastin stains highlighted residual venous elastic lamina confirming obliterative phlebitis. Immunohistochemistry staining revealed an increased IgG4/IgG ratio, and there was no malignancy identified. The patient was not treated with immunosuppressive therapy and he was asymptomatic twenty months postoperatively.

CONCLUSION(S)
IgG4-RD can manifest in various ways, including obstruction in the gastrointestinal tract and abdominal mass mimicking malignancy. It is important to consider IgG4-RD in the differential diagnosis of an obstructive gastrointestinal mass, especially if the patient has other manifestations of IgG4-RD. In the absence of other manifestations, preoperative diagnosis is challenging, and IgG4-RD of the colon may continue to be a diagnostic pitfall.

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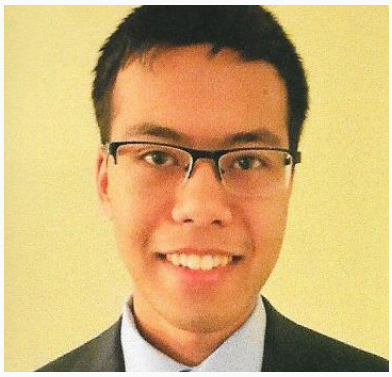

GAMMA-DELTA T LYMPHOCYTE RESPONSES TO PRIMARY EBV & CMV INFECTION IN UGANDAN INFANTS

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BACKGROUND/OBJECTIVES

Gamma-delta T lymphocytes (GD T cells) constitute a minority of the total T cell compartment in humans, but are unique from conventional alpha-beta T lymphocytes in that they are not restricted by classical major histocompatibility complex molecules. GD T cells develop early in gestation and are thought to be important in the control of early-life infections. The fetus and infant are highly susceptible to viral infections, such as those caused by human cytomegalovirus (CMV) and Epstein-Barr virus (EBV), resulting in significant mortality and morbidity. We investigated the changes in GD T cell populations and their T cell receptors (TCRs) in infants to elucidate the possible functions of GD T cells in immune responses to viral infection.

METHOD(S)

Peripheral blood mononuclear cells (PBMCs), plasma, and oral swabs were isolated from infants of mothers with known human immunodeficiency virus (HIV) status that were enrolled in the Primary Herpesvirus in Children Study (PHICS), a longitudinal birth cohort study of primary human herpesvirus (HHV) infections in Kampala, Uganda. CMV and EBV viral loads were assessed by real-time polymerase chain reaction (PCR) of the oral and plasma specimens. Serology was performed using enzyme-linked immunoassays to identify infant immune status to CMV and EBV in the plasma. Flow cytometry was performed on unstimulated PBMCs using T, B, and natural killer (NK) / GD T cell staining protocols. After stimulation of PBMCs with CMV- and EBV-specific antigens, proliferation using the CFSE assay and cytokines IL-2, IFN-gamma, and TNF-alpha were measured. Finally, RNA was extracted from unstimulated PBMCs and will be analyzed using high-throughput sequencing (HTS) of the CDR3 repertoire of gamma and delta TCR chains to quantify GD T cell populations and responses in CMV- and EBV-infected and -uninfected infants.

RESULT(S)

20 CMV and 19 EBV primary infections were identified in infants, as defined by PCR evidence of high oral shedding or viremia and validated by serology. Many infants also displayed low-level, transiently positive oral PCRs in the absence of infection, both preceding confirmed infections and in those who never developed viremia. Preliminary flow cytometric analysis revealed significant expansions of GD T cell populations in some but not all CMV-infected infants, including some with transient infections. Lymphocytic expansion correlated with other immune parameters, such as NKG2C-positive NK cells and B cells.

CONCLUSION(S)

EBV was acquired significantly earlier in infants exposed to HIV via their mothers, compared to those who were unexposed. Transient "blips" of HHV infection were very common in the PHICS cohort and have not been described in previous literature. GD T cell expansions in CMV-infected infants may suggest that these lymphocytes represent one important mechanism by which humans can fight viral infection in early stages of immune development. Further computational analysis of flow data for additional lymphocytes (T, B, NK) and GD TCR sequencing may help reveal patterns in host immune responses to early-life HHV infection or novel GD T cell subpopulations in humans.

USING MACHINE LEARNING TO PREDICT ASPARTATE AMINOTRANSFERASE (AST) TEST RESULTS

BACKGROUND/OBJECTIVES
Previous studies have observed a significant correlation between aspartate aminotransferase (AST) and other liver enzymes. It was proposed that AST testing may only be indicated in selected clinical scenarios; however, the validity of the research methodology was challenged. Herein we adopted a machine learning approach to rigorously study the relationship between AST and other liver enzymes to confirm the predictability of AST.

METHOD(S)
We extracted six months of inpatient and outpatient liver enzyme data to train regression algorithm models for AST result prediction, based on results from other liver biochemical tests. We compared predicted and measured AST values, and calculated the proportion of non-elevated AST results that could be eliminated based on the model proposed, as well as the cost savings realized.

RESULT(S)
Results of other liver enzymes, namely alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT), successfully predicted whether or not AST results would be elevated, achieving an area under the curve (AUC) as high as 0.902. ALT alone achieved an AUC of 0.89 in predicting AST abnormality. If AST testing were reflexed only when the probability of AST elevation exceeded 10%, half of AST testing could be eliminated, while missing 8.8% of elevated AST results (representing 2.4% of total AST requests).

CONCLUSION(S)
Substantial information predicting the likelihood of AST abnormality are captured by the results of other liver enzymes, especially ALT. We have demonstrated how machine learning can be applied to a large laboratory dataset to selectively eliminate AST requests which have no added clinical benefit. Locally in the province of British Columbia, this approach could result in annual cost savings of $85,000.

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CHARACTERIZING NEUROPROTECTIVE ROLES FOR ARYL-HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR EXPRESSION AND NEURONAL PAS 4 DOMAIN PROTEIN IN MODELS OF MULTIPLE SCLEROSIS

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BACKGROUND/OBJECTIVES
Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system (CNS), characterized by disruption of the myelin sheath, axonal loss and neurodegeneration which leads to disability. The aryl-hydrocarbon receptor nuclear translocator 2 (ARNT2) is a transcription factor that can dimerize with other transcription factors to drive gene regulation in response to developmental and environmental stimuli. ARNT2 is largely limited to the CNS, is highly expressed in neurons and has been found essential for CNS development and protective in models of ischemia. ARNT2 binds to neuronal PAS domain protein 4 (Npas4) in the healthy brain where complexes regulate brain-derived neurotrophic factor (BDNF), a major mediator of neuronal and axonal health. We hypothesized that Npas4 and ARNT2 expression was dysregulated over the course of experimental autoimmune encephalomyelitis, the animal model of MS. We found Npas4 and ARNT2 lowest at peak clinical disease, and proposed their deletion would decrease cell viability through BDNF reductions. We examined ARNT2 and Npas4 regulation and influence on cell viability in neurons and astrocytes, the primary sources of these factors in the CNS.

METHOD(S)
Spinal cord tissues from EAE mice were analyzed at several points over the course of disease for ARNT2 RNA and protein expression by qPCR, western blotting and immunohistochemistry, respectively. Astrocyte morphology, viability and BDNF expression was tested in vitro in response to serum deprivation and following siRNA-mediated knockdown of ARNT2. Neuronal viability and BDNF expression was tested in vitro following siRNA-mediated knockdown of Npas4. BDNF, ARNT2 and Npas4 expression was examined by immunocytochemistry and qPCR.

RESULT(S)
ARNT2 expression is significantly decreased in EAE mice at peak disease, both in astrocytes and neurons. In culture, astrocytes express negligible amounts of ARNT2 and BDNF. Serum deprivation significantly increases ARNT2 and BDNF message and protein within 12-24 hours, and changes astrocyte morphology to a more ramified and activated phenotype. Notably, ARNT2 knockdown in astrocytes does not affect BDNF expression or viability. Adult neurons constitutively express moderate to high levels of ARNT2 RNA and protein. In response to oxidative stress, ARNT2 protein levels are rapidly increased, independent of de novo RNA production. Oxidative stress drives de novo neuronal expression of Npas4 and increases BDNF. Notably, knockdown of ARNT2 or Npas4 in adult neurons does not affect BDNF expression or viability in steady state or in response to stressors.

CONCLUSION(S)
In contrast to developmental studies and settings, regulation of BDNF by Npas4 and ARNT2 is not the primary neurotrophic property attributed to these proteins. Our findings suggest that other partners for Npas4 and ARNT2 exist and drive trophic support in the CNS. Identifying these partners and their downstream targets may pinpoint novel targets for intervention to afford neuroprotection and limit disability in MS.

APPLICATION OF THE PROACTIVE MOLECULAR RISK CLASSIFIER OF ENDOMETRIAL CANCER (PROMISE) IN YOUNG WOMEN

BACKGROUND/OBJECTIVES
Endometrial carcinoma (EC) is the most prevalent gynecologic malignancy [1,2] and the sixth most prevalent cancer in women globally [3]. The Proactive Molecular Risk Classifier of Endometrial Cancer (ProMisE) uses targeted molecular analysis for enhanced stratification of EC. This EC classifier has been validated in a large population-based case series inclusive of all age groups. Here, we use ProMisE to assess a retrospective cohort of young women (age <50) from across British Columbia diagnosed with EC.

METHOD(S)
We assessed a retrospective cohort of 171 women (age 29-49, median 43) from across British Columbia diagnosed with EC between 1997-2014. We sequenced the exonuclease domain of polymerase epsilon (POLE) and assessed p53 and mismatch repair (MMR) immunohistochemistry for subsequent molecular stratification. Disease-specific, progression-free, and overall survival were evaluated for clinical and molecular features.

RESULT(S)
ProMisE molecular classification of 171 EC in young women revealed 35 (20%) were MMR deficient, 18 (11%) were POLE mutant, 5 (2.9%) were p53 abnormal, and 113 (66%) were p53 wildtype. Age at diagnosis was the youngest in the p53wt group: 22% < 35, representing 86% of all diagnoses in this age group, and 25% between 36-40), and all p53abn cases were in the 41-49 age bracket (median age 48). Although most patients were obese or morbidly obese, the patients with MMR deficient tumors were more likely to have normal body mass index (BMI) (35%) compared to patients with POLE mutant (7%) or p53 wild type tumors (4%)($p<0.001$). MMR deficient tumors were more likely to be advanced stage (stage 3 or 4) at presentation, compared to POLE mutant or p53 wildtype tumors (31% versus 11% or 4%, $p<0.001$).

CONCLUSION(S)
Endometrial carcinomas arising in women less than 50 are predominantly of p53 wildtype molecular subtype, but with a significant minority of MMR deficient and POLE mutant tumors. The MMR deficient EC are less likely to be associated with obesity/increased BMI, and are more likely to be advanced stage. The ProMisE molecular classifier can identify subsets of young women with EC whose tumors differ with respect to risk factors and natural history, information important in deciding whether to pursue conservative management with preservation of fertility, and important consideration in this subset of patients with EC.

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REPETITIVE CLOSED-HEAD IMPACT MODEL OF ENGINEERED ROTATIONAL ACCELERATION INJURY IN RATS INCREASES IMPULSIVE CHOICE, ALTERS THE DOPAMINERGIC MESOLIMBIC PATHWAY AND GENERATES WHITE MATTER TAU PATHOLOGY

Supervisor:
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BACKGROUND/OBJECTIVES
Mild traumatic brain injuries (mTBI) accounts for up to 90 percent of all TBIs. A significant number of patients experience enduring symptoms after mTBI that include chronic traumatic encephalopathy (CTE), a condition characterized by headaches to increased psychiatric symptoms including impulsivity. Modelling translatable animal models to assess mTBI has proven to be challenging due to the heterogeneity of symptoms. Our group recently developed the Closed-Head Impact Model of Engineered Rotational Acceleration (CHIMERA) model of mTBI in mice, which generates a highly consistent mild injury defined by behavioral and histopathological measures. However, behavioral assessments have been limited to tasks of motor and memory function that mice can perform. To examine psychological deficits observed after mTBI, we used the delay discounting task, a measure of impulsive choice, to assess the effects of repeat closed-head injuries in rats subjected to five CHIMERA mTBIs spaced two weeks apart.

METHOD(S)
Rats were trained to perform the delay discounting (DD) task. In this task, rats choose between an immediate small reward (in a form of a sugar pellet) versus a larger delayed reward (in a form of 4 sugar pellets). After baselines were yielded, subjects were randomized for TBI and sham animals, and behavioral tests followed injury/sham procedure in subsequent weeks. Immunohistochemistry at 3 weeks post injury was performed and included staining for Iba1 to detect microglia, and CP13 to detect phosphorylated tau in areas of the brain involved in the mesolimbic reward pathway. Entire coronal sections were imaged, and quantification was performed using Image J by cell count per area.

RESULT(S)
The TBI group displayed significantly increased impulsivity through the DD task; rats subjected to TBI switch to the immediate reward sooner as the delay for the larger reward increases. Immunohistochemical examination of CP13-positive p-tau in grey matter showed an intriguing increase in pre-tangle-like neurons in the nucleus accumbens shell and orbitofrontal cortex, but not in the basolateral amygdala and olfactory tubercle. In addition, the optic tract showed significant increase of p-tau by CP13 staining in TBI animals compared to sham controls, indicating white matter changes consistent with CTE. White matter area such as optic tract and corpus callosum showed increase in Iba-1-positive microglia and argyrophilic fibres by silver staining, indicating microgliosis and axonal injury. Corpus callosum thickness was also significantly reduced in TBI group compared to shams, suggesting white matter degeneration.

CONCLUSION(S)
These observations support a selective effect of repetitive CHIMERA TBI on the dopaminergic mesolimbic reward pathway consistent with behavioral observations of increased impulsive choice without alterations in overall motor behavior. We are the first to demonstrate altered impulsive choice after cumulative concussive-like injury in rats. Although the mechanisms driving this phenomenon remain to be determined, axonal damage may be a key factor.

TARGETING MRNA TRANSLATION IN MYCN AMPLIFIED PEDIATRIC NEUROBLASTOMA

BACKGROUND/OBJECTIVES
The MYC oncogenes contribute to more than 50% of all human cancers, but their therapeutic targeting has proven challenging. *MYCN* amplification in childhood neuroblastoma (NB) determines aggressive disease and high mortality, underlying the need for novel and effective therapies. MYC-driven transformation is energy demanding and impairs cell survival under nutrient deprivation (ND), a characteristic stress condition within the tumor microenvironment. We recently identified eukaryotic Elongation Factor 2 Kinase (eEF2K) as a pivotal mediator of the adaptive response of tumor cells to ND. We therefore hypothesized that eEF2K facilitates the adaptation of *MYCN* amplified NB to ND, and that inhibiting this pathway can impair tumor progression.

METHOD(S)
Initially, immunohistochemistry for key molecules in the pathway (p-eEF2 Thr56) was performed on NB tissue microarrays (TMAs) to link results with clinical outcome. Effects of eEF2K genetic inactivation on cell survival were evaluated in vitro in NB cell lines under nutrient deprivation. Cell viability and cellular apoptosis were assessed by MTT assay, PI staining and western blotting for cleaved caspase 3. Finally, NB xenografts in mice, either fed ad libitum or kept under caloric restriction, were used to confirm in vitro observations.

RESULT(S)
Analyzing publicly available genomic databases and tissue microarrays, we found that high eEF2K expression and activity are strongly predictive of poor outcome in NB (p<0.001), and correlate with *MYCN* amplification (p<0.001). Inhibition of eEF2K significantly decreases survival of *MYCN* amplified NB cell lines in vitro under ND. Combination of eEF2K knockdown and caloric restriction determines a twofold growth decrease of *MYCN* amplified NB mouse xenografts. Finally, eEF2K inactivation significantly attenuated the ability of tumor cells to engage fatty acid oxidation under ND, suggesting a link between eEF2K, *MYCN* transformation and lipid metabolism.

CONCLUSION(S)
EEF2K represents a critical mediator for the adaptive response of *MYCN* amplified NB to acute metabolic stress, and is therefore a promising therapeutic target. Future studies will combine eEF2K pharmacological inhibition with caloric restriction mimetics, as eEF2K activity appears to be critical under ND.

Supervisor:

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IDENTIFICATION OF NOVEL BIOMARKERS FOR MESONEPHRIC-DERIVED CARCINOMAS

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BACKGROUND/OBJECTIVES
Female adnexal tumors of probable Wolffian origin (FATWOs) and mesonephric carcinomas of the endometrium and cervix are rare gynecologic tumors that lack robust evidence-based and tumor-specific treatment regimens. These tumors can be challenging to diagnose due to their rarity and morphologic similarities to other ovarian and uterine tumors. FATWOs and mesonephric carcinomas both arise from developmental remnants of the male mesonephric duct. Due to their common tissue of origin, it is likely that they have protein markers in common that can be used to distinguish both cancers from other gynecologic malignancies. Candidate biomarkers will be identified and tested for their potential use as diagnostic markers of FATWOs and mesonephric carcinomas.

METHOD(S)
A whole proteome screen comparing 9 endometrial mesonephric tumors to 54 endometrial tumors of various subtypes was performed to identify potential protein markers that are more highly expressed in mesonephric tumors. Antibodies targeting protein candidates are being used in immunohistochemical staining of FATWOs, endometrial mesonephric carcinomas, normal tissues, and other endometrial tumours.

RESULT(S)
Differentially expressed proteins between mesonephric and endometrial tumors were identified and we chose EEF1A2, GSK3B, and GSTM3 for follow up studies. EEF1A2 stained 8 of 9 mesonephric tumors and 2 of 8 FATWOs but suffered from a lack of specificity. The immunohistochemical staining conditions for GSK3B is currently being optimized. GSTM3 shows male-tissue-specific expression and stained 7 of 9 mesonephric tumors and 5 of 8 FATWOs. The sensitivity and specificity of these antibodies as diagnostic markers of FATWOs and mesonephric carcinomas are being investigated.

CONCLUSION(S)
We have identified several biomarkers that can be used to support diagnosis and identify potential therapeutic targets for mesonephric-derived carcinomas.

UNDERSTANDING THE ROLE OF PPP2R2A IN ER-POSITIVE BREAST CANCER CELLS

BACKGROUND/OBJECTIVES
Our previous study of 2,100+ breast cancer (BC) patients identified recurrent deletions in the PPP2R2A gene, which encodes a regulatory subunit of Protein Phosphatase 2A (PP2A), a serine/threonine phosphatase enzyme which plays key roles in cell proliferation, apoptosis, and signal transduction. Copy loss number correlated with loss of mRNA, suggesting that deletion was driving the change in expression. These deletions were found to be over-represented in the luminal B sub-type of BC, characterized by estrogen receptor (ER+) expression. Using a breast cancer cell line and inducing PPP2R2A knockdown (KD), we found that KD resulted in increased levels of ER phosphorylation, differential binding of ER to target DNA sequences, and differential expression of ERα response genes. In this study, we sought to identify the effect of mutations on pSer118/167 on the ER-transcription factor complex in terms of whether their mutations could recapitulate the effects of PPP2R2A knockdown on ER cofactor levels and ER binding sites.

METHOD(S)
We performed transfection on T47D (ER+) cells cultured in RPMI growth medium utilizing siPPP2R2A with RNAiMAX. The cells were then ligand activated with 100nM B-estradiol. Western blot analysis checked for efficiency of knockdown and levels of ER cofactors. For immunoprecipitation, lysates were pulled down with ER (HC-20 Rabbit or F-10 Mouse) Antibodies. To simulate mutant ERs, GFP plasmids with mutant residues (S118A and S118E) were transfected into the cells. A set of experiments involving S118E, S118A, and siPPP2R2A were performed to determine if the mutant residues could recapitulate the effects of PPP2R2A KD, looking into ER cofactor levels and binding sites.

RESULT(S)
Our Western Blot densitometric analysis indicated that protein levels of the cofactors FOXA1, SPDEF, and p300 in ER complexes precipitated from T47D cells increased upon PPP2R2A knockdown. Additionally, phospho-S118 levels increased as well. Therefore, we speculate that a S118E mutant simulating a constitutively phosphorylated residue would lead to similar increases in ER cofactor levels. Additionally, we possess ChIP-seq data for ER binding sites of both the S118 and S167 residues in an unaltered ER and Chip-seq data for ER binding sites of a PPP2R2A KD cell. Presumably, given that our data shows that phospho-S118 increases and phospho-167 decreases in a PPP2R2A KD scenario, ChIP-seq performed on a phosphor-S118 and dephosphorylated S167 mutant would resemble that of the PPP2R2A KD ChIP-seq.

CONCLUSION(S)
Our data supports that PPP2R2A KD results in differentially expressed ER response genes, shifts in ER binding sites, differential ER cofactor recruitment and changes in the phosphorylation states of ER and SPDEF. This data supports our preliminary mechanism in which PPP2R2A knockdown leads to an alteration in phosphorylation of ER, leading to a change in ER complex composition, which ultimately affects the genes expressed in PPP2R2A KD ER+ breast cancer cells. Increased understanding of PPP2R2A will provide information for future prognosis and avenues for therapeutic treatment.

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ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR 2 EXPRESSION CHARACTERIZES THE REMYELINATING AND REPAIR POTENTIAL OF OLIGODENDROCYTE DIFFERENTIATION IN A MODEL OF MULTIPLE SCLEROSIS

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BACKGROUND/OBJECTIVES
Multiple sclerosis (MS) is a chronic inflammatory and demyelinating neurodegenerative disorder characterized pathologically by alterations in the vasculature, inflammatory infiltrates, demyelination, glial scarring, oligodendrocyte (OL) loss, and axonal degeneration. Improving myelination processes in OLs could decrease axonal loss and ultimately translate to repair and an improved prognosis for MS patients. Aryl hydrocarbon receptor nuclear translocator 2 (ARNT2) is a basic-helix-loop-helix transcription factor that binds with partners of the same family to drive transcription of proteins essential for neuronal development and axonal health. We are the first to describe its expression in glia including astrocytes and OL, the primary support cells of the nervous system, and hypothesize that reductions in ARNT2 may favor OPC/OL maturation and myelination to repair damaged tissue.

METHOD(S)
The immortalized OPC cell line Oli-neu was examined prior to and following maturation with dibutyryl cyclic adenosine monophosphate (dbcAMP). Primary neuronal-enriched cortical cultures and OPC were derived from rat and mouse cortices at E18. Immunocytochemistry (ICC) to detect myelin proteins 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase) and myelin-associated glycoprotein (MAG) was used to assess OL maturation and relative ARNT2 expression. Immunohistochemistry was used to compare ARNT2 expression in tissues from healthy mouse spinal cords to spinal cords of mice induced with experimental autoimmune encephalomyelitis (EAE), the animal model of MS.

RESULT(S)
In healthy mice, ARNT2 was localized to OL and OPC (Olig2+) as well as GFAP+ astrocytes. Increases in Olig2+ cells as well as reactive astrocytes (GFAP+/Olig2+ cells) were observed in animals at peak clinical disease. Approximately 50% of Olig2+/GFAP- cells in the healthy CNS expressed ARNT2; OL expression of ARNT2 was reduced by almost half at peak disease in the grey matter. In mixed cortical cultures, we observed mature OL with numerous CNPase+ processes and negligible ARNT2 expression. Treatment with dbcAMP increased myelin production and cell branching. . Immature Oli-neu had relatively high ARNT2 expression. Expression of ARNT2 was significantly lower in matured Oli-neu, suggesting ARNT2 is a marker of immature or proliferating OL or OPC both in vitro and *in vivo*.

CONCLUSION(S)
Our studies are the first to characterize ARNT2 expression by glial populations in the healthy and diseased CNS. ARNT2 depletion is associated with cell differentiation and myelination Continued studies to examine the direct contributions of ARNT2 to proliferative and maturation processes in OL and the identification of binding partners and downstream targets will be essential to evaluate ARNT2 as a potential therapeutic target in MS.

VALIDATION OF NOVEL LAB-DEVELOPED LOOP-MEDIATED ISOTHERMAL NUCLEIC ACID AMPLIFICATION (LAMP) ASSAY FOR HERPES SIMPLEX AND VARICELLA ZOSTER VIRUSES FOR CLINICAL USE IN INFECTED CHILDREN

BACKGROUND/OBJECTIVES
Viral infections are a common cause of morbidity in children, particularly in those with weakened immune systems. Rapid diagnosis of infections is important to direct optimal use of anti-virals and determine the best methods of patient isolation. The polymerase chain reaction (PCR) has been very useful for detecting these pathogens. However, PCR's can only be run efficiently once per day. More recently, a rapid, sensitive and cheap alternative has been described – the loop mediated isothermal amplification assay, or LAMP. With this assay we have the opportunity to report results in about an hour and potentially replace PCR. But before such a change can be implemented, careful validation of LAMP is necessary to ensure accurate results for patient care. The objective of this study is to determine if LAMP is comparable to PCR in terms of sensitivity and specificity for the detection of HSV-1, HSV-2, and VZV using clinical samples.

METHOD(S)
A total of 142 patient samples collected from vesicular lesions or ulcerations of the skin between 2010 and 2017 from BC Children's Hospital were analyzed. These clinical samples were previously tested with PCR, culture, and/or a commercial direct fluorescence assay (DFA). Of the 142 samples selected, 68 negative and 26 positive known HSV-1 samples, 68 negative and 22 positive known HSV-2 samples, and 61 negative and 23 positive known VZV samples were tested to compare with LAMP. A novel probe-based method developed in-lab at BCCH by placing a fluorescent tag on a loop primer was used. Positive and negative controls were run weekly plus pUC19 internal control. The LAMP mixture consisted of Chelex resin and Master Mix containing Bst polymerase, buffer, primers, and DNA template. The reaction was performed using a real-time fluorimeter, the ESEQuant TS2.6 instrument at 65˚C for 20 minutes. The data was analyzed based on the following criteria: 1) A culture positive was accepted as positive. 2) The PCR result was taken to be correct. 3) If DFA only was available, the result was compared to LAMP and accepted as correct if both were in agreement. 4) If the DFA and LAMP did not agree, PCR was performed for discrepant analysis, and PCR was taken as correct.

RESULT(S)
Compared to the original clinical result, the sensitivity of LAMP for HSV-1, HSV-2, and VZV were 100%, 95.5%, and 92.3%, respectively. The specificity of LAMP for HSV-1, HSV-2, and VZV were 100%, 100%, and 100%, respectively. Following discrepant analysis and repeat of assays, the sensitivity and specificity of VZV were 96.3% and 100%.

CONCLUSION(S)
LAMP performed well as a simple, inexpensive, and rapid diagnostic tool with high sensitivity and specificity for the specific detection of VZV, HSV-1, and HSV-2. These results suggest that LAMP could be useful clinically for patient management and can be applied in low-resource settings. With further confirmation of results using a commercialized kit, LAMP may become a routine laboratory test for the diagnosis of HSV and VZV, similar to influenza rapid diagnostic tests. LAMP may replace PCR as the mainstream instrument of use at BC Children's Hospital in the very near future.

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ATTENUATION OF GRANZYME B REDUCES TISSUE DAMAGE AND IMPROVES FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD INJURY

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BACKGROUND/OBJECTIVES
Traumatic spinal cord injury (SCI) affects over ten thousand people per year and exerts a significant physical, sociological and economic burden. SCI can be sub-divided into two phases: initial mechanical injury and secondary immune-mediated insult. Secondary injury involves inflammatory, glial, and vascular changes that exacerbate tissue damage and broaden lesion size. Identifying the pathogenic mechanisms of secondary SCI may lead to novel strategies for modulating immune-mediated damage and promoting functional recovery. Granzyme B (GzmB) is a serine protease traditionally associated with T-lymphocyte mediated apoptosis. It is now recognized that GzmB accumulates extracellularly in a broad range of inflammatory states and contributes to matrix protein degradation, endothelial permeability, and impaired healing. Elevated GzmB has recently been observed in a rat model of SCI, however the role of GzmB in secondary SCI is not understood. We hypothesized that GzmB accumulation following SCI contributes to increased inflammation, loss of white matter, and impaired functional recovery in a murine thoracic spinal cord contusion model of SCI.

METHOD(S)
GzmB Knockout (KO) or C57BL/6 (wild-type) mice (3 months old) were subjected to thoracic spinal cord contusion injury and monitored for 6 weeks post-injury. Locomotive recovery was assessed in injured mice using the Basso Mouse Scale (weekly assessment) and Rotarod test (biweekly assessment). At the time of sacrifice, mice were transcardially perfused with PBS followed by 4% PFA to fix tissue. Spinal cord lesions were isolated and sectioned for histological analyses. Eriochrome Cyanine R was used to quantify the amount of white matter in the injured spinal cords. Antibodies against decorin, an extracellular matric (ECM) protein associated with Glial scarring, were used to assess extracellular GzmB activity in SCI lesions. Quantification of white matter sparing was performed in ImageJ analysis software by manual outlining of the EC positive space. A second cohort of mice were maintained for 1-week post injury. Mice were perfused with PBS and lesions were flash frozen. RNA was isolated from whole tissue using Trizol and inflammatory cytokine expression was assessed using quantitative PCR.

RESULT(S)
Increased GzmB was observed in spinal cord lesions of mice at 5 days post-injury. GzmB KO mice exhibited improved functional recovery compared to controls. This was supported by histological analysis, demonstrating an increase in white matter sparing at the epicenter and rostral to the injury. GzmB deficiency did not affect the TNF-alpha and IL-1-beta transcript levels.

CONCLUSION(S)
GzmB may contribute to secondary tissue damage, myelin loss, and functional impairment following SCI. Inhibition of GzmB offers a novel therapeutic approach for the prevention of secondary injury following traumatic SCI.

LIPOPOLYSACCHARIDE INHIBITS INNATE LYMPHOID CELLS TYPE 2 (ILC2)-MEDIATED ALLERGIC LUNG INFLAMMATION

BACKGROUND/OBJECTIVES
Allergic lung inflammation is a type 2 inflammation driven by overproduction of type 2 cytokines due to inhaled allergens. Upon allergen encounter, lung epithelium gets damaged and produces alarmin cytokine IL-33, which activates innate lymphoid cells type 2 (ILC2s). Activated ILC2s produce type 2 cytokines IL-5 and IL-13 that leads to type 2 inflammation characterized by airway hyper-reactivity and mucus overproduction. According to the hygiene hypothesis, infections by microbes induce a type 1 immune response that prevents the development of type 2 immune responses such as allergy and asthma. We have found that lipopolysaccharide (LPS), a component of outer membrane in gram negative bacteria, inhibits ILC2 activation. This effect is dependent on both Toll-like receptor (TLR) 4 and IFNγ. The goal of this project is to understand the molecular and cellular mechanisms behind the inhibition of ILC2 activation by microbial products.

METHOD(S)
We used relevant genetic mouse models and treated them with LPS in models of IL-33 induced allergic lung inflammation. We subsequently used techniques such as flow cytometry, in vitro cell culture and ELISA to analyze cell proliferation and expression of cytokines.

RESULT(S)
In mice treated with LPS, we found higher numbers of IFNγ+ Natural Killer (NK) cells, Natural Killer T (NKT) cells and ILC1s. Intranasal injection of IFNγ inhibited IL-33 induced activation of ILC2s and eosinophilic lung inflammation. LPS is known to induce IL-12 production by myeloid population, and IL-12 has been shown to activate NK, NKT cells and ILC1s. Treatment with IL-12 had a similar effect on ILC2s as treatment with LPS and resulted in inhibition of IL-33 induced allergic lung inflammation.

CONCLUSION(S)
These results show that LPS stimulates myeloid populations to secrete IL-12, which activates NK cells, NKT cells and ILC1s. These populations secrete IFNγ, which subsequently inhibits ILC2-mediated allergic lung inflammation.

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UTILITY OF DIFFERENTIAL OLIGOCLONAL BANDING PATTERN IN THE DIAGNOSIS OF MULTIPLE SCLEROSIS

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BACKGROUND/OBJECTIVES
Multiple sclerosis (MS) is a heterogeneous, immune-mediated disease of the central nervous system characterized by inflammation, demyelination and axonal degeneration. Cerebrospinal fluid (CSF) analysis of oligoclonal bands (OCBs) is often ordered during the workup of MS patients as a useful adjunct investigation. At the Vancouver General Hospital (VGH) laboratory, OCBs are currently reported as either present or absent. However, there is much diversity in the banding pattern, including the number and intensity of OCBs, the clarity of OCB margins, and whether banding is also seen in serum. A previous study noted that clinically definite MS (CDMS) patients exhibited only a delta pattern OCB (strongly staining, usually ≥4 OCBs), and none displayed a theta pattern OCB (weakly staining, usually 2 or 3 bands). The aims of our study are: (1) to further validate whether the presence of theta banding precludes a diagnosis of MS; (2) to differentiate banding patterns by the number, intensity, and discreetness of OCBs, and the presence vs. absence of serum bands; and (3) to evaluate the diagnostic utility of these findings for MS.

METHOD(S)
From January 1st 2012 to December 31st 2013, all patients with positive OCB results reported by the VGH laboratory were identified and matched with the original isoelectric focusing/immunofixation gels. The banding pattern (theta vs. delta), the number of OCBs present, the intensity and discreteness of OCBs, and the presence or absence of serum bands, were recorded by 2 study team members blinded to the subjects' diagnosis. A retrospective chart review was then conducted to document the subjects' basic demographics, final diagnosis, and results of other CSF investigations.

RESULT(S)
134 patients with positive OCBs were included in the study, with 66 subjects (49%) diagnosed with CDMS. Theta pattern OCBs were observed in 23% of the CDMS cohort. CDMS patients were more likely to have >12 ($p<0.005$), intensely-stained ($p<0.05$) OCBs. Non-CDMS patients were more likely to have 2-3 OCBs ($p<0.01$). The clarity of OCB margins, and the presence vs. absence of serum bands, were not useful in distinguishing CDMS from non-CDMS. The likelihood of a CDMS diagnosis increased with the number of OCBs present, reaching a peak positive predictive value (PPV) of 0.74 if >12 OCBs were seen in a female patient; this was further increased to 0.81 if no serum bands were seen. In the male cohort, neither the number of OCBs, the intensity of OCBs, nor the presence vs. absence of serum bands, were particularly useful in predicting CDMS development.

CONCLUSION(S)
Contrary to previous reports, theta pattern OCBs were seen in CDMS patients. Rather than reporting OCB results using a binary classification system, we found the provision of number (+/- intensity) of OCBs observed, along with a comment on the presence vs. absence of serum bands, may provide more useful information to clinicians, especially for female patients undergoing neurological investigations. The VGH laboratory plans to modify its reporting practice based on the findings from this study.

GENOMIC SCREEN OF CISPLATIN RESISTANCE GENES IN MUSCLE-INVASIVE BLADDER CANCER USING GENOME-WIDE CRISPR KNOCKOUT SCREEN

BACKGROUND/OBJECTIVES
Neoadjvuant chemotherapy (NAC) followed by radical cystectomy in patients with bladder cancer has been shown in multiple trials and meta-analyses to improve five-year survival, and is therefore currently the first-line standard of care in patients. However, 60% of patients are inherently resistant to NAC at the time of cystectomy. While several mechanisms of cellular resistance to cisplatin have been proposed, the complete landscape of genetic modification remains largely unknown. The main objective of this study is to take a functional genomic approach to identify genes that, when knocked out, induce a cisplatin resistant phenotype.

Several mechanisms of cellular resistance to cisplatin have been proposed, however, the data presented thus far is conflicting at best and the molecular effectors facilitating cisplatin resistance remain largely unknown. In order to address this gap in knowledge, we take advantage of a pooled genome-wide CRISPR knock-out library (Brunello) as well as a CRISPR-based activation library (SAMv2) targeting 19,114 protein coding genes with 76,441 synthetic guide RNAs (sgRNAs) which allows for an unbiased screen.

Our central hypothesis is that using a genome-wide CRISPR knockout library, we can identify specific gene targets that confer cisplatin resistance in bladder cancer cell lines

METHOD(S)
The genome-scale CRISPR/Cas9 knockout and transcriptional activation screens begin with packaging the plasmid library into lentivirus followed by transduction into bladder cancer cell lines to generate stably expressing cell lines. Cisplatin selection pressure is applied to select for cells that have acquired beneficial genetic perturbations, and at the end of treatment, genomic DNA is harvested. The sgRNA regions are amplified from the genomic DNA and analyzed by next-generation sequencing and subjected to statistical analysis (MAGeCK) to determine candidate genes. Candidate genes are then validated for their involvement in cisplatin resistance using various methods of analysis, including but not limited to testing of individual sgRNA knockouts and cisplatin toxicity assays, targeted sequencing to determine indel formation, transcript up and down regulation by qPCR and protein expression level analyses in cisplatin-resistant and naïve cell lines by western blot. Final validations are then to be carried out in vivo using patient-derived xenograft (PDX) and orthotopic mouse models.

RESULT(S)
Based on our preliminary screen, we have identified 38 potential candidate genes which may be involved in the cisplatin resistance - including regulators of epithelial-to-mesenchymal (EMT), calcium sginalling, mtiochondria-mediated cell death and cell cycle regulators. We performed the MAGeCK analysis on our data and picked candidates based on $p>0.005$ and a false discovery rate (FDR) of less than 10%. Of these, Filamin A Interacting Protein 1-Like (FILIP1L) was the top candidate and is known to control the wnt pathway and EMT in ovarian cancer – thereby influencing cisplatin resistance.

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MODERATE-SEVERE TRAUMATIC BRAIN INJURY ELICITS INJURY-DEPENDENT NEUROPATHOLOGICAL, BIOCHEMICAL, AND ELECTROPHYSIOLOGICAL CHANGES IN A BIOFIDELIC MURINE MODEL OF IMPACT-ACCELERATION HEAD INJURY

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BACKGROUND/OBJECTIVES
CHIMERA (Closed Head Impact Model of Engineered Rotational Acceleration) is a recently described murine model of traumatic brain injury (TBI) that, at the mild level (0.7J), primarily produces diffuse axonal injury (DAI) characterized by white matter inflammation and axonal damage (Namjoshi et al, 2017). CHIMERA was specifically designed to reliably generate a variety of TBI severities using precise and quantifiable biomechanical inputs in a non-surgical, user-friendly platform. The objective of this study was to define the upper limit of single impact moderate-severe CHIMERA (sCHIMERA) injury with the use of a recently engineered 3D-printed interface that enables impacts up to 2.5J. In addition to characterizing the upper limit of injury, we wished to define the injury-dependent relationship between biomechanical inputs and neurological, behavioral, electrophysiological, neuropathological, and biochemical outcomes.

METHOD(S)
Wild-type male and female mice aged 5-7 months were subjected to a single CHIMERA TBI at 2.5J. Control animals were subjected to a sham procedure. The following assays were carried out post-TBI: loss of righting reflex latency (immediately post-injury); neurological severity score (1h); field recordings in slice (6h); Iba-1, GFAP, and CP-13 immunohistochemistry (6h, 2d); Evans blue extravasation (6h, 2d); cytokine, neurofilament-light, and tau biochemistry (6h, 2d). A small pilot of animals underwent susceptibility weighted magnetic resonance imaging (MRI) 6h post-injury.

RESULT(S)
We report that single sCHIMERA impacts induce injury-dependent changes in the form prolonged loss of consciousness post-TBI, as well as , grey matter microgliosis, blood-brain barrier (BBB) extravasation, and decreased post-synaptic response in the hippocampus through electrophysiology. Intriguingly, we show tight positive correlations between TBI blood biomarkers of interest, namely tau protein and neurofilament, and increases in brain cytokine levels at 6h. Using MRI, we see evidence of hemorrhagic activity at 6h post-injury, though this aspect of the study is currently under-powered.

CONCLUSION(S)
Our data extend the validation of CHIMERA as a biofidelic animal model of head injury and establish working parameters to guide future investigations of the mechanisms underlying the pathological outcomes induced by mechanical trauma.

METHODOLOGICAL CONSIDERATIONS TO PREPARE HIGH-DENSITY LIPOPROTEIN PARTICLES WITH OPTIMAL FUNCTION IN CEREBROVASCULAR HEALTH AND ALZHEIMER'S DISEASE

BACKGROUND/OBJECTIVES
Most Alzheimer's disease (AD) patients have cerebrovascular dysfunction in addition to the parenchymal amyloid beta (Abeta) plaques and neurofibrillary tangles that constitute the neuropathological hallmarks of AD, and there is great interest in understanding the vascular contributions to dementia. Plasma high-density lipoprotein cholesterol (HDL-C) levels are inversely associated with AD in several human epidemiological studies, and HDL can protect cerebral vessels from Aβ-induced dysfunction in experimental models.
Objective: The objective of this study is to compare the function of HDL isolated from healthy human donors by various methods to determine how isolation method may alter HDL composition and affect functions relevant to cerebrovascular health.

METHOD(S)/ RESULT(S)
We compared HDL isolated by density gradient ultracentrifugation to non-HDL lipoprotein depletion with polyethylene glycol (PEG) and observed that HDL isolated by PEG-depletion was no longer able to induce nitric oxide production in primary human brain-derived endothelial cells, suppress Abeta-induced monocyte adhesion to these cells, or to promote Abeta transport across three-dimensional bioengineered human blood vessels. Both HDL preparations had equivalent functions with respect to cholesterol efflux, inhibition of TNFalpha-induced monocyte adhesion, and suppression of Aβ accumulation in bioengineered human blood vessels.

CONCLUSION(S)
The vasoprotective functions of HDL that are most relevant for cerebrovascular health and dementia risk remain to be fully elucidated. Our study shows that the method by which HDL particles are prepared can affect specific activities of importance to AD, thereby establishing the basis to develop new HDL functional assays for the cerebrovasculature.

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DECIPHERING THE ROLE OF EOSINOPHILS AND B CELLS IN LUNG CANCER AND BREAST CANCER LUNG METASTASIS

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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 eosinophils (Eo) and B cells. Eo are innate immune cells that exert cytotoxic effector functions through the release of secretory granules and participate in tissue homeostasis and immunity. B cells can act as antigen-presenting cells in the lung, as well as differentiate into plasma cells. Eo have been shown to support B cells in the blood, but their relationship within the lung is unclear. Despite the prevalence of Eo and B cells in the lung, the role of Eo and B cells in lung cancer is largely unexplored. The Bennewith lab has previously found that mice with elevated lung Eo and B cells are protected from lung colonization in a model of breast cancer lung metastasis. **We hypothesize that Eo and B cells play a protective role in lung cancer development.**

METHOD(S)

In collaboration with Dr. Kelly McNagny, we used IL-5Tg transgenic mice that over-express IL-5 and have a systemic expansion of Eo, ddGATA transgenic mice which are Eo-deficient, and ddGATA/IL-5Tg double-transgenic mice (excess IL-5 but no Eo) to study lung cancer. Lewis Lung carcinoma (LLC) cells were injected intravenously (IV) into transgenic mice. After three weeks, we harvested lungs and used flow cytometry to quantify immune cell subsets in the lungs. Additionally, we used clonogenic assays to quantify LLC cell lung colonization.

RESULT(S)

We confirmed that naive ddGATA and ddGATA/IL-5Tg mice have no lung Eo. In contrast, IL-5Tg mice have a 100-fold expansion of Eo in the lungs, and these Eo express higher levels of the Eo activation marker CD11b compared to wild-type (WT) mice. Naive IL-5Tg and ddGATA/IL-5Tg mice had an increased proportion of lung B-1 B cells, as well as an increase in the expression of the apoptosis-inducing cell surface molecule FasL. The absence of Eo in ddGATA mice did not impact lung colonization of LLC cells. Though there was a substantial expansion of Eo in the lungs of IL-5Tg mice compared to WT mice, there was no change in the number of lung-infiltrating Eo three weeks after IL-5Tg and WT mice were injected IV with LLC cells. IL-5Tg mice injected IV with LLC cells had an increase in the total number of lung-infiltrating Bconv 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.

CONCLUSION(S)

Though Eo may play an anti-tumorigenic role in the presence of excess IL-5, the absence of Eo in ddGATA mice did not result in an increase in lung tumor burden. This suggests that Eo need to be activated and expanded to exert an anti-tumorigenic effect, or that the expansion of B cells in IL-5Tg mice is responsible for the decrease in lung colonization in IL-5Tg mice relative to WT mice. Illuminating the specific roles Eo and B cells play in cancer progression will allow us to better understand the interplay between host immune cells and malignant cells and could reveal new avenues of cancer immunotherapy development.

CHIMERA REPETITIVE MILD TRAUMATIC BRAIN INJURY INDUCES LONG-TERM PATHOLOGICAL AND PTSD-LIKE BEHAVIOUR IN APP/PS1 MICE

BACKGROUND/OBJECTIVES
The annual incidence of traumatic brain injury (TBI) is over 2.5 million in the US, with over 3-5 million people living with residual problems. Moderate and severe TBI survivors have high rates of long-term disability and increased risk of neurodegenerative disease. On the other hand, the symptoms of mild TBI (mTBI, the most common form of TBI) usually resolve within weeks of injury. However, some patients may still present symptoms in long-term, which is known as the "Post-concussive syndrome". In addition, repetitive exposure to mTBI has been linked to a neurodegenerative condition called chronic traumatic encephalopathy, which is characterized by tau deposits at sulcal depths and around cerebral vessels, and can exhibit amyloid deposition (50%).

METHOD(S)
We reported the acute outcomes (6 hr -14 d) of two mTBI (0.5J, 24 hr apart) in the APP/PS1 amyloidogenic mouse model (6-mo or 13-mo), using the Closed-Head Injury Model of Engineered Rotational Acceleration (CHIMERA). We observed that age at injury, in addition to genetic predisposition to AD, is crucial in determining acute outcomes: TBI induced cognitive deficits and A-beta deposition of APP/PS1 mice in an age-dependent manner; and post-TBI neuroinflammation was exacerbated in 6-mo APP/PS1 but blunted in 13-mo APP/PS1. In the current study, we extended our investigation time point up to 8-mo post-injury.

RESULT(S)
We found that mice with mTBI had prolonged white matter microgliosis (Iba1) and degenerative injury (silver) up to 8-mo, and they showed increased risk-taking behaviour (Elevated plus maze). These injury outcomes were not different between APP/PS1-TBI and WT-TBI mice. However, long-term fear memory (passive avoidance) was chronically intensified in APP/PS1 mice only, which also showed deficits in extinction of fear memory, suggesting post-traumatic stress disorder (PTSD)-like phenotype. In addition, spatial learning (Barnes maze) was most impaired in APP/PS1-TBI mice, and reversal spatial learning was only impaired in APP/PS1-TBI mice, suggesting deficits of spatial learning and cognitive flexibility. Neither Aβ nor tau levels in brain homogenates were changed by TBI 8-mo post-injury.

CONCLUSION(S)
Our findings suggest that two mild TBI are sufficient to induce long-term neuropathological and behavioral changes up to 8-mo post-injury, without chronic effects on Aβ or tau. In addition, TBI may exacerbate chronic cognitive deficits to a greater extent in predisposed to amyloid deposition. These findings have implications on the long-term disease progression and management of repetitive mTBI.

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CHARACTERIZATION OF THE NOVEL CANDIDATE TUMOR SUPPRESSOR GENE SHPRH IN LUNG CANCER DEVELOPMENT

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BACKGROUND/OBJECTIVES
Lung cancer is the leading cause of cancer related death worldwide, mainly due to late stage diagnosis. Early detection of lung cancer is essential in order to improve patient outcomes. Using comprehensive genomic characterization of a panel of lung adenocarcinomas (LAC), our group has identified a candidate tumor suppressor gene (TSG), SHPRH, based on its high frequency and "two-hit" pattern of disruption. SHPRH is located on chromosome 6q, a genetic locus associated with familial lung cancer susceptibility, and is an evolutionarily conserved E3 ligase that mediates crucial processes related to DNA repair. Our genomic data identified frequent, biallelic inactivation of SHPRH in LACs, highlighting it as a candidate TSG. Here, we seek to determine if SHPRH is a tumor suppressor involved in the initiation and progression of LAC and if so, characterize its mechanism of action in this process.

RESULT(S)
Assessment of lung cancer cell lines has demonstrated that those with biallelic inactivation of SHPRH have diminished protein levels. Subsequent experiments determined that SHPRH reconstitution in these cell lines inhibits growth and colony formation while no effects are observed in cell lines without SHPRH inactivation. In reciprocal experiments, inactivation of SHPRH in normal human peripheral (small) airway epithelial cells using CRISPR/Cas9 targeting induced cell transformation as determined by colony formation in soft-agar. In addition, preliminary data suggests that loss of SHPRH can accelerate tobacco carcinogen-induced lung cell transformation further suggesting a tumour suppressive role for SHPRH in lung tumorigenesis. Due to its functional role in regulating DNA repair we next aimed to determine if loss of SHPRH was associated with DNA instability. Assessment of in-house and publicly available genomic data from LACs revealed that those with mutation or copy number loss of SHPRH had significantly greater chromosomal instability compared to LAC with normal SHPRH. Furthermore, patients with LAC harbouring SHPRH inactivation have significant worse disease-free survival compared to those without suggesting that this increased instability may influence patient outcome.

CONCLUSION(S)
Our work demonstrates that SHPRH is a potential tumor suppressor in human LAC. We propose a model whereby decreased expression of SHPRH impairs the capacity to sense and repair DNA damage leading to continued cell proliferation and survival in the presence of DNA breaks that result in the accumulation of genetic alterations that drive cancer progression. In addition, as SHPRH lies within the lung cancer susceptibility locus on chromosome 6q, it is possible that its inactivation could lead to increased lung cancer risk by promoting an impaired response to DNA damaging agents such as cigarette smoke. Thus, once validated in our in vivo model systems and familial lung cancer cohorts, SHPRH status may be useful as a risk and early detection marker for both familial and sporadic LAC.

HYPOXIA DEVELOPS IN HUMAN AND MURINE LYMPH NODES DURING B CELL-DRIVEN IMMUNE RESPONSES TO TUMOR ANTIGENS

BACKGROUND/OBJECTIVES
The lymphatic system plays dual, yet opposing roles in cancer progression. Lymph nodes (LNs) are the earliest and most common site of metastasis formation in cancer patients, but also serve as hubs of antigen presentation and immune response development against tumor-associated antigens. Anti-tumor immunotherapy provides durable control of advanced disease in a fraction of cancer patients, but such responses rely on the establishment of a pre-existing immune response against the primary tumor, and it is unknown what conditions in the tumor-draining lymph node (TDLN) promote the development of effective anti-tumor immunity. Upon antigenic stimulation, lymphocytes proliferate within the T cell paracortex or B cell rich germinal centers of the responding LN, where molecular cues dictate their differentiation into distinct effector or memory cell subsets. We hypothesized that regions of low oxygen (hypoxia) developed during lymphocyte expansion in TDLNs, which would affect the phenotype of differentiating immune cells, and consequent efficacy of anti-tumor immune responses.

METHOD(S)
We used flow cytometry and immunofluorescence microscopy to quantify hypoxia, germinal centre development, and B cell sub-types in inguinal and axillary LNs draining orthotopic 4TO7 mammary tumors. TDLNs from triple-negative breast cancer patients with lymph node negative disease were obtained from the BCCA Tumor Tissue Repository and analyzed for hypoxia.

RESULT(S)
Approximately 60% of tumor-bearing mice developed hypoxia in the B cell cortex of inguinal and axillary TDLNs, while LNs draining the mammary fat pad (MFP) in naïve tumour-free mice were hypoxia-free. We hypothesized that the levels of hypoxia in TDLNs reflected the extent of adaptive immune activation against tumor-associated antigens in the primary tumor. We injected lethally irradiated or heat-killed cells into the MFPs, and found that tumor antigens from dying cells were sufficient to induce germinal center hypoxia within the B cell cortex, which correlated with the levels of activated, germinal center B cells induced by the two modes of cell kill. In LNs draining established tumors, hypoxia was associated with more antibody-secreting B cells (ASCs) rather than B memory cells, despite similar levels of germinal center B cells, suggesting that TDLN hypoxia promotes preferential B cell differentiation towards an ASC phenotype. In TDLN samples from breast cancer patients, we detected the hypoxia-inducible protein CA9 specifically within their germinal center reactions, which is the first clinical observation to suggest that humans can develop germinal center hypoxia in response to tumor antigens.

CONCLUSION(S)
Our results suggest that hypoxia develops in human and murine TDLNs, and reflects the extent of adaptive immune activation against the primary tumor. The work presented describes a novel role for hypoxia in the development of anti-tumor immune responses within the LN, and raises the possibility that TDLN hypoxia can help predict positive treatment outcome in clinical settings.

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TDP-43 PROTEINOPATHIES

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BACKGROUND/OBJECTIVES
The transactive response DNA-binding protein of 43 kDa (TDP-43) is a ubiquitously expressed nuclear protein, involved in RNA processing and metabolism. This protein has been associated with several neurodegenerative disorders, which are collectively referred to as TDP proteinopathies. In TDP proteinopathies, TDP-43 can be the major component of pathological aggregates or be observed in conjunction with another proteinopathy, defining primary and secondary TDP proteinopathies, respectively. To date, the mechanism of TDP-43 pathogenesis remains elusive. To gain insight into the mechanism of TDP pathology it may be helpful to explore similarities and differences of the various TDP proteinopathies. Thus, the objective of this review was to synthesize current experimental evidence related to primary and secondary TDP proteinopathies.

METHOD(S)
We performed a review of peer-reviewed literature on TDP proteinopathies. We focused on TDP-43 histopathological characteristics of primary and secondary pathologies. Additionally, we compared epidemiological data and clinical findings in individuals with secondary proteinopathies, with and without TDP-43.

RESULT(S)
Abnormal TDP-43 aggregation has been documented in over 20 conditions including several forms of dementia and motor neuron disease. Features of pathological TDP-43 include cytoplasmic mislocalization and loss of normal nuclear localization, proteolytic cleavage, misfolding, and aggregation. The primary TDP proteinopathies are frontotemporal degeneration, amyotrophic lateral sclerosis, and Perry syndrome. TDP-43 inclusions in secondary TDP proteinopathies, include aggregates in conjunction with tau in Alzheimer's disease, chronic traumatic encephalopathy, hippocampal sclerosis, and corticobasal degeneration, and alpha-synuclein in Parkinson's disease and dementia with Lewy bodies. We observed pathological TDP-43 to form three main spatial patterns within the brain: limbic distribution, diffuse distribution, and in association with motor neurons. In secondary TDP proteinopathies, a substantial portion of concomitant TDP-43 aggregates, were not found within the primary pathological aggregates. Additionally, the presence and extent of TDP-43 co-pathology positively correlated with age, disease comorbidity, and disease severity for Alzheimer's disease, argyrophilic grain disease, progressive supranuclear palsy, and Parkinson's disease dementia. Abnormal TDP-43 aggregation has also been found in cognitively normal elderly individuals but rarely in individuals under 65 years of age.

CONCLUSION(S)
Findings support age-assisted aggregation of TDP-43. Moreover, symptoms of TDP pathology may not present until TDP-43 accumulation passes a certain threshold. Given the correlation between age, disease severity, and disease comorbidity, TDP-43 likely plays an important role in disease pathology even when present as a secondary co-pathology.

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF NORMAL GYNECOLOGICAL TISSUE: IMPLICATIONS FOR THE PATHOGENESIS AND PREVENTION OF GYNECOLOGIC CANCERS

BACKGROUND/OBJECTIVES
Collectively, ovarian and uterine cancers account for 7.7% of cancer related deaths in Canadian women. The comparison of the immunohistochemistry (IHC) profiles of tumours with their normal tissue counterparts can give insight into the pathogenesis of cancers from potential cells of origin that share a similar IHC profile, and it can help understand the functional/differentiation state of the tumour as it relates to normal cell types. Therefore, the broad goal of this study was to examine the expression of multiple proteins by IHC in normal premenopausal and postmenopausal gynecologic tissues (ovary, fallopian tube, endometrium and endocervix) to gain a better understanding of their potential roles in the diagnosis, pathogenesis, and therapy of gynecologic cancers originating from these tissues.

METHOD(S)
Cases were selected based on surgery for indications other than endometrial or tubo-ovarian cancer. The following anatomic sites were studied: ovary, fallopian tube (fimbriated and non-fimbriated regions), endometrium, and endocervix. Premenopausal tissue samples (from both proliferative and secretory phases of the menstrual cycle) were obtained from women under 40 years of age. Postmenopausal tissue samples were obtained from women aged over 60 years. A tissue microarray (TMA) was constructed using samples from each anatomic site from five proliferative phase cases, five secretory phase cases, and five post-menopausal cases. The TMA was stained for 16 markers: ER, PR, AR, FOXL2, CD10, p53, p63, p73, p40, HNF1b, CTH, HAND2, ASS1, PAX8, OVGP1, and calretinin. Secretory, ciliated and stromal cells were separately scored, when present. In addition, sections of normal fallopian tube fimbria from 27 premenopausal and 24 postmenopausal women were stained for p53 to identify and quantify so-called p53 signature lesions, which are considered latent precursors of tubo-ovarian high-grade serous carcinoma (HGSC). All scoring was performed by a gynecologic pathologist. Scores for each of the markers were quantified for each phase of the menstrual cycle. Data analysis was performed using Excel and R software.

RESULT(S)
p53 signature lesions were identified in 3/27 (11%) of premenopausal women and 10/24 (42%) of postmenopausal women (p=0.012). Of note from the other markers, ASS1 was highly specific for secretory cells in the fallopian tube, and p73 was a marker of ciliated cells. FOXL2 was expressed specifically expressed in stromal cells throughout the gynecologic tract.

CONCLUSION(S)
The preliminary data suggests that p53 lesions are significantly more common in postmenopausal compared to premenopausal women. This may indicate that there is a window of opportunity to prevent the development of p53 lesions as a mechanism of preventing high grade serous ovarian carcinoma. Additionally, our preliminary findings from the other markers analyzed on the TMA suggest potential diagnostic uses and raise important questions related to the pathogenesis of gynecological cancers.

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SHORTER CELL SUBSET TELOMERES IN HIV SLOW PROGRESSORS THAN IN HIV NON-SLOW PROGRESSOR WOMEN

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BACKGROUND/OBJECTIVES

Although shorter leukocyte telomere lengths (TL) of people living with HIV have been reported in numerous studies, TL investigations in HIV-relevant immune subsets remain scarce. Immune aging, including subset-specific TL attrition and imbalance in senescent/proliferative CD8 T cell distributions, likely link HIV infection with premature age-related comorbidities, even among people on stable cART. It is unknown whether this link exists for HIV slow progressors (SP), in whom studies on immune subset TL are lacking. Our objective was to characterize TL within T and B cells of SP to determine whether their innate ability to control HIV disease progression also protects against HIV-modulated immune aging. We hypothesized that TL shortening and expansion of the senescent CD8 T cell subset would be less severe in SP compared to cART-controlled HIV+ non-slow progressors (NSP) with undetectable viral load.

METHOD(S)

Live PBMCs were obtained from cART-controlled NSP and HIV- women enrolled in the CARMA cohort, as well as SP from the Canadian Cohort of HIV+ Slow Progressors. Groups were matched 1:1:1 for age. CD4 T cells, proliferative (CD28+), senescent (CD28-) CD8 T cells, and (CD19+) B cells were sorted by FACS, and their relative TL measured by multiplex qPCR. Groups were compared using Mann-Whitney or χ^2 tests.

RESULT(S)

Women (n=35/group) ranged in age from 27 to 60 years and were closely matched for age and ethnicity; SP and NSP had similar CD4 counts. All NSP were undetectable, while 15/35 SP were ART-experienced, including 2 on cART at visit. Cells were sorted from all matched groups, and TL data were available for n=20-31, as matched trios were excluded if any subsets contained too few cells. Shorter TL was observed in SP proliferative CD8 T, senescent CD8 T, and B (but not CD4 T) cell subsets, compared to both NSP ($p<0.047$, n=20-31) and HIV- ($p=0.002$) participants. Within CD8 T cells, TL was shorter in senescent compared to proliferative cells among SP (n=34, $p<0.001$) and NSP (n=27, $p=0.027$) but not HIV- controls (n=22, $p=0.13$). Finally, the senescent CD8 T cell compartment was also expanded in SP (median CD28- to CD28+ ratio=1.67) compared to NSP (0.66, $p<0.001$) and HIV- (0.45, $p<0.001$).

CONCLUSION(S)

Contrary to our hypothesis, these data strongly suggest that cellular aging, at least within CD8 T and B subsets, may be accelerated among SP compared not only to HIV-, but also NSP women. These results stress the importance of cART treatment and viral suppression in SP.

MICROFLUIDIC ISOLATION OF DRUG-RESISTANT PLASMODIUM FALCIPARUM PARASITES TO ENABLE RAPID TARGET IDENTIFICATION OF ANTIMALARIAL DRUG CANDIDATES

BACKGROUND/OBJECTIVES
Red blood cells (RBCs) measure 8 µm in diameter, but must deform through constrictions as small as 2-3 µm while traveling through microvasculature. This ability to deform is compromised by certain disease pathologies, including malaria. The ability to sort RBCs based on deformability could therefore be used to isolate different types of pathological cells. In *Plasmodium falciparum* (*Pf*) malaria, reduced deformability of infected RBCs (iRBCs) arises from parasites' metabolism of hemoglobin, resulting in accumulation of toxic heme. *Pf* reduces the loss of RBC deformability through biocrystallization of heme into inert crystal hemozoin. Antimalarials, such as chloroquine (CQ), disrupt this process in order to cause heme accumulation, which induces oxidative stress resulting in the loss of host RBC deformability and facilitate host-mediated clearance. Recently our group developed the Microfluidic Ratchet device which can sort RBCs based on their deformability. We showed that decreased iRBC deformability is a property of nearly all clinical antimalarials. **Here, we hypothesize that deformability based cell sorting could be used to separate iRBCs harboring drug-resistant Pf parasites in order to expedite target identification for novel antimalarials by genome sequencing.**

METHOD(S)
We employ the Microfluidic Ratchet mechanism to sort RBCs through a matrix of tapered constrictions that gradually decrease in size from the bottom to top of the sorting matrix. RBCs are separated into one of twelve distinct outlets based on their ability to deform. To separate iRBCs into distinct outlets based on drug resistance we use partially CQ resistant DD2 and fully CQ susceptible 3D7 *Pf* strains. The sensitivity of the device to separate drug resistant mutants in a mixed population is tested by sorting mixtures of 3D7 and DD2 parasites after drug treatment. Sorted parasites will be genotyped and the strain will be confirmed using *Pf* CQ resistance transporter and multidrug resistance genes, *pfcrt* and *pfmdr1*.

RESULT(S)
Device validation with beads in varying sizes demonstrates consistent sorting based on size across repeated experiments. Phenazine Methosulfate (PMS) treatment was used to model oxidative damage resulting from *Pf* infection and resulted in a clear shift of the distribution of iRBCs towards more rigid outlets, correlating with PMS treatment. We used the device to sort RBCs infected with the two strains of *Pf* after exposure to CQ for 4 hours. The distribution of iRBCs in outlets dramatically shifts towards the more rigid fraction for CQ sensitive 3D7, but not partially resistant DD2 strain

CONCLUSION(S)
We developed a Microfluidic Ratchet device which can sort RBCs based on their deformability. We show that iRBCs harboring CQ-resistant *Pf* parasites can be sorted into distinct fractions from CQ-sensitive parasites. This capability has the potential to accelerate antimalarial drug development by enabling the isolation of drug-resistant *Pf* parasites in order to facilitate rapid target identification through genome sequencing.

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EXPLORING PREDICTIVE BIOMARKERS OF RESISTANCE IN BREAST CANCER PRE-CLINICAL MODELS AT SINGLE CELL GENOMICS AND TRANSCRIPTOMIC LEVEL

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BACKGROUND/OBJECTIVES

Breast cancer is an ecosystem of genetically diverse evolving clones, which emerges in time and space. The dynamics of cells may be conceptualized via the notion that cells of different phenotypes (expression states) and/or genotypes can be described in relation to each other by descent, known as a "**clone**". This has vast implications for cancer biology and therapeutics with respect to drug resistance. A major obstacle to successful treatment is the recurrence of cancer cells with acquired resistance. Recent advances in DNA and RNA sequencing at single cell level have enabled us to explore and identify mutations across the genome and illuminate the expressional differences among the proliferating cells. Our approach is to exploit the principles of natural selection affecting tumor cell dynamics under drug selection. We are aiming to evaluate the genomic and transcriptomic determinants of cellular fitness and to dissect diagnostic approaches that incorporate clonal information leading to drug resistance. We selected first, the conventional chemotherapeutic drugs, for example, Taxols, Anthracyclines, and Platinums. Secondly, some new targeted approaches for e.g. PI3 Kinase, CX-5461 and PARP inhibitors etc. "Conventional" chemotherapies expected to exhibit non-genomic resistance whereas targeted therapies may manifest genomic resistance. We hypothesize that distinct clonal genotypes and *cellular expressional states may predict biomarkers of sensitivity and resistance to therapy in breast cancer*.

METHOD(S)

Molecular determinants of the resistance then determined by using single cell genome and transcriptomes and confirming them with population-based sequencing.

1. **Patient derived xenografts (PDX)**: Serial transplantation of breast cancer patient's tumor in immune-deficient NRG-mice and treated (Rx) with cytotoxic drugs (Rx=>re-transplantation=>Rx=>re-transplantation) till they exhibit resistance.
2. **Whole genome single cells sequencing (SC-WGS)**: The tumor tissue is chopped and enzymatically digested to spot for single cells/nuclei on piezo-based cell dispenser. This method is set up as a co-function of Aparicio lab.
3. **Single cells RNA sequencing (10X Genomics)**: We have started optimizing protocols for various batch effect among samples according to 10X genomics standard protocols.

RESULT(S)

One of the TNBC, SA604 showed reproducible sensitivity to Paclitaxel but resistance to Doxorubicin. Another TNBC, SA609, showed a di-morphic response to lower doses of Paclitaxel. Sequencing results have just started receiving.

CONCLUSION(S)

SA604-TNBC is the best candidate for treatment re-transplants to create a resistant tumor and SA609-TNBC could have two types of populations that might be defined by their genotypes or their expressional states. **Significance:** This study provides a methodological framework to explore biomarker identification. This study plan conceptually has a potential to identify some promising targets in breast cancer.

DESIGNING HYBRIDIZATION PROBES FOR TARGET ENRICHMENT AND DEEP SEQUENCING OF HIGHLY DIVERSE VIRAL GENOMES

BACKGROUND/OBJECTIVES
Shotgun metagenomic sequencing is a promising tool for clinical microbiology. By allowing unbiased sequencing of nucleic acids in various specimens, it can identify and characterize pathogens that would otherwise go undetected by conventional diagnostic assays. Viruses, however, remain a challenge for shotgun sequencing. The vast size discrepancy between viral and host genomes limits the proportion of viral nucleic acids in samples. This hinders deep sequencing of viruses on many next-generation sequencing platforms, restricting specific strain identification and surveillance of viral evolution. One popular technique for enriching rare targets before sequencing involves capturing them with complementary biotinylated DNA oligomers called hybridization probes. The main obstacle to applying this technique to viral target enrichment is the large genome sequence diversity of many viral species. This greatly expands the target space the probes must cover, complicating the selection of complementary probe sequences while limiting the size of the probe panel. To address this challenge, we built a bioinformatic pipeline for designing efficient probe panels against diverse viral targets. We tested our pipeline against the highly diverse hemagglutinin gene of the influenza A virus, selecting human, swine, and avian strains due to their importance in seasonal epidemics, severe zoonotic outbreaks, and growing pandemic threats.

METHOD(S)
We obtained 44 385 influenza A virus hemagglutinin gene sequences from the Influenza Research Database, representing a target space of 77 Mb. Strains with redundant sequences were removed, leaving 29 285 strains and a design space of 50 Mb. Using custom Python scripts and the Vsearch tool, we enumerated all 120mers in the design space, clustered them using a threshold of 90% identity, and ranked the clusters by size. Representative centroid sequences were extracted from the largest clusters as candidate probes, and we simulated their ability to hybridize with the target strains using additional custom scripts and BLASTn. These scripts also provided statistics on the hybridization simulation and extracted poorly-covered target sequences for subsequent, iterative designs.

RESULT(S)
Using a minimal panel of 2 000 probes, our simulation captured over 85% of the hemagglutinin gene sequence from 26 030 distinct influenza A virus strains (89% of the distinct target strains). Iterative design, where additional probes were designed solely from target sequences poorly-covered by previously design panels, improved performance. Phylogenetic analysis revealed extensive coverage across clinically important hemagglutinin subtypes in human, swine, and avian strains. We also observed excellent coverage of a key virulence marker in avian strains that is responsible for severe outbreaks in poultry flocks that expose humans to zoonotic disease.

CONCLUSION(S)
This bioinformatics pipeline allows for the efficient design of hybridization probe panels against diverse viral genomes. This will assist target enrichment for deep sequencing of viral genomes in many clinical applications.

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

Supervisor:
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BACKGROUND/OBJECTIVES

Cancer is the leading cause of death in Canada and is responsible for 30% of all deaths. Metastasis remains the cause of >90% of cancer related mortality. Malignant cells that comprise primary tumor are heterogenous and during disease progression selection of certain tumour cells occur as means to adapt and survive. 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 disease progression is important to develop new therapeutic strategies for cancer metastasis. Previous studies showed that metastases are initiated by rare tumour cells that are able to leave the primary tumour and survive as well as to colonize at the secondary organs. Also, it has been suggested that interactions between clones contribute to their survival. However, which clones of certain characteristics are capable of surviving outside of primary site and colonizing at distant site is still unknown. Here we propose to investigate the clonal dynamics and genomic profiles of human breast tumour xenograft mouse model to understand the mechanism of disease progression and metastasis.

Hypothesis: We hypothesize that specific genes and transcripts within certain clones which contribute to metastasis can be identified by dissecting clonal relationships during cancer progression.

Main Questions: Q1. Which clones from primary tumour succeed in metastasis? Q2. What are the differences between primary tumor and metastasis? And Q3. What are the differences between macro-metastasis and micro-metastasis in terms of clonal dynamics, genomic and transcriptomic profiles? Q4. Are there specific genomic profiles of metastatic cells at different metastatic sites?

Experimental Plan: Tumour cells from serially passaged human patient-derived tumor sample will be transplanted into mammary fat pad of mice. Tumours will be removed when it reaches certain size and mice will be monitored and allowed to grow metastasis. After 8 weeks or when mice present clinical signs of distress from metastasis such as respiratory distress, abdominal distension, and palpable metastatic lesions, mice will be euthanized and examined for the evidence of metastasis. Clonal population of primary tumour and metastasis will be analyzed by measuring single nucleotide variant and copy number aberrations using single cell analysis. Single cell RNA sequencing will be carried out to compare transcriptomes of primary tumor, macro-metastasis and micro-metastasis.

Significance: While the majority of cancer patients are dying from metastasis, there is no effective treatment strategy to overcome or prevent metastatic disease. Complex process of cancer progression hinders effective targeting. It is imperative to understand the mechanism of cancer metastasis. This research will not only enhance the understanding of which clones of primary tumour end up in metastasis and what properties are responsible for tumour cell survival in distant organ, but also identify potential treatment targets for cancer metastasis.

DETERMINING THE FUNCTIONAL ROLE OF REPRESSOR ELEMENT-1 SILENCING TRANSCRIPTION FACTOR CO-REPRESSOR 1 (RCOR1) IN DIFFUSE LARGE B CELL LYMPHOMA

BACKGROUND/OBJECTIVES
Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (30-40%) and is fatal without rapid treatment following diagnosis. Despite recent breakthroughs in the characterization of the disease using genomics and transcriptomics approaches, approximately a third of people with DLBCL receiving the standard therapeutic regiment of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP) experience relapse and are not cured. The biological heterogeneity of clinical responses remains to be fully elucidated. A recent study by our lab analyzing genomic variations and gene expression patterns of 139 diagnostic DLBCL samples from human subjects treated with R-CHOP revealed that deletion of the restriction element 1-silencing transcription factor co-repressor 1 (RCOR1) gene was correlated with poor 5 year progression free survival. Reduction of expression of RCOR1, which encodes a global regulator of neuronal genes in non-neuronal cells, defined a gene signature involving biological pathways such as downregulation of histone deacetylation. Aberrant histone modification activity has been suggested to dysregulate oncogenic signaling pathways such as the PI3K/AKT/mTOR and NF-kB pathways leading to their constitutive activation in B cell lymphomas. As such, our objective is to further investigate the molecular and genomic mechanisms of RCOR1 biology which potentially contribute to DLBCL pathogenesis.

METHOD(S)
To determine the intrinsic interactions involving the RCOR1 co-repressor in a B cell context, we co-immunoprecipitated RCOR1 with various histone modifying enzymes such as lysine-specific demethylase 1A (KDM1A) and histone deacetylase 2 (HDAC2) from human DLBCL cell line lysates. Next, we generated RCOR1-deleted human DLBCL cell lines using CRISPR/Cas9 genome editing technology in order to model and to assess proliferation, cell cycle progression and R-CHOP sensitivity.

RESULT(S)
Co-immunoprecipitation and western blot analysis demonstrated that RCOR1 directly interacts with both KDM1A and HDAC2 in DLBCL cell lines. In addition, KDM1A and HDAC2 also form an interaction in DLBCL cell lines. Screening of CRISPR/cas9 genome edited single cell isolated colonies using Sanger sequencing generated 26 heterozygous RCOR1 mutant clones. We are currently developing a bi-allelic knockout model to determine if changes in proliferation, cell cycle progression and R-CHOP sensitivity can be observed in RCOR1-deficient DLBCL.

CONCLUSION(S)
We confirm that RCOR1 forms complexes with the histone modifying enzymes KDM1A and HDAC2 and further establish that this phenomenon occurs in a B cell lymphoma context. Our preliminary results raise the possibility of RCOR1 involvement in epigenetic modifications and regulatory functions in DLBCL. Ongoing studies in our lab will aim elucidate these potentially novel mechanisms.

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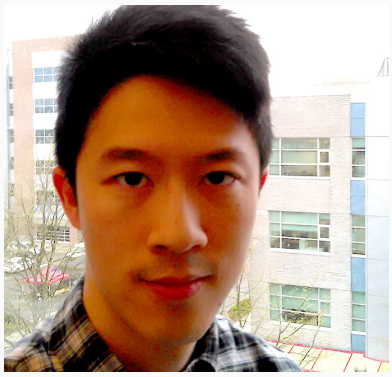

FUNCTION AND PREVALENCE OF HOST-DERIVED COAGULATION FACTORS ON THE VIRUS SURFACE

Supervisor:
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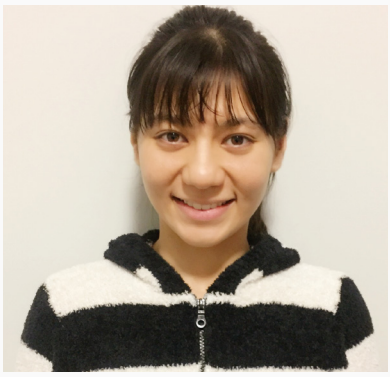
BACKGROUND/OBJECTIVES
Numerous viruses are known to alter the hemostatic system. To explain this link, we have shown that the envelope of three herpesviruses acquire the initiators of coagulation from the host, tissue factor (TF) and anionic phospholipids (aPL). Our model virus, herpes simplex virus 1 (HSV1), was shown to utilize TF to enhance infection. HSV1-encoded glycoprotein C (gC) has also been implicated in FX activation. This study investigates the role of gC in coagulation as well as the ubiquity of viral TF and aPL on enveloped viruses.

METHOD(S)
FX activating roles of viral TF and gC were assessed by chromogenic and plasma clotting assays. Immunogold electron microscopy was used to simultaneously visualize TF, aPL, and a virus-specific marker on virus surfaces.

RESULT(S)
In plasma, HSV1 and dengue virus induced TF-mediated clotting. Viral TF was required for optimal FX activation and was essential for HSV1 gC-mediated enhancement. In purified systems, recombinant gC could enhance Factor VIIa-mediated FX activation in the absence of TF. Both TF and aPL were incorporated into HSV1 and DENV particles.

CONCLUSION(S)
Virus surface TF function is enhanced by gC to contribute to FX activation and clot formation. TF presence on HSV1 and dengue virus suggests the ubiquity of TF on enveloped viruses, supporting the targeting of viral TF as a broad-spectrum anti-viral agent.

EVALUATING NORMAL TISSUE TOXICITY RESULTING FROM INHIBITORS OF RADIATION-INDUCED-DAMAGE-REPAIR IN MURINE MODELS



BACKGROUND/OBJECTIVES
One of the major factors driving radiation resistance and therefore regrowth in tumours after curative radiotherapy is the presence of hypoxia (poor oxygenation). Hypoxia reduces radiation-induced DNA damage. Tumour cells are able to repair this damage thus several inhibitors of DNA damage repair are in development including inhibitors to DNA-dependent protein kinase (DNA-PK). DNA-PK is an enzyme involved in repairing non-homologous end-joining of DNA double strand breaks. These drugs would be combined as sensitizers with other DNA damaging agents to increase sensitivity. However, they may inhibit DNA repair in all tissues therefore sensitization of normal cells that are unavoidably within the normal tissue margin of a radiation beam are still dose-limiting. My lab is currently developing novel DNA-PK inhibitors that are selectively activated under hypoxic conditions, hypoxia activated DNA-PK inhibitors (haDNAPKis), which would increase radio-sensitivity of tumours without affecting the typically well-oxygenated, normal cells.We aim to evaluate the toxicity of DNA-PK inhibitors in normal tissues, including the intestines, kidney, thymus, liver, tongue, bone marrow, in combination with radiation. Indicators of toxicity include cell death, damage and reduced proliferation. We hypothesize that hypoxia activated inhibitors will have reduced toxicity relative to systemic DNA-PK inhibitors in the presence or absence of radiation in normal tissue of mice.

METHOD(S)
Preliminary investigation was done to assess demonstration of hypoxia and proliferation in tumours and under normal physiology in normal tissues. Rag2M mice model was implanted subcutaneously with HCT cell line. Tissues were collected from groups (1. radiation, 2. untreated control) and compared for evidence of toxicity. The mice were injected with bromodeoxyuridine(brdu) and pimonidazole(pimo) 2 hours prior to sacrifice. Tissues including tumour kidney, thymus, liver, intestines, tongue and bone marrow were collected and frozen immediately followed by OCT embedding. Immunohistochemistry analysis of brdu (s-phase cells), pimo (hypoxia) staining were performed to assess the overall effects of treatments on cell proliferation and oxygenation.

RESULT(S)
Without treatment, the kidney and small intestines demonstrated varying degrees of hypoxia. Thymus, intestines, bone marrow, and the liver were found to have substantial proliferative properties under normal physiology. However, the liver and bone marrow were shown to have extensive cross-reactivity to immunohistochemistry antibodies; most likely due to the massive cell population as well as blood mixing. The thymus, kidney, and small intestines serve to be good indicators of hypoxia and proliferation. This finding will be re-confirmed by repeating the assay.

CONCLUSION(S)
Based on the preliminary findings, we may narrow future toxicity evaluation to a few representative tissues that demonstrate significant treatment response. Planned experiments will consist of utilizing these representative tissues, with radiation, to evaluate response of DNAPK inhibitors in comparison to hypoxia-activated inhibitors.

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UNCOVERING IMMUNE CHANGES THAT PROMOTE IMMUNE EVASION IN EARLY LUNG CANCER

Supervisor:
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BACKGROUND/OBJECTIVES
Lung cancer is the leading cause of cancer-related deaths in Canada. Late diagnosis partially attributes to high mortality, when the disease is widespread. The immune system is a key area of interest, for its tumor-promoting and suppressive roles. Multiple studies have assessed immune cells and cytokines in advanced tumors; however, little is known about those during initiation and early progression. Identifying immune changes during these stages of tumor development may yield powerful clinical tools; these include biomarkers for early detection and targets for chemoprevention. My study aims to identify changes in immune modulatory cytokines during oncogenic-driven transformation of lung epithelial cells. I hypothesize that oncogenic-driven transformation upregulates cytokines that promote immune evasion.

METHOD(S)
I will use genetically engineered mouse models to establish lung adenocarcinoma tumors. These models are driven by temporally-regulated oncogenes (KRAS, EGFR) identified in patients, and accurately represent human lung cancer tumorigenesis. I will induce oncogene expression via doxycycline-treated food; micro computed tomography will be used to monitor tumor progression. As this model usually develops advanced tumors by six months (26 weeks), I will sacrifice ten animals at 0, 2, 4, 8, 12, and 24 weeks. Hyperplastic, premalignant, and adenocarcinoma tissue will be collected and compared to control mice, sacrificed at parallel time points. I will also collect peripheral blood (PB), bronchoalveolar lavage (BAL), and lung tissue at these time points; cytokines will be isolated from PB plasma, BAL supernatant, and lung lysate. Multi-colour flow cytometry and the Luminex platform will be used to identify and quantify cytokines. ELISA assays will be used to validate results from both platforms. This analysis will provide insight on cytokine profiles pre-tumor and post-tumor initiation. Cytokines of interest will be assessed in vitro for their impact on immune cell phenotype, migration, and proliferation. Flow cytometry and cell-specific markers will be used to measure changes in immune cell phenotypes; transwell migration assays to evaluate chemotaxis of multiple immune cell populations; and standard growth curves and cell cycle analyses to measure proliferation. These assays will demonstrate direct impacts of cytokine changes on effector immune cells.

RESULT(S)
None at this time.

CONCLUSION(S)
None at this time.

ANALYSIS OF XIST AS A SEX-SPECIFIC MIRNA SPONGE IN NON-SMALL CELL LUNG CANCER

BACKGROUND/OBJECTIVES
Genes on the X chromosome are differentially present between sexes, and XIST (X-inactivated specific transcript) is a well-studied nuclear lncRNA required for equilibration of gene expression on each female X to the singular X in males. In cancer, there are conflicting reports regarding the role of additional XIST-mediated gene regulation, many of which propose that XIST regulates protein-coding genes by sponging singular inhibitory miRNAs. However, important biological considerations, including sex and localization of miRNA and XIST transcripts, are often ignored in these models, besides the effect of a shared miRNA pool.

METHOD(S)
We perform a comprehensive unbiased analysis of the role of XIST in lung adenocarcinoma (LUAD) through positive regulation of protein coding genes. To find genes regulated by XIST sponging of miRNAs, we correlate all Ensembl-annotated genes with XIST expression levels in female LUAD (n=307). Using a specialized algorithm based on binding energies and sequence homology, we assessed the potential binding of all annotated miRNAs against DMX genes and XIST. We then determine the best candidates for sponging by XIST using XIST-high and XIST-low systems and validate the presence of these candidate miRNAs in the nucleus.

RESULT(S)
Our analysis yielded 543 genes that may be defended from miRNAs by XIST (DMX genes) (Rho>0.4, p <0.05). 804 miRNAs were found to target both XIST and DMX genes. We then compared the changes in miRNA-DMX relationships in XIST-high (female) and XIST-low (male) systems, and discovered 13 miRNA-DMX gene pairs, 4 of which involve miRNA with known nuclear localization.

CONCLUSION(S)
Previous studies have implicated miRNA-XIST interactions, but are limited in biological context (including cellular localization, sex-specific gene expression, and multisite binding). By analyzing the transcriptome in female and male LUAD, we identify genes defended from miRNA by XIST (DMX). We show that the XIST-miRNA-DMX sponging axis is affected by expression of sex-specific genes and number of shared miRNA binding sites on DMX genes. We also find that miRNAs targeting exonic regions of XIST are more strongly anti-correlated with DMX gene expression. Importantly, we identify that the specific set of miRNAs that mediate the XIST-DMX gene axis and are enriched in the nucleus, co-localizing with XIST.

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CHARACTERIZING THE LANDSCAPE OF ONCOFETAL SMALL NON-CODING RNAS IN THE LIVER

Supervisor:

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BACKGROUND/OBJECTIVES

Liver cancer is one of the deadliest forms of cancer worldwide. Developmental pathways originally repressed in normal adult tissues have been shown to become re-activated in tumours and associate with aggressiveness. Small non-coding RNAs (sncRNAs) are key regulators of gene expression, with major roles in cancer development. Thus, this study aims to identify sncRNAs similarly expressed in fetal liver and liver tumors, providing new insights of molecular drivers in liver cancer.

METHOD(S)

Briefly, sncRNAs expression was deduced using a previously published sequence analysis pipeline. The expression levels of fetal liver samples (n=10) and a tumor/non-malignant paired cohort (TCGA, n=47) were compared. sncRNAs that were not expressed in non-malignant samples and had similar expressions in both fetal and tumor tissues were classified as oncofetal sncRNAs. Finally, the biological functions of these molecules were deduced using gene target prediction and pathway enrichment analyses.

RESULT(S)

In this study, we have characterized known sncRNAs and discovered 198 novel miRNA sequences, expanding the human liver miRNA transcriptome by 9,3%. In non-malignant tissue, these novel miRNA sequences display increased tissue specificity, distinguishing liver from other tissue types. Additionally, we identified a subset of 45 sncRNAs and one novel miRNA with similar expression levels between fetal and tumour tissues, but absent in non-malignant adult liver. Target prediction and pathway enrichment analyses revealed the involvement of these sncRNAs in key liver-specific cellular processes.

CONCLUSION(S)

Our study provides a comprehensive characterization of the sncRNA transcriptome in liver tissues, uncovering new and promising regulatory networks for liver tumour biology. In particular, the discovery of previously-undescribed miRNAs may aid to the development of novel diagnostic and therapeutic targets for the management of liver cancer patients.

INVESTIGATING THE EFFECTS OF TP53 MUTATIONS ON LENALIDOMIDE RESISTANCE IN DEL(5Q) MYELODYSPLASTIC SYNDROME PATIENTS

BACKGROUND/OBJECTIVES

Myelodysplastic syndromes (MDS) are a heterogeneous group of myeloid malignancies characterized by dysplastic bone marrow and cytopenias, occurring in up to 40,000 Canadians in 2016. del(5q) MDS is the most common cytogenetic abnormality in MDS and the drug, ***Lenalidomide (LEN)***, is the first line of therapy. 60-80% of patients respond to LEN, but around half relapse within 2-3 years. Mechanisms of resistance are not well understood. Deep sequencing of patient samples by our lab and others of patient marrow show mutually exclusive occurrences of TP53 and RUNX1 mutations in ~70% patients at relapse. RUNX1 is a transcription factor involved in hematopoiesis. CRISPR/Cas9 knockout (KO) of ***RUNX1*** in the del(5q) MDS cell line, MDS-L, reduces LEN sensitivity and p53 dependent transcription. TP53 encodes a transcription factor that also acts in the cytoplasm. p53 localizes at the endoplasmic reticulum (ER) and interacts with sarco/ER Ca2+-ATPase (SERCA) pumps to regulate Ca2+ fluxes and apoptosis. Ca2+ signaling regulates stem cell differentiation, and LEN acts through elevating intracellular Ca2+ levels. There is a strong correlation between ***TP53*** mutations and LEN resistance, but a causal relationship has not been demonstrated. ***Hypothesis***: LEN sensitivity in del(5q) MDS cells requires p53 and RUNX1 localization at the ER, where they cause calcium deregulation to induce apoptotic signaling and terminal differentiation. Mutations in either protein cause LEN resistance.

METHOD(S)

Aim 1- Determine if wild-type p53 is required for LEN sensitivity in del(5q) MDS: CRISPR-Cas9 technology was used to KO TP53 in MDS-L cells. In parallel, a TP53 mutant with a dominant negative effect (p53-DN) on transcription was lentivirally expressed in parental MDS-L cells. KO clones and transduced cells were treated with LEN in methylcellulose for CFCs (colony forming cell assay). Aim 2- Determine how expression of patient TP53 mutations affects LEN response. Patient p53 mutants were lentivirally co-expressed with GFP in TP53 KO clones and the same experiments in (Aim 1) were conducted.

RESULT(S)

Aim 1- MDS-L cells expressing p53-DN did not have significantly increased colony formation with LEN treatment compared to cells expressing empty vector control. Of the 4 validated p53 CRISPR KO clones, only one had reduced LEN sensitivity (no significant reduction in colony formation with LEN treatment). ***Aim 2***- Over-expression of only one of the two patient TP53 mutants reduces LEN sensitivity, and only in one of the LEN-sensitive TP53 CRISPR KO clones.

CONCLUSION(S)

p53-dependent transcription is not required for LEN sensitivity in MDS-L cells. Results regarding the effect of TP53 KO and TP53 patient mutations on LEN sensitivity are inconclusive. We will examine the nature and functional effects of the mutations introduced by CRISPR/Cas9 and the mutations found in the patients for a deeper understanding of the role of TP53 mutations in LEN resistance in del(5q) MDS.

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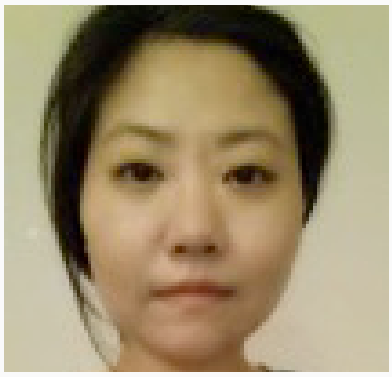

TOWARDS UNIVERSAL BLOOD - ENZYMATIC CONVERSION OF BLOOD GROUP A ANTIGENS

Supervisor:
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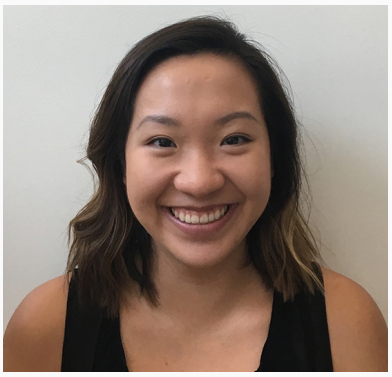
BACKGROUND/OBJECTIVES
In transfusion therapy, unintentional mismatching of red blood cells (RBC) for blood groups remains one of the most common causes of serious and sometimes fatal adverse reactions. Scarcity of RBCs with certain blood groups is another important transfusion challenge. The ABH blood group specificity is determined by the terminal monosaccharides. Enzymatic removal of these sugars would uncover universally acceptable H-antigen (O group RBCs). Enzymatic conversion of group A RBCs has lagged behind due to a lack of very active, specific glycosidases and the more complex nature of A antigens. In this research two novel glycosidases with improved kinetic properties and high specificities for the A antigen are studied to efficiently convert A group RBCs to universal O group RBCs with improved conversion conditions compared to previously reported glycosidases.

METHOD(S)
Whole blood samples from A type donors were collected, washed and diluted to 10% hematocrit in PBS buffer (pH7.4). The washed RBCs were treated with the highly active new glycosidases. The rate of conversion of type A RBCs to universal O type RBCs by the new glycosidases is enhanced using a neutral macromolecular crowder; 40kDa dextran (300 mg/mL). These novel glycosidases were tested under a range of conversion conditions including different enzyme concentrations, temperatures, buffers, and incubation time. The efficiency of the different conversion conditions and the characterization of the enzyme-converted O (ECO) RBCs were evaluated by gel column-based micro typing system (MTS) cards against A antigen, traditional agglutination technique against A and H antigens, and blood group serology.

RESULT(S)
The results from anti A micro typing system cards and traditional agglutination assays against A and H antigens showed that A type RBCs were efficiently converted to O RBCs. The conversion of A type to O type RBCs with these novel glycosidases was achieved using more cost-efficient quantities of enzymes (5 ug/mL with Dextran and 10 ug/mL without Dextran) and under gentler conditions including neutral pH and short incubation time at a wide range of temperatures when compared to conventional glycosidases. During the blood group serology study, the ECO RBCs reacted with some group O and B sera, a common observation for ECO RBCs.

CONCLUSION(S)
Our novel glycosidases converted A type to O type RBCs efficiently. However, ECO-RBCs still reacted with some group O and B sera reflecting the more complex nature of A type antigens and maybe the changes in the glycocalyx structure. The biochemical nature of enzymatic conversion of A type RBCs using these novel glycosidases has not been fully clarified. Further studies regarding the relevance of the sera cross matching reactivity towards ECO-RBCs is ongoing. With these novel glycosidases it is now possible to efficiently remove immunodominant A antigens from the intact RBCs at neutral pH, low temperature, and at enzyme concentrations much lower than previously reported. This enzymatic removal of major antigens has a potential to greatly advance the field of transfusion/transplantation medicine.

ROUTINE CLINICAL USE OF MASS SPECTROMETRY FOR QUANTIFICATION OF AMYLOID-BETA 1-40 AND 1-42: PRE-ANALYTICAL AND ANALYTICAL CONSIDERATIONS



BACKGROUND/OBJECTIVES
Towards implementation in a routine clinical setting, we previously developed an automated high performance liquid chromatography—triple quadrupole mass spectrometry (HPLC-MS/MS) method for quantification of amyloid-beta peptides 1-40 and 1-42 in cerebrospinal fluid (CSF). This assay has been validated following Clinical and Laboratory Standards Institute guidelines in an accredited hospital laboratory. With clinical implementation in mind, we evaluated pre-analytical and analytical factors to optimize assay performance and develop criteria for sample acceptance/rejection.

METHOD(S)
Human CSF was obtained from the biobank of the Clinic for Alzheimer's Disease and Related Disorders and St. Paul's Hospital, Vancouver, Canada. Amyloid-beta peptides in CSF were concentrated by solid phase extraction (SPE), followed by HPLC-MS/MS analysis. To identify a robust and reproducible SPE workflow, various wash and elution buffers were tested. In development of an automated workflow, we explored pre-treatment strategies to reduce amyloid-beta absorption to the 96-well plates. Spike and recovery experiments were used to determine interference from hemolysate (to model a traumatic lumbar puncture), and the effect of total protein content (0 – 20 g/L) on amyloid-beta recovery. A comparison was performed to assess analytical performance of the manual versus automated specimen handling workflow (n = 40).

RESULT(S)
SPE efficiency was improved by 57 %, compared to a previously published protocol, using two 4 % phosphoric acid washes and an elution buffer of 75:15:10 by volume of acetonitrile, water, and ammonium hydroxide. Pretreating plates with bovine serum albumin extended the possible storage window in the auto-sampler from 10 minutes to over 1 hour and 30 minutes (enabling delayed analysis). A maximum of 5 % whole blood contamination met the pre-specified acceptance criteria (expected value +/-15 %) for both amyloid-beta 1-40 and 1-42. Amyloid-beta recovery demonstrated a generally decreasing trend with increasing total protein concentration from 0.2 to 20 g/L. The method comparison revealed comparable accuracy between manual and automated workflows with a median difference of -1.64 %.

CONCLUSION(S)
We automated the workflow of our HPLC-MS/MS method and optimized key analytical steps to reduce variability associated with multiple operators and support ease of use in a clinical environment. We also identified criteria for specimen acceptance/rejection given the variability of CSF samples submitted to clinical laboratories for routine analysis.

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OPTIMIZING A PROTEOMIC WORKFLOW FOR THE ISOLATION OF CELL SURFACE TERMINI

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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.

METHOD(S)
To enrich for cell surface 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 that have unprotected amine groups— i.e., not labeled with biotin— were tagged with undecanal, an 11-carbon aldehyde. Tagging peptides with undecanal imparts the peptide with additional hydrophobicity. This increase in hydrophobicity was used in the latter stages of sample preparation as a means of excluding hydrophobic peptides from Tandem Liquid Chromatography Mass Spectrometry (LC-MS/MS) analysis and negatively selecting for the biotin-labeled peptide.

RESULT(S)
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. So far, we have shown that cell surface proteins can be successfully biotin-labeled. Using flow cytometry, we have demonstrated that 99% of Jurkat cells can be labeled after incubation with 2mM biotin. Additionally, peptides that resulted from trypsin digestion can be successfully tagged with undecanal, and that these undecanal-tagged peptides can be excluded from further analysis using reversed phase chromatography. Future work will focus on optimizing the entire workflow on Jurkat cells, using limited (1-5 million cells) starting material, before moving on to analyze clinical samples.

CONCLUSION(S)
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.

GENOMIC CHARACTERIZATION OF ADULT-TYPE GRANULOSA CELL TUMOURS OF THE OVARY: ARE THERE FEATURES OF IMPORTANCE BEYOND FOXL2?

BACKGROUND/OBJECTIVES
Adult granulosa cell tumour (AGCT) accounts for 90% of sex cord-stromal tumours of the ovary. Our research team previously identified a single somatic missense mutation (c.402C>G; pC134W) in the transcription factor, forkhead box protein L2 (FOXL2) in 97% of AGCTs. As this mutation is pathognomonic, it has transformed the ability to accurately diagnose challenging cases. AGCTs can have an unpredictable clinical course and up to one-third of patients relapse, leading to increased mortality. Thus, identification of patients with increased risk for relapse is important to ensure proper follow-up for disease management.

METHOD(S)
We performed whole genome sequencing (WGS) on ten AGCTs and found that 20% of cases had a mutation in the bone morphogenetic protein 7 (BMP7), a TGF-beta subfamily gene that is involved in the regulation of folliculogenesis and steroidogenesis. To validate WGS results, we designed an amplicon-based assay for targeted resequencing of 40 genes of interest in our international extension cohort of 300 cases.

RESULT(S)
We found that 4 of 81 cases (4.9%) harbour a mutation in BMP7, including two cases with a novel hotspot mutation. We hypothesize that the TGF-beta signaling pathway and in particular BMPs contribute to the progression of AGCT. To test this hypothesis, we first developed a cell model from primary human granulosa-lutein cells obtained from patients undergoing oocyte retrieval. We are using lentiviral transduction of either $FOXL2^{WT}$ or $FOXL2^{C134W}$ with telomerase as we previously discovered frequent activating TERT promoter mutations in AGCT. Currently, we are treating both the $FOXL2^{WT}$ and $FOXL2^{C134W}$ cell models with various BMPs and other TGF-beta proteins to measure differences in hormone production, cell proliferation and apoptosis. The pathogenicity of the BMP7 mutation will be assessed in this model along with the potential to target this pathway.

CONCLUSION(S)
Overall, our findings suggest that BMPs and specifically *BMP7* likely contribute to the development of AGCT.

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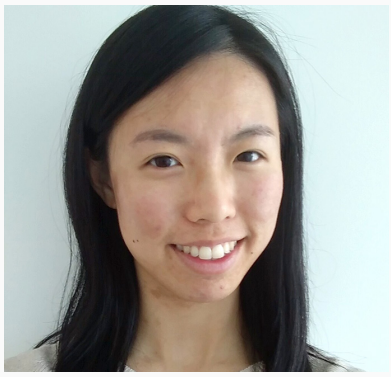

INCREASING APOE IN PRIMARY HUMAN BRAIN PERICYTES DOES NOT MODIFY MIGRATION IN A SCRATCH-WOUND ASSAY

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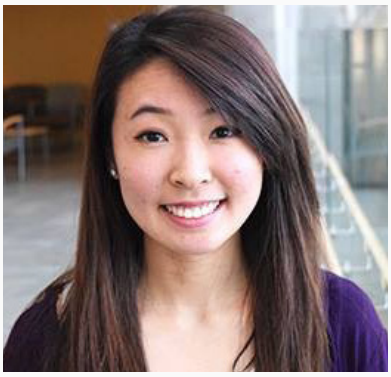
BACKGROUND/OBJECTIVES
Pericytes are contractile cells that surround endothelial cells and play crucial roles in the microvasculature. Within the brain pericytes maintain the blood brain barrier (BBB) homeostasis, and lost of pericyte coverage due to migration reduces BBB integrity in Alzheimer's disease (AD). Pericytes also produce apolipoprotein E (ApoE), whose genetic isoforms confer different risks for developing AD, with apoE2 being protective and apoE4 being detrimental relative to apoE3. ApoE knockdown in murine pericytes was previously reported to promote pericyte migration, which could be rescued by exogenous apoE3 but not apoE4. As pericyte loss or migration away from the endothelial layer is thought to compromise its protective function and leaves the brain vulnerable to damage, compounds that modulate pericyte apoE expression and function may decrease pericyte migration and protect the BBB. Here we tested that hypotheses that pharmacologically increasing ApoE levels and lipidation would decrease migration of human pericytes, and that apoE genotype will influence migration.

METHOD(S)
We treated primary pericytes from three donors genotyped as *APOEe3/3*, *APOEe3/4* and *APOEe4/4* with the ApoE-modulating compound GW3965. We observed pericyte migration in a wound-healing assay through the IncuCyte live-cell imaging system, and measured secreted apoE levels with ELISA.

RESULT(S)
First, we confirmed that GW3965 increases the levels of secreted apoE and ATP-binding cassette transporter A1 (ABCA1), a transporter that loads lipids onto apoE. Second, we observed that GW3965 has no effect on pericyte migration in all pericyte donors, despite increased apoE.

CONCLUSION(S)
Overall, this suggests that excess apoE does not modify migration of human pericytes in a wound healing assay

USE OF A CARDIAC-TARGETING APPROACH ENHANCES UPTAKE OF POLYMER THERAPEUTICS IN CARDIOMYOCYTES



BACKGROUND/OBJECTIVES
Disorders of hemoglobin, such as sickle cell anemia and thalassemia, are an increasing global health concern. Patients with such disorders receive regular blood transfusions as a life sustaining treatment. Unfortunately, a consequence of chronic blood transfusion is excess iron burden leading to a condition known as transfusion-associated iron overload. Excess iron enables the production of reactive oxygen species leading to oxidative damage of cells. Further, as the human body lacks an iron excretion pathway iron accumulates in vital organs, notably, the liver and the heart, leading to organ damage and failure. The current standard of care is chelation therapy using small molecule chelators which suffer from short circulation time, low iron excretion efficiency, and adverse side effects. Currently, no methods are available to remove iron from specific organs. Macromolecular chelators have demonstrated advantages of multivalent presentation of targeting ligands and chelating moieties. Further, it has been previously reported that macromolecular approaches have enhanced circulation time and reduced toxicity compared to small molecules. Recently, we have identified two candidate molecules (CM1 and CM2) for cell-specific delivery to cardiomyocytes. We ***hypothesize*** the use of these CMs will enhance specificity of chelation towards the heart and will be effective system for the treatment of cardiac iron overload.

METHOD(S)
CM1 and a FITC dye molecule were conjugated to amine functionalized polymer. The system was characterized through H-NMR and UV/VIS spectrometry for confirmation of CM conjugation. Monocultures of rat cardiomyocytes and co-cultures of human stem cell-derived cardiomyocytes and human fibroblasts were incubated with FITC-labelled polymer conjugated to peptide (PCM1) for 2 hours at varying concentrations. Data was compared with FITC-labelled control polymer without CM1 as a control. Assessment of cellular uptake was then conducted through flow cytometry and confocal imaging experiments.

RESULT(S)
Flow cytometry experiments demonstrate greater cellular uptake in rat cardiomyocytes by CM1 conjugated to polymer compared the control. These results were validated using confocal imaging. Further, energy-dependent mechanisms of uptake are demonstrated by minimal uptake of the PCM1 system at 4°C. Finally, confocal imaging of cellular uptake in co-cultures demonstrates modest cell-selectivity towards human cardiomyocytes over fibroblasts.

CONCLUSION(S)
Preliminary results demonstrate that CM1 improves uptake of polymer in rat cardiomyocytes with indication of an energy-dependent uptake process. Further detailed study on cellular trafficking of the nanosystems will carried out to understand the difference in cellular distribution and iron binding in targeted carriers vs non-targeted carriers. The development of a novel chelating system that targets the heart would significantly enhance therapeutic efficacy and decrease the risk of cardiac failure in transfusion patients. This study will provide novel insight into the mechanisms of cardiac-targeted polymeric systems for site-specific chelation.

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DENGUE VIRUS INDUCED PROTEIN SYNTHESIS IN PLATELETS

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BACKGROUND/OBJECTIVES
According to the World Health Organization, about 50% of the world's population lives in regions where dengue virus (DENV) is endemic. Approximately 400 million people are infected yearly, of which nearly half are asymptomatic despite high virus titers. The most common clinical manifestation observed in all stages of DENV infection is the significant reduction of platelet (PLT) number (thrombocytopenia). However, the mechanism by which DENV induces thrombocytopenia is not well understood. DENV is an RNA virus which is able to reproduce in various host cells including in PLTs which are enucleate blood constituent. Currently, there are pathogen reduction technologies (PRTs) that employ photoreactive agents and ultraviolet lights to inactivate viruses and reduce the risk of transfusion related transmission. In the presence of PLTs the rate of DENV reduction was significantly lower suggesting that PLTs may play a protective role on viral-RNA. A recent study showed that PLTs synthesize proteins that may protect their RNA from PRT induced damage. Furthermore, our lab demonstrated that DENV can bind and enter PLTs, and utilize their simple cellular machinery to replicate and assemble infectious virus progeny. To mitigate DENV induced thrombocytopenia and alleviate transfusion related virus transmission, we utilize qualitative proteomics approach to follow newly synthesized proteins in PLTs during activation with physiological agonists, direct interaction with DENV and during PRT treatment. Hypothesis: Translation of PLT and viral-RNA will be triggered by DENV-PLT interaction.

METHOD(S)
DENV serotype 2 will be propagated in Vero (African green monkey kidney epithelial cells) and purified by differential density sedimentation. PLT will be treated with physiological agonists, viable and UV-inactivated DENV to dissect the effects of DENV on platelets independent of virus protein translation. The effect of PRT will be investigated using Mirasol technology (riboflavin and UV) treatment. Newly translated proteins will be selected and captured using biotinylated puromycin (a protein synthesis inhibitor), which is incorporated into the growing peptide during translational elongation. The biotin-puromycin tagged translatome will be captured with streptavidin magnetic beads. High performance liquid chromatography coupled to tandem mass spectrometry will be used to separate and identify nascent PLT and DENV peptides. Validation of alterations in the protein profile will be performed by western blot, flow cytometry and immunofluorescence confocal microscopy. Significance: The proteomics data collected will have future implication on antiviral design to attenuate DENV infection, mitigate clinical symptoms of thrombocytopenia and alleviate transfusion linked transmission of DENV. Furthermore, discovery of a biomarker may assist in the quality control of platelet transfusion products by the identification of critical proteins found to be affected by DENV.

ANTITHROMBOTICS WITHOUT BLEEDING SIDE EFFECTS: INHIBITION OF PROTHROMBOTIC POLYANIONS WITH POLYMERIC INHIBITORS

BACKGROUND/OBJECTIVES
Abnormal blood clot formation (Thrombosis) is a common pathology underlying ischemic heart disease, ischemic stroke, venous thromboembolism (VTE) and is invariably associated with sepsis and cancer. It is the leading cause of mortality worldwide. Although current drugs are effective in reducing thrombosis, bleeding is the major side effect associated with their use. These drugs target key players in the coagulation cascade resulting in a hemostatic imbalance favoring bleeding. Recently, important mediators of thrombosis have been identified, including cell-free DNA, Polyphosphates, RNA and Neutrophil extracellular traps (NETs). The commonality among these mediators is that, they are negatively charged macromolecules that are shown to activate the contact pathway of coagulation. NETs are released from neutrophils when activated by microbial or inflammatory stimuli. These web like structures are composed of cell-free DNA, histones and antimicrobial proteins. They have been shown to trap and kill microorganisms, playing a critical role in host defense. NETs are extensively produced during condition of sepsis to combat overwhelming bacteremia. Studies have shown that, NETs not only entrap and clear pathogens but also, activate coagulation proteins initiating thrombosis. Similarly, cell-free DNA is a major contributor to thrombosis in cancer patients. My objective is to develop polymeric inhibitors with optimized cationic charge density and binding constants to selectively bind and neutralize anionic NETs and cell-free DNA in in vitro, ex-vivo and in mouse models of sepsis and cancer. The ***hypothesis*** is that, newly designed inhibitors could be used to target and inhibit prothrombotic actions of cell-free DNA and NETs without the risk of bleeding.

METHOD(S)
Neutrophils isolated from human blood and are activated with phorbol-12-myristate-13-acetate(PMA) to release NETs. The formation of NETs is confirmed by fluorescence microscopy. Cell-free DNA is obtained from the Genomic DNA isolated from mammalian cells or blood. High throughput thrombin generation assay (measures the amount of thrombin generated with time) in human plasma is used to screen the library of inhibitors for identifying lead candidates. Further, lead candidates are investigated for their biocompatibility and antithrombotic potential using thromboelastometry in whole blood.

CONCLUSION(S) / RESULT(S)
Results from the clotting studies shows that, genomic DNA is pro-coagulant and this effect is reversed by the treatment with our inhibitors. Congruent with previous studies, we show that, NETs are formed when neutrophils are activated by PMA. Fluorescence microscopy studies show the binding of alexa-488 labeled inhibitors with NETs. This indicates that, our inhibitors might be able to neutralize the negative charge of DNA in NETs and prevent the pro-coagulant potential of NETs. Our approach to target cell-free DNA and NETs –recently identified mediators of thrombosis will have the potential to reverse thrombosis and minimize risk of bleeding.

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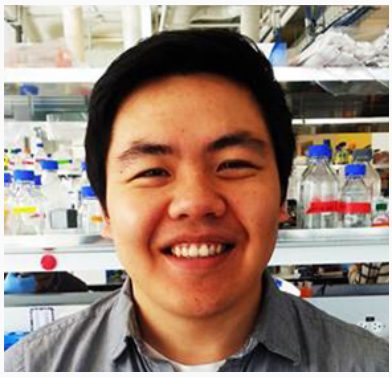

INTERNALIZATION MECHANISM OF MALARIA PARASITE PROTEIN IN CANCER CELLS

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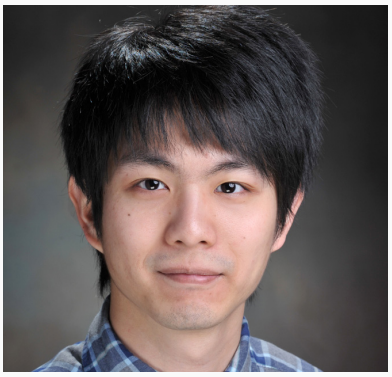
BACKGROUND/OBJECTIVES
The limited clinical efficacy of anti-cancer drugs is a direct consequence of their non-selective nature. In order to improve their efficacy, targeted therapy approaches are needed. One of the major strategies is to conjugate drugs to a delivery vehicle that displays selective binding to tumor cells. Our lab seeks to advance a novel targeting approach, taking advantage of a malaria protein (rVAR2) that binds with high affinity to oncofetal chondroitin sulfate A (ofCSA) specifically present on the placenta and cancer cells. In addition to binding to cancer cells, rVAR2 is internalized into the cell. Internalization is complicated process and depends on the nature of the antigen. Different mechanisms of internalization that could be involved are clathrin and caveolae mediated endocytosis, as well as macropinocytosis. Once in the cell, the release of the drug payload from the protein is dependent on the cellular compartment (affected by pH, Proteases etc.). As such it is equally important to study the intracellular trafficking of rVAR2 in cells. Data from this project will build a foundation for future designs of rVAR2-drug conjugates that will maximize drug efficacy and reduce toxicity.

METHOD(S)
Different cancer cell lines were incubated with rVAR2 at different time points and was investigated by confocal, live cell, and electron microscopy as well as western blotting. Specific fluorescent dyes, tracers, and antibodies were used to determine the intracellular localization of rVAR2. Inhibitors of internalization pathways was also used to determine the route of entry.

RESULT(S)
Our data show that rVAR2 binds different cancer cells within 5 min and gets rapidly internalized into intracellular vesicular structures. Furthermore, rVAR2 is internalized through micropinocytosis and not clathrin or caveolin mediated. Inside the cells, rVAR2 is localized to the early endosome within 2hrs, however it does not reach the lysosome after 4hrs.

CONCLUSION(S)
Due to its ability to specifically target cancer cells, rVAR2 is a novel malaria protein that can be exploited as a therapeutic tool against cancer. In order to maximize rVAR2's potential as a delivery tool, we elucidated its internalization mechanism and showed that rVAR2 is actively internalized into cancer cells via micropinocytosis. However, its intracellular trafficking remains to be seen.

AN IMPROVED MICROSCALE N TERMINI ENRICHMENT TECHNIQUE WITH ACTIVATED MAGNETIC BEADS AND HYDROPHOBIC TAGGING



BACKGROUND/OBJECTIVES
N and C termini are start and endpoint, respectively, of a protein. They provide useful information in foreseeing important functions and characteristics of a protein. Modifications at the protein N termini, which contribute to protein complexity and are essential in cellular regulation and signaling, can be resulted after specific proteolytic processing. Additionally, abnormal proteolysis is closely related to various cancers and developmental defects. Therefore, there is a growing interest in the proteomic field in identifying N-terminal peptides and related modifications. Understanding changes in the protein N-termini can be a critical step in achieving personalized medicine. However, most N termini enrichment techniques require a large sample amount (> 1mg proteins). Hence, there is a necessity to improve sample handling strategy for N-terminome analysis on 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.

METHOD(S)
To develop and optimize an N termini enrichment 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).

RESULT(S)
Results from quantitative fluorometric peptide assays suggest that undecanal tagging reaction under 10% acetonitrile overnight was effective and efficient. Based on preliminary results, 1839 N termini from 10,000 HeLa cells (2µg of protein) have been identified in 1 hour of LC-MS/MS analysis time. The number of total peptides, acetylated and dimethylated N termini and their abundance before and after the enrichment are compared. It is found that the abundance of dimethylated N termini increased from 20% to 50% in enriched samples, indicating successful enrichment from current method. The current enrichment strategy has been further applied without bovine serum albumin depletion in bone marrow interstitial fluid to observe proteome changes before and after treatment.

CONCLUSION(S)
A negative enrichment protocol to isolate protein N-termini from as low as 2µg proteins has been developed. This microscale N-terminome analysis will be especially useful in studying rare and precious clinical samples. The improved N termini detection could potentially aid discovering new information related to proteolytic pathways and neo-termini selection as specific drug targets. Therefore, this method is highly valuable in cancer biology research and biomarker development.

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GRANZYME B CLEAVES TENASCIN-C: ROLE IN CHRONIC WOUND HEALING

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BACKGROUND/OBJECTIVES
Chronic wounds, defined as wounds that do not heal within 3 months, affect up to 6.5 million people in the USA alone, costing more than $25 billion per year. Treatments are limited and new therapeutic options are needed. Granzyme B (GzmB) is a serine protease traditionally associated with perforin-dependent, lymphocyte-mediated apoptosis. However, GzmB is now being redefined with additional extracellular roles in inflammation and negative tissue remodeling during wound healing through its role in extracellular matrix (ECM) cleavage. GzmB is significantly elevated in chronic, non-healing wounds including diabetic wounds. Multiple studies have identified ECM that are cleaved by GzmB including decorin, fibronectin, laminin and fibrinogen. Many of these substrates are important for tissue remodeling following injury. Tenascin-C (TN-C) contributes to normal wound healing by augmenting cell migration, proliferation and cellular signaling. In the present study, we investigated whether Tenascin-C is cleaved by GzmB in a murine Apolipoprotein E (ApoE)-KO model of delayed healing. ***Hypothesis*. TN-C is cleaved in chronic wound healing and contributes to impaired healing.**

METHOD(S)
Identification of protease substrates was generated by terminal amine isotopic labing of substrates (TAILS). An in vitro cleavage assay was used to confirm GzmB-mediated TN-C cleavage. TN-C protein levels were determined by immunohistochemistry in excisional wounds in ApoE knockout mice fed on a high fat diet (HFD), providing a model of chronic age-impaired healing, and compared to equivalent wounds in wild-type mice (acute healing model).

RESULT(S)
Human keratinocytes were grown to confluency. Supernatant, from cultured keratinocytes exposed to GzmB, were subjected to analyses to identify substrates cleaved by GzmB. Mass spectrometry quantification of the sample was performed by isotopically labeling N-termini peptides compared to an untreated sample. Bioinformatic analysis of the data eliminated background proteolysis from the targeted cleavage products. TAILS analysis ranked TN-C highly as a substrate. A TN-C cleavage assay confirmed GzmB cleaves TN-C in vitro and this effect was attenuated with GzmB-specific inhibition. Although negligible, TN-C expression was observed in unwounded skin, TN-C was up-regulated at the wound margin of excisional wounds in both HFD ApoE-KO and wild-type mice. However, TN-C expression appeared to be reduced in the HFD ApoE knockout mice wounds, which correlated to an elevation in GzmB expression in the same region of tissue.

CONCLUSION(S)
TN-C is cleaved by GzmB. There is increased TN-C cleavage in chronic compared to acute wounds during wound healing, likely due to increased GzmB-mediated cleavage. Studies are now underway to identify the contribution of GzmB-mediated TN-C cleavage to the healing of chronic wounds.

EXPLORING THE FUNCTIONAL RELATIONSHIP BETWEEN CAPICUA (CIC) AND ATAXIN-1-LIKE (ATXN1L) IN GLIOMA

BACKGROUND/OBJECTIVES
Oligodendroglioma (ODG), a molecularly defined subtype of glioma, is a treatment responsive, slow growing tumour strongly associated with IDH mutation and 1p19q co-deletion. Mutations in CIC, located on chromosome 19q, have been found in up to 70% of IDH mutated, 1p19q co-deleted ODGs; suggesting that loss or altered function of CIC may be crucially associated with ODG's unique biology. CIC and ATXN1L have previously been implicated in neurodegeneration. However, this interaction has not been studied in cancer. Our objective is to better understand the nature of this relationship and their potential role in tumour biology.

METHOD(S)
CIC and ATXN1L interaction was confirmed using immunoprecipitation followed by Western blot. siRNA knockdown of ATXN1L was performed to determine the effect of ATXN1L loss on CIC function. CIC and ATXN1L knockout (KO) cell lines were generated using CRISPR/Cas9 gene editing technology, then underwent gene expression profiling using Affymetrix microarray and differential expression (DE) analysis. DE analysis was also performed on patient lower grade glioma (LGG) RNAseq data publicly available through The Cancer Genome Atlas (TCGA) using samples which harbored aberrations in either CIC or ATXN1L, but not both.

RESULT(S)
Immunoprecipitation confirmed CIC and ATXN1L interaction while siRNA knockdown of ATXN1L resulted in derepression of CIC target genes ETV1/4/5 which was in agreement with our ATXN1L KO cell lines. DE analysis and comparison of differentially expressed genes of CIC KO and ATXN1L KO cell lines resulted in 110 and 299 shared differentially expressed genes between CIC KO and ATXN1L KO cell lines. Further, gene set enrichment analysis of our KO cell lines and patient TCGA ODGs found as part of the LGG cohort converged upon activation of the MAPK pathway and dysregulation of central nervous system development. Lastly, copy number losses of both CIC and ATXN1L were discovered in the remaining portion of TCGA LGG samples. DE analysis of CIC or ATXN1L loss and WT TCGA LGG samples resulted in 132 shared differentially expressed genes and enrichment of gene sets related to cell cycle regulation such as E2F targets and Rb downregulation.

CONCLUSION(S)
CIC and ATXN1L are functional interactors which regulate similar gene sets both in our in vitro cell systems and in TCGA patient data. Loss of either interactor appears to lead to dysregulation of the MAPK pathway in both our KO cell lines and in TCGA ODGs, whereas, in the remaining TCGA LGG samples, loss of either CIC or ATXN1L lead to dysregulation of the cell cycle through upregulation of E2F and downregulation of Rb. Our data suggests that CIC-ATXN1L may be general regulators of the cell cycle and loss of either interacting partner may lead to similar transcriptomic changes.

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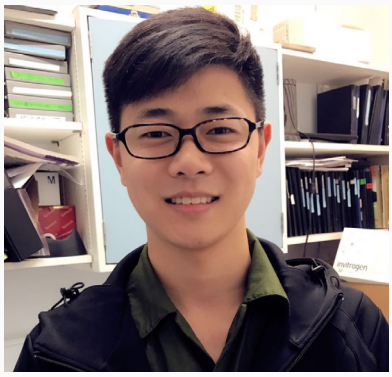

COXSACKIEVIRUS B3-INDUCED REDUCTION OF INTERCALATED DISC PROTEINS IN VIRAL PATHOGENESIS

Supervisor:
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BACKGROUND/OBJECTIVES
Viral myocarditis is an inflammatory disease of the heart muscle, which is a major cause of sudden unexpected death in young patients. Among many types of viruses, coxsackievirus B3 (CVB3) is one of the most common pathogens of this disease. However, the mechanism by which CVB3 induces cardiac damage has not been fully elucidated. Particularly, the involvement of destruction of cardiomyocyte intercalated disks (ICD) by viral infection is still poorly understood. ICDs are substantial connections maintaining cardiac structure and mediating signal communication among cardiomyocytes. Deficiency in ICD components, such as desmosomes, fascia adherens, and gap junctions, leads to heart dysfunction. Our previous research showed that miR-21 is increased by CVB3 infection in cardiomyocytes and we further demonstrated that the CVB3-induced miR-21 triggers cardiac damage by interfering with ICDs. In the experiments, we discovered that several important proteins within ICDs decreased during CVB3 infection and some of them were reduced even more upon miR-21 treatment of the CVB3-infected cells. For instance, alpha-E-catenin were decreased by both CVB3 infection and miR-21 transfection; however, gamma-catenin (plakoglobin) was decreased in CVB3 infection but not by miR-21 treatment alone. Thus, we **hypothesise** that in CVB3 infection, gamma-catenin is decreased by transcriptional inhibition, viral-protease-mediated cleavage or proteasome-mediated degradation, while alpha-E-catenin is decreased due to upregulation of miR-21, which contributes to ICDs destruction and development of myocarditis.

METHOD(S)
We used Western blot to detect the protein levels and potential cleavage products of gamma-catenin and alpha-E-catenin in cells infected with CVB3. Also, proteasome inhibitor MG132 was used to treat the CVB3- infected cells, and then we compare the Western blot result of treated group with that of untreated group to determine the role of protein degradation in the decrease of these two proteins. Meanwhile, cellular RNA was extracted from CVB3 infected HeLa cells, and q-PCR analysis was performed to detect the expression of gamma-catenin and alpha-E-catenin at transcriptional level.

RESULT(S)
Results showed that no cleavage bands of gamma-catenin or alpha-E-catenin were detected in the cell lysis after CVB3 infection; and after treating the cell lines with MG132, the expression level of both alpha-E-catenin and gamma-catenin were increased in both sham- and CVB3-infected cells; The mRNA level of alpha-E-catenin in CVB3 infected cells showed no significant difference compared to the uninfected cells.

CONCLUSION(S)
Decreased protein levels of alpha-E-catenin and gamma-E-catenin in CVB3 infection is, at least in part, due to proteasome-mediated degradation.

AXL RECEPTOR TYROSINE KINASE REGULATES THE EXPRESSION OF APOLIPOPROTEIN E IN HUMAN ASTROCYTES

BACKGROUND/OBJECTIVES
Alzheimer's disease (AD), the cause of 60% to 70% of overall dementia, is a chronic neurodegenerative disease. One of the hallmarks of AD is the accumulation of amyloid plaques in the brain. Apolipoprotein E (apoE), which carries lipids in the brain in the form of lipoproteins, plays an undisputed role in AD pathophysiology. The APOE gene is the most highly associated susceptibility locus for late onset AD, and has well-established associations with amyloid deposition and clearance from the brain. We and others have shown that lipidation of apoE can assist amyloid clearance, raising interest in augmenting apoE function as a proposed therapeutic strategy for AD. As deciphering functions of apoE in brain aging and AD will require research tools to manipulate apoE levels and lipidation, a high-throughput phenotypic screen was conducted using CCF-STTG1 human astrocytoma cell line to identify small molecules that could upregulate apoE secretion. A set of small molecules, Axl receptor tyrosine kinase modulators, has been identified as positive hits. ***The objective of this study is to dissect the mechanism of action (MoA) of Axl modulators to upregulate apoE expression in human astrocytes.***

METHOD(S)
To determine if the Liver X Receptor (LXR) activity is required in apoE upregulation by modulating Axl, LXR knock-out (KO) mouse embryonic fibroblasts (MEF) cells were treated with Axl modulators and apoE mRNA level was determined by RT-qPCR. Immunoblotting analysis was used to confirm the ability of Axl modulators to promote Axl receptor cleavage and stabilize the intracellular domain (ICD). To investigate the role of Axl-ICD in apoE homeostasis, AXL-/- CCF-STTG1 cells were generated using CRISPR-Cas9 method. Various Axl isoforms including WT Axl, kinase-dead Axl mutant, Axl-ICD, and N-terminal fragment of Axl were stably reconstituted in AXL-/- CCF-STTG1 cells. Baseline secretion of apoE across cell lines was measured using human apoE ELISA.

RESULT(S)
Axl modulators were able to significantly upregulate apoE mRNA expression in LXR-KO MEF cells suggesting LXR-independent MoA. The compounds promoted Axl receptor cleavage and ICD production. AXL-/- CCF-STTG1 had significantly lowered apoE baseline compared to WT cells. Reconstitution of ICD-containing Axl isoform in AXL-/- CCF-STTG1 cells significantly elevated apoE baseline expression indicating the critical role of AXL-ICD in apoE homeostatsis.

CONCLUSION(S)
We have identified Axl modulators as LXR-independent apoE agonists. The intracellular domain of Axl has been determined to be the apoE driving force. This study has discovered a novel apoE regulatory pathway which is previously unknown and could potentially provide new insights into the role of apoE in AD.

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EVALUATION AND OPTIMIZATION OF COLD STORED PLATELETS IN TRANSFUSION

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BACKGROUND/OBJECTIVES

For every 4 platelet units collected, 1 unit will be thrown away due to expiration or storage induced lesion. Platelet unit wastage costs our health care system over 5 million dollars per year. Currently, platelet concentrates (PCs) are stored at room temperature (22°C) under constant agitation for seven days. This short storage life is the primary cause of platelet wastage. Furthermore, room temperature stored platelets (RPs) are prone to microorganism contamination and growth. Cold stored platelets (CPs) have been proposed as a potential storage method to extend platelet storage life by limiting microorganism growth and preserving platelet hemostatic function. Recent studies have shown that CPs have superior hemostatic function compared to RPs *in vitro* and *in vivo*. CPs have better responses to agonists, and can form stronger blood clots compared to RPs. However, the effect of cold storage on platelet biology is not well established. In addition, refrigeration is known to drastically reduce the number of platelets during storage, and thus limiting therapeutic effectiveness. Our lab, as well as other groups, has discovered that CPs are sensitive to the specific storage solution, cooling mechanism and storage container. We **hypothesize** that by modulating storage parameters, we can improve the quality of CPs.

METHOD(S)

Platelet concentrates will be produced using standard buffy coat method by Canadian Blood Services. In order to remove the effect of donor variability, we will use a 'pool and split' model. The effect of storage container size and fill volume will be tested. Various sized sterile storage bags will be filled with different volumes of platelets and stored at 4°C. These platelets' *in vitro* characteristics, such as glucose consumption, changes in pH, count and morphology scores, will be compared to CPs stored in standard storage bags as well as RPs. Platelet activation will be assessed by flow cytometry. Rotational thromboelastometry (ROTEM) will be used to assess haemostatic function of the stored platelets.

RESULT(S)

We expect cold storage to have a significant impact on the metabolism of CPs. We will most likely see reduced glucose consumption and smaller changes in pH in CPs compared to RPs. Furthermore, we expect to see an increased platelet activation measured by CD62P expression on the cell surface. ROTEM results should show CPs have more haemostatic activity compared to RPs. By manipulating the storage bag size and fill volume, we hope to see reduced platelet loss during storage in the cold. Furthermore, we hope that the optimum bag size and volume will have a positive impact on the platelet morphology.

CONCLUSION(S)

By exploring platelet storage parameters, we hope to show that CP quality can be improved. This will allow us to develop new storage protocols to be applied in clinics for CP storage. Ultimately we want to demonstrate that by optimizing these storage parameters, we can improve upon the current platelet storage practice by extending storage life and eliminating platelet storage lesion.

SMOKING AND HIGH HIV VIREMIA DAMAGE MITOCHONDRIAL DNA, AND MAY ACCELERATE MTDNA AGING

BACKGROUND/OBJECTIVES
People living with HIV appear to experience accelerated aging. The accumulation of somatic mitochondrial DNA (mtDNA) point mutations is believed to be a marker of biological aging, and has been implicated in many age-associated diseases that manifest prematurely in HIV+ individuals. Current theories of aging describe both de novo mutations, as well as the clonal expansion of pre-existing mutations, as potential mechanisms for the accumulation of mtDNA mutations. In this study, we quantified somatic mtDNA substitutions (low frequency, ≤2%) which may represent de novo mutations, and heteroplasmic mtDNA substitutions (higher frequency, > 2%), that may have resulted from clonal expansion. Both types of mutations were hypothesized to increase with older age, tobacco smoking, and HIV infection.

METHOD(S)
Participants in this cross-sectional study were HIV+ (n = 92, 12 < 19y) and HIV- (n = 72, 13 < 19y) females enrolled in the CARMA cohort, not infected with hepatitis C or B virus, and either current or never (but not past) smokers. Whole blood DNA was extracted and somatic mtDNA substitution mutation rates/10,000bp were quantified via next generation sequencing with primer IDs. Univariate associations between our mtDNA measures (occurrence of heteroplasmy and somatic mtDNA mutations rates) and age, smoking, or HIV were examined, along with other possible confounders Variables important univariately ($p < 0.1$) were considered for inclusion in multivariable models. Age, smoking status and HIV status were included in all models, as per our a priori study design.

RESULT(S)
The raw (median [IQR] (range) blood mtDNA mutation rates for 92 HIV+ (12 < 19y) and 72 HIV- (13 < 19y) individuals aged 1-62 years was 0.52 [0.32 – 0.84] (0.00 – 3.97 mutations/10,000bp. Age was similar between the two groups, as was the number of current smokers. In a model of adult participants (n = 139) that included age, smoking status, and HIV status, being older ($p = 0.003$) and having high peak HIV viremia (≥100 000 copies/ml vs. HIV-) ($p = 0.045$) were independently associated with higher somatic mtDNA mutation rate. With respect to heteroplasmy among all participants (n = 164), there was no association with HIV, but older age ($p = 0.006$) and being a current smoker ($p < 0.001$) was independently associated with the presence of mtDNA heteroplasmy. Interestingly, we observed an interaction between age and smoking whereby non-smokers show an increase in heteroplasmy as they age ($p = 0.004$), while current smokers show a decline ($p = 0.025$).

CONCLUSION(S)
Both somatic and heteroplasmic blood mtDNA substitutions increased with age, which is consistent with the theory of aging and suggest that our assay works. Exposure to high HIV viremia is also associated with somatic mtDNA mutations which may explain accelerated aging in at least some people living with HIV. In contrast, smoking seems to influence the clonal expansion of mutations rather than promote de novo mutations. This too may be in keeping with the knowledge that smoking promotes age-related diseases.

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HIGH-DENSITY LIPOPROTEINS REDUCE AMYLOID-BETA-DEPOSITION IN A NOVEL IN VITRO MODEL OF THE HUMAN BRAIN VASCULATURE

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BACKGROUND/OBJECTIVES
Alzheimer's Disease (AD) is the leading cause of senile dementia with over 44 million affected persons and an economic burden of over $600 billion. Amyloid plaques, consisting of deposited beta-amyloid (Ab), are a neuropathological hallmark of AD. As cardiovascular risk factors increase dementia risk, major pathways that regulate Ab clearance from the brain involve the cerebrovasculature, and most AD patients have cerebrovascular amyloid, it is clear that cerebral vessels play a major role in AD pathogenesis.

METHOD(S)
Here we describe a novel human experimental platform to investigate the cerebrovascular contribution to AD, in which three dimensional perfusible cerebral blood vessels are engineered in a scaffold-directed dynamic pulsatile flow bioreactor system from primary human endothelial cells (EC) and smooth muscle cells (SMC), with or without astrocytes, to form bipartite and tripartite human vessels, respectively. Histological analyses confirm correct vascular anatomy with an EC lumen surrounded by layers of SMC in bipartite vessels. Tripartite vessels include antelumenal astrocytes surrounding the SMC and EC cells.

RESULT(S)
Using this novel biofidelic platform, we showed that high-density lipoproteins (HDL) reduce both Ab accumulation within the tissue and Ab-induced monocyte binding. Interestingly whereas HDL reduce Ab-induced monocyte binding through scavenger receptor (SR)-BI, HDL reduce vascular Ab accumulation independently of SR-BI but rather by maintaining Ab in a soluble state.

CONCLUSION(S)
Taken together, our results establish the utility of human engineered cerebral vessels as highly innovative in vitro platform to study key mechanistic questions relevant to lipoprotein and AD.

TARGETING PRODUCTION OF A FAST-FORMING PROTEOTYPIC PEPTIDE FOR RAPID QUANTIFICATION OF APOLIPOPROTEIN A1 IN PLASMA BY LC-MS/MS

BACKGROUND/OBJECTIVES
Apolipoprotein A1 (apoA1) is the major protein component of high-density lipoprotein particles in plasma, comprising up to 70% of the total protein mass. Clinically, concentrations of apoA1 are used in risk assessment of cardiovascular events. Clinical laboratories commonly use nephelometric or turbidometric methods to measure apoA1, which can be costly, require a relatively large volume of sample and prone to interferences common to immunoassays. An alternative methodology, liquid chromatography–tandem mass spectrometry (LC-MS/MS) has been previously applied to the measurement of apoA1; however, uptake of these methods for routine clinical testing has not been realized due to complex and time-intensive sample preparation workflows. Toward the design of an assay suitable for implementation in a clinical laboratory, we simplified the sample preparation workflow. By optimizing digestion conditions and monitoring digestion profiles of nine apoA1 proteotypic peptides, we previously identified an peptide demonstrating rapid and stable digestion kinetics. Herein we describe the design and validation of a simple and rapid quantitative LC-MS/MS assay targeting this fast-forming peptide.

METHOD(S)
For the external calibrators, the proteotypic peptide was synthesized with concentration assigned by HPLC and amino acid analysis (AAA). We used a C-terminal 13C/15NArg labeled peptide as the internal standard (IS), and human EDTA plasma as quality controls (QC1 = 0.23, QC2 = 0.75, QC3 = 1.07 g/L, values assigned by LC-MS/MS). Samples were diluted in phosphate-buffered saline and added to ammonium bicarbonate buffer containing the IS. Samples were denatured at 99 °C for 10 min. Samples were then cooled to room temperature and N-tosyl-L-phenylalanine chloromethyl ketone treated trypsin was added for digestion at 37 °C for 20 min. All steps are additive and no separation is required. The method validation followed Clinical & Laboratory Standards Institute guidelines, which, briefly, includes assessment of sensitivity, precision, accuracy, linearity, interferences, and stability.

RESULT(S)
Using our rapid digest protocol, proteotypic peptide THLAPYSDELR was selected for quantitation of apoA1. The AAA-assigned calibrators ranged from 0.005 – 0.300 g/L, and with plasma specimens subjected to a 10-fold dilution, the clinically reportable range was 0.05 – 3.00 g/L. QCs were stable through at least 4 freeze-thaw cycles and could be left at room temperature for at least 7 days. Method comparison (n = 40) of our LC-MS/MS method to the Siemens BNII nephelometry assay revealed the following linear regression: LC-MS/MS = 0.70 × nephelometry – 0.09, R2 = 0.9148, CIslope: 0.63,0.78. Studies are ongoing to assign the LC-MS/MS calibrators against the certified reference material. The intra-assay precision was 11.38%, 6.45%, and 7.76% and the inter-assay precision was 12.08%, 5.88%, and 12.29% for QC1, QC2, and QC3, respectively.

CONCLUSION(S)
By streamlining sample preparation and optimizing conditions of denaturation and digestion, we were able to develop a simple and rapid LC-MS/MS method for quantitation of apoA1 in human plasma.

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DEVELOPING SAMPLE PREPARATION WORKFLOWS AND MULTIPLE REACTION MONITORING PARAMETERS IN THE DESIGN OF A MASS SPECTROMETRY METHOD FOR DETECTION OF ALPHA-SYNUCLEIN IN CEREBROSPINAL FLUID

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BACKGROUND/OBJECTIVES
Alpha-synuclein is the primary component of Lewy bodies, which are the major pathological finding in the brains of individuals with Parkinson's disease dementia, dementia with Lewy bodies and associated neurodegenerative disorders. Immunometric analyses suggest that alpha-synuclein in cerebrospinal fluid is a promising biomarker of Lewy body pathology; however, these assays are not appropriate for use in routine care. Our goal is to develop a clinical quality diagnostic tool for quantitation of alpha-synuclein in cerebrospinal fluid. We are using a selective technique called multiple reaction monitoring mass spectrometry to achieve our objective. Alpha-synuclein is present in relatively low abundance (~70 pM) in cerebrospinal fluid and our challenge is to design a workflow with adequate sensitivity.

METHOD(S)
Assay development included: (1) establishing an efficient digestion protocol for recombinant alpha-synuclein, (2) optimization of the multiple reaction monitoring parameters and (3) development of a sample preparation workflow suitable for downstream mass spectrometry analysis. We developed digestion conditions by submitting aliquots of alpha-synuclein in 50 mM ammonium bicarbonate for overnight digestion at 37 °C by either TPCK or sequencing grade trypsin. For optimization of the multiple reaction monitoring assay, we performed precursor ion (Q1) and product ion (Q3) scans. By this approach, an ion is selected, fragmented and product ions are sorted by mass to charge ratio. Transitions were optimized by varying collision energy and declustering potential. For sample preparation workflows, we screened various sample clean-up methods using recombinant alpha-synuclein spiked in ammonium bicarbonate or human cerebrospinal fluid: (i) immunoprecipitation, (ii) solid-phase extraction, (iii) thermal enrichment, (iv) acid precipitation and (v) ammonium sulfate precipitation.

RESULT(S)
We experimentally identified eight tryptic peptides from recombinant alpha-synuclein using our multiple reaction monitoring method. We improved digestion efficiency by 59% by substituting TPCK trypsin for sequencing grade trypsin. Using recombinant alpha-synuclein in ammonium bicarbonate with no enrichment/clean-up, we were able to achieve a lower limit of detection of 11 pM with a signal-to-noise greater than 3 when monitoring tryptic peptides from residues 61-80 and 81-96. The lower limit of detection for recombinant alpha-synuclein spiked into human cerebrospinal fluid was 10,000-fold higher (100 nM), and thus we explored strategies to improve analytical sensitivity. In comparing sample preparation workflows, we found that by immunoprecipitation, we were able to achieve a signal-to-noise of 5 by enrichment of 10 nM recombinant alpha-synclein in human cerebrospinal fluid.

CONCLUSION(S)
We have developed a multiple reaction monitoring assay for alpha-synuclein and we are now exploring alternative enrichment strategies for improving analytical sensitivity in cerebrospinal fluid. Improving sample preparation and analysis is a critical component of assay development and we are currently optimizing the workflow to reach the pM range necessary.

LARGE 7 CM PARATHYROID CYST IN A 14 YEAR OLD BOY: CASE REPORT AND REVIEW OF LITERATURA

BACKGROUND/OBJECTIVES
Parathyroid cyst is rare, with fewer than 300 adult cases and only 7 pediatric cases reported. Most are non-functioning but a minority causes hypercalcemia due to parathyroid hormone (PTH) production. The pediatric clinical differential diagnosis includes branchial cleft cyst and cystic degeneration of thyroid. PTH can be found in the cyst aspirate, but tissue biopsy enables definitive diagnosis

METHOD(S)
A 14 year old boy had 3-month history of a growing left anterior neck mass, with no associated pain, dyspnea, dysphonia, or apnea. Thyroid function tests were normal. Clinical exam showed a 6 cm cystic lesion at the left lateral border of thyroid gland. Ultrasound and computerized tomography showed a simple cyst in the lateral left thyroid lobe, and right tracheal deviation. Left hemithyroidectomy and cyst excision were performed. Intraoperatively, no sinus tract was identified and the left superior parathyroid was not positively identified.

RESULT(S)
Grossly there was a ~7 cm thin-walled cyst with smooth inner lining. Residual thyroid tissue was present superomedially. The cyst fluid was clear and colourless. Microscopically, lining cells were simple cuboidal to low columnar with clear cytoplasm, positive for GATA3 and negative for TTF-1. Conversely, the adjacent mildly compressed benign thyroid epithelium was positive for TTF-1 and negative for GATA3. These findings allowed for definitive diagnosis of benign parathyroid cyst.

CONCLUSION(S)
Large parathyroid cyst is very rare, and its presence in children is even less common and mimics other pediatric cystic neck lesions. Immunohistochemistry for GATA3 and TTF-1 is useful to identify this very rare entity.

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EVALUATION OF A TWO-STEP ALGORITHM FOR THE DIAGNOSIS OF C. DIFFICILE INFECTION IN CHILDREN: IS IT DIFFERENT FROM ADULTS?

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BACKGROUND/OBJECTIVES
We evaluated a two- step algorithm for the diagnosis of *C. difficile* infection (CDI) in children.

METHOD(S)
Based on low CDI prevalence, the study was conducted over two periods; **period 1** (5 months) where samples were tested using both GeneXpert (Cepheid Xpert C. difficile assay) and C. Diff Quik Chek Complete dual-antigen EIA and **period 2** (26 months) where only GeneXpert positive samples were tested using EIA.

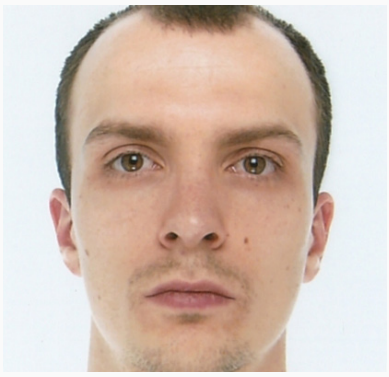
RESULT(S)
During period 1, of a total of 223 samples, 38 were positive by GeneXpert (17%). Using a two-step algorithm, 80% would have been reported based on EIA results (166 negative and 14 positive) with 39 discrepant. Four patients were positive by GeneXpert, but negative by EIA. Clinical review showed that 3 met the CDI case definition, but there were no records for the fourth one.

During period 2, 235 were positive by GeneXpert, but only 221 were available for further testing. By EIA, 89 (40%) were positive, 122 (55%) discrepant and 10 (5%) were falsely negative. Clinical review of the 10 negatives showed that 3 didn't meet the case definition, 4 met the definition and there were no records for the remaining 3.

Compared to GeneXpert, the EIA-antigen, glutamate dehydrogenase (GDH), has a sensitivity of **96%**, positive predicative value (PPV) of **65%**, specificity of **90%** and negative predictive value (NPV) of **97%**. The EIA-toxin has PPV of **88%,** specificity of **99%** and NPV of **88%**, but, unsurprisingly, sensitivity of **40%**.

CONCLUSION(S)
This study demonstrates that, as in adults, the use of C. Diff Quik Chek Complete as an initial screening test for the diagnosis of *C. difficile* infection in children is accurate, rapid and cost effective.

ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR-2 AND NEURONAL PAS DOMAIN PROTEIN 4 EXPRESSION IN CORTICAL NEURONS IS INFLUENCED BY INFLAMMATORY MEDIATORS IN VITRO AND IN MODELS OF MULTIPLE SCLEROSIS



BACKGROUND/OBJECTIVES
MS is a chronic inflammatory neurodegenerative disorder characterized pathologically by alterations in the vasculature, inflammatory infiltrates, demyelination, glial scarring, oligodendrocyte loss, and axonal damage and loss. ARNT2 and Npas4 are both members of the basic helix-loop-helix (bHLH)-Pas transcription factor family that form heterodimers to regulate target gene expression in response to environmental and physiological signals. Npas4 is almost exclusively expressed in neuronal cells and regulates genes that control inhibitory synapse development and synaptic plasticity, a "master switch" to calm down over-excited cells. ARNT2 is largely limited to the CNS and kidney and has been found essential for CNS development and protective in models of ischemia. ARNT2 is recognized to be the major heterodimeric partner of Npas4 in the healthy brain where complexes regulate brain-derived neurotrophic factor (BDNF) transcription, a major mediator of physiological functions including activity-dependent functions, axonal growth, as well as neuronal and axonal survival.Using experimental autoimmune encephalomyelitis (EAE, the animal model of MS), we showed that the expression of ARNT2, NPAS4 and BDNF decreases over the course of the disease in mice and are lowest at peak disease. We hypothesize ARNT2 and NPAS4 influence neuronal function and survival in inflammatory settings by regulating the expression of BDNF.

METHOD(S)
Enriched neuronal cultures from rat embryonic cortices were processed for RNA or examined by immunocytochemistry. Absolute cDNA copy number was determined using standard curves of plasmid containing genes of interest. mRNA levels were normalized to housekeeping genes (β-actin and GAPDH). Npas4/ARNT2/BDNF protein expression were quantified at the single cell level using MAP2 as a neuronal specific marker. Neuronal cultures were treated with stressors and stimuli including H2O2, glutamate and KCl.

RESULT(S)
ARNT2 and BDNF are constitutively expressed in neurons *in vitro* while NPAS4 expression is low or negligible. Both oxidative stress and glutamate, used to model inflammation, have no effect on ARNT2 message but influence protein post-transcriptionally. Npas4 gene and protein expression is driven early and is followed closely by enhanced expression of BDNF. 85-95% siRNA knockdown of ARNT2 RNA and protein in neurons for up to three days has no apparent influence on cell viability or morphology in mature neuronal cultures. Notably, ARNT2 knockdown also has no effect on BDNF message or protein.Npas4 siRNA almost completely abrogates induction of Npas4 RNA in response to a potent excitatory stimulus, potassium chloride (KCl) but also has no effect on steady state BDNF RNA or protein nor the otherwise increased BDNF RNA and protein in response to KCl.

CONCLUSION(S)
The constitutive expression of ARNT2 in neurons is non-essential for neuronal survival in vitro, as BDNF generation is not dependent upon ARNT2 alone nor pairing with Npas4 in adult neurons. Our results emphasize the need to characterize roles for ARNT2 and NPAS4 in maintaining neuronal and axonal health including downstream targets that are independent of BDNF.

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Author Index - Abstract Page

Author Index - Abstract Page

Author Index - Abstract Page

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