THE UNIVERSITY OF BRITISH COLUMBIA

PATHOLOGY DAY
ABSTRACT BOOK

JUNE 1, 2023

– Attention Pathday attendees!

Please note that on June 1st, the DHPLC will be open all day for drop-ins, hosted by Jen Kong and Gang Wang. Although this is not officially a part of Pathday, it is an excellent opportunity to participate in a scavenger hunt activity. Additionally, there are several activities planned within the David Hardwick Pathology Learning Centre, including: four medical mysteries written as short case-based learning, a scavenger hunt, and a talk from 2-3 pm.

Don't miss out on the fun!

Dr. Zu-hua Gao, Professor and Department Head

MESSAGE FROM THE HEAD

As I welcome all of you to this annual Pathology Day, I am also reflecting on the resilience of our outstanding research scientists and academic physicians, our excellent graduate students, resident physicians, postdoctoral and clinical fellows during the COVID-19 pandemic. Despite the resource limitation and ever-increasing competitiveness, our department's grant funding increased from 22.5 million in 2021 to over 33.8 million in 2022 and our number of Canadian Research Chairs increased from 3 to 5.

We published 814 peer-reviewed high impact articles in 2022, and won 22 prestigious research awards. Congratulations to all of you! Your cutting-edge research work is making a huge difference in improving the health of Canadians and beyond and saving lives!

Pathology Day is a significant event in the department calendar as it provides us with an opportunity to showcase and celebrate the wide spectrum of scholarly activities undertaken by our trainees and faculty. This gathering allows us to recognize the outstanding contributions in research and in service given by all members of the department. Pathology Day gives us the chance to connect, socialize, and to share and learn more about each other, as well as gaining an appreciation for the breadth of scholarly activities that take place in our department.

We are very lucky this year to be able to invite two remarkable individuals to participate in the program, Dr. Jayachandran Kizhakkedathu from our department will give the James Hogg Lecture, while Dr. Jana Lipkova, from the University of California is our Keynote Speaker.

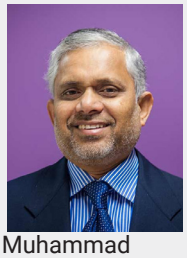
I would like to extend my sincere thanks to the members of the organizing committee for this event, including Dr. Muhammad Morshed, Dr. Catherine Hogan, Dr. Shazia Masud, Tetiana Povshedna, Dr. Tony Ng, Dr. Suzanne Vercauteren, Heleena Mistry, Joyce Zhang, Dr. Spencer Martin, Fiona Zhang and Rana Minab, as well as all the other individuals whose efforts make the day a success.

Hoping you all have a wonderful Pathology Day!

Acknowledgements

Pathology Day is a team effort and we would like to extend our thanks to everyone who contributed to the 2023 edition. Heleena Mistry and Genevieve MacMillan 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.

COMMITTEE 2023


Muhammad Morshed – Co-chair


Catherine Hogan – Co-chair


Suzanne Vercauteren


Shazia Masud


Tony Ng

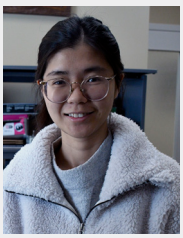

Spencer Martin


Heleena Mistry


Tetiana Povshedna


Joyce Zhang


Jamie Lee


An Li Zhang


Rana Minab

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:

VOLUNTEERS

Staff:

Genevieve MacMillan, Debbie Bertanjoli, Shelby Douglas, Jen Xenakis, Ivy Zhang, Heather Cheadle, Shelley Berkow

Grad Students:

Spencer Martin, Tania Povshedna

Students:

Athena Li (BMLSc), Eva Su (BMLSc), Dana Go, Nadine Plett, Ghazal Sokhanran

Judges:

Abstract Review: Corree Laule, Nevio Cimolai, Shazia Masud, Jefferson Terry, Lucy Perrone, David Grynspan, Sakara Hutspardol, Eric McGinnis, Karina Rodriguez-Capote, Agatha Jassem, Ayesha Vawda

Oral Presentation Judges: Maria Victoria Monsalve, Leandro Venturutti

Poster Presentation Judges: Shazia Masud, Jefferson Terry, Taylor Pobran, Kerstin Locher, Elizabeth L. McKinnon, Natalie Prystajecky, Andre Mattman

thank you

FEATURED SPEAKERS

Keynote Speaker:

Dr. Jana Lipkova, Assistant Professor, University of California / Postdoctoral Researcher, Harvard Medical School

Title: "AI-based Diagnostics in Computational Pathology"

James Hogg Lecturer:

Dr. Jay Kizhakkedathu, Tier 1 Canada Research Chair in Immunomodulation Materials and Immunotherapy, Professor, Pathology and Laboratory Medicine, Medicine, Michael Smith Foundation for Health Research Scholar, Associate Member, Department of Chemistry

Title: "Novel Anti-thrombotic Agents without Bleeding Risk"

Guest Lecturer::

Dr. Ali Bashashati, MSFHR Scholar, Director of AI & Bioinformatics OVCARE, UBC/BC Cancer/VCHRI; Assistant Professor, Dept of Pathology & Laboratory Medicine and School of Biomedical Engineering, UBC; Adjunct Professor, Electrical & Computer Engineering Dept, UBC

Title: "AI in Pathology at UBC: Progress and Outlook for Cancer Biomarker Discovery"

CONFERENCE OUTLINE

TIME	SPEAKER(S) DHCC 1-020
7:55am – 8:05am	WELCOME AND OPENING REMARKS: DR. GAO

ORAL PRESENTATIONS BY STUDENTS & RESIDENTS

GRAD STUDENTS DHCC 1-020		RESIDENTS DHCC Rm. 2263	
8:10am – 8:25am	Emily Kamma	8:10am – 8:25am	Calvin Ka-Fung Lo
8:25am – 8:40am	Ali Khajegili Mirabadi	8:25am – 8:40am	Conor Broderick
8:40am – 8:55am	Forouh Kalantari	8:40am – 8:55am	Karina Chornenka

TIME	SPEAKER(S) DHCC 1-020
9:00am – 9:20am	**Guest speaker:** Dr. Ali Bashashati **Title:** "AI in Pathology at UBC: Progress and Outlook for Cancer Biomarker Discovery"
9:20am – 9:35am	Power Pitch Talks
9:35am – 9:50am	BREAK
9:50am – 10:30am	**James Hogg Lecturer:** Dr. Jay Kizhakkedathu **Title:** "Novel Anti-thrombotic Agents without Bleeding Risk"

ORAL PRESENTATIONS BY STUDENTS & RESIDENTS

GRAD STUDENTS DHCC 1-020		RESIDENTS DHCC Rm. 2263	
10:40am – 10:55am	Honor Cheung	10:40am – 10:55am	Reed Huber
10:55am – 11:10am	Jennifer Cooper	10:55am – 11:10am	Spencer Martin
11:10am – 11:25am	Katlyn Richardson	11:10am – 11:25am	Toby Schmitt

11:35am – 1:35pm	POSTER SESSION & LUNCH - ICORD

ORAL PRESENTATIONS BY STUDENTS & RESIDENTS

GRAD STUDENTS DHCC 1-020		RESIDENTS DHCC Rm. 2263	
1:45pm – 2:00pm	Lauren Forgrave	1:45pm – 2:00pm	Yu-Yu (Tami) Lin
2:00pm – 2:15pm	Michael Lane		
2:15pm – 2:30pm	Ryan Chan		
2:30pm – 2:45pm	John Perrier		

TIME	SPEAKER(S) DHCC 1-020
2:50pm – 3:10pm	**Selected Presentation (PDF):** Farhia Kabeer **Title:** "The evolution of high grade serous ovarian cancer under the pressure of non-cytotoxic and cytotoxic treatment"
3:15pm – 4:15pm	**Keynote Speaker:** Dr. Jana Lipkova **Title:** "AI-based Diagnostics in Computational Pathology"
4:30pm – 6:30pm	**AWARDS PRESENTATION, DRINKS & CANAPES, AND NETWORKING - ICORD**

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ORAL PRESENTATIONS

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2	**Ali Khajegili Mirabadi** (grad student)	GRAPH-STRUCTURED PYRAMIDAL WHOLE SLIDE IMAGE REPRESENTATION Ali Khajegili Mirabadi[1], Graham Archibald[1], Amirali Darbandsari[3], Alberto Contreras-Sanz[4], Ramin Nakhli[1], Maryam Asadi[1], Allen Zhang[2], Peter C. Black[4], Blake Gilks[2], Gang Wang[2,4], Hossein Farahani[1,2], Ali Bashashati[1,2]
3	**Forouh Kalantari** (grad student)	MODELING THE DEVELOPMENT OF CLEAR CELL OVARIAN CARCINOMA (CCOC) USING ORGANOID CULTURE Forouh Kalantari, Dawn Cochrane[2], Yemin Wang[2], Kieran Campbell[2], Germain Ho[2], Winnie Yang[2], Genny Trigo-Gonzalez[2], Maxwell Douglas, Jessica McAlpine[2], David Huntsman[1,2]
4	**Calvin Ka-Fung Lo** (resident)	EVALUATING THE ACCURACY OF THE MBT LIPID XTRACT KIT FOR ASSESSING COLISTIN RESISTANCE IN COMPARISON TO BROTH MICRODILUTION Calvin Ka-Fung Lo[1], Jennifer Bilawka[2], Willson Jang[2], Marc G Romney[1, 2], Aleksandra Stefanovic[1, 2], Christopher F Lowe[1, 2]
5	**Conor Broderick** (resident)	HUMAN ENHANCING TECHNOLOGY IN THE MICROBIOLOGY LABORATORY: ANALYTICAL PERFORMANCE AND VALIDATION OF METASYSTEMS FOR DETECTION OF RESPIRATORY MYCOBACTERIAL INFECTIONS AND IMPLEMENTATION CONSIDERATIONS Claudine Desruisseaux[1, 2], Conor Broderick[2], Kim Sy[1], Gaurav Barot[1], Duang-Jai Garcia[1], Kerstin Locher[1,2], Charlene Porter[1], Mélissa Caza[2], Valery Lavergne[1,2], Marthe K. Charles[1,2]
6	**Karina Chornenka** (resident)	INCRING BRAIN MATURITY IN INFANTS BORN WITH CARDIOTHORACIC DEFECTS USING NEURODEVELOPMENTAL TIME WINDOWS OF HYPOXIC-ISCHEMIC PATHOLOGY Karina Chornenka[1], Christopher Dunham[2]
7	**Honor Cheung** (grad student)	USING LIGHT SHEET MICROSCOPY TO INVESTIGATE THE ROLE OF APOLIPOPROTEIN E4 IN TRAUMATIC VASCULAR INJURY Honor Cheung [1,2], Mehwish Anwer [1,2], Jianjia Fan [1,2], Wai Hang Cheng [1,2], Carlos Barron [1,2], Cheryl L. Wellington [1,2]
8	**Jennifer Cooper (**grad student)	APOE4 CARRIER STATUS MODIFIES ALZHEIMER'S DISEASE PLASMA BIOMARKER CONCENTRATIONS IN HEALTHY ADULTS OVER 85 YEARS OLD Jennifer Cooper[1], Mohammad Ghodsi[1], Sophie Stukas[1], Stephen Leach[2], Angela Brooks-Wilson[2], Cheryl Wellington[1,3,4]
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21	**Rachel Floyd**	SIMULTANEOUS DETECTION AND QUANTIFICATION OF SARS-COV-2, INFLUENZA A, INFLUENZA B, AND RESPIRATORY SYNCYTIAL VIRUS FROM WASTEWATER AS A POPULATION-LEVEL SURVEILLANCE TOOL Jennifer Kopetzky[1], Rachel Floyd[2], Tracy Lee[1], Christine Tchao[1], Frankie Tsang[1], Agatha Jassem[1,3], Sarah Mansour[1,3], Natalie Prystajecky[1,3]
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#1

Emily Kamma

SUPERVISOR(s): DR. JACQUELINE QUANDT

A NOVEL MOUSE MODEL OF PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS BASED ON A FAMILIAL NR1H3 MUTATION PRESENTS WITH AN ALTERED MYELOID PHENOTYPE AND A FAILURE TO RECOVER FROM DISEASE

Emily Kamma
(grad student)

Emily Kamma[1], Pierre Becquart[1], Curtis Wong[1], Carles Vilariño-Güell[2], Jacqueline Quandt[1]

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ABSTRACT

Background/objectives: Multiple sclerosis (MS) is an inflammatory neurodegenerative disease that often develops to a highly disabling progressive phase. A mechanistic understanding of progression is limited by the lack of animal models that recapitulate human progression. We developed a novel mouse model carrying a nuclear receptor subfamily 1 group H member 3 (Nr1h3) mutation identified in two families with severe and rapidly progressive MS. Nr1h3 encodes a transcription factor whose targets include macrophage expressed proteins that regulate reverse cholesterol transport and dampen inflammation. We aim to define the role of Nr1h3 in the pathophysiological mechanisms involved in increased risk, severity, and progression in MS.

Methods: Transcriptome sequencing, immunohistochemistry, and flow cytometry were used to characterize splenic immune cells in heterozygous (HET) and homozygous (HOM) Nr1h3 mutant and wild-type (WT) mice. Enzyme-linked immunosorbent assays were used to assess serum levels of CD5 antigen-like (CD5L), a predominantly macrophage-secreted target of NR1H3 that modulates inflammation and lipid metabolism. Mice were immunized with the MOG 35-55 experimental autoimmune encephalomyelitis (EAE) preclinical model of MS. Mice were assessed for disability, histopathology, and lipid accumulation in the central nervous system (CNS) at peak (day 17) and chronic (day 50). Flow cytometry was used to characterize CNS resident and infiltrating cells at peak EAE.

Results: Nr1h3 HET and HOM vs. WT mice showed lower splenic expression of CD5L, CD163, and CD209b, which are myeloid associated proteins linked to lipid homeostasis and reparative macrophage phenotypes, respectively. In mutant mice, splenic macrophages had reduced CD5L staining, and fewer macrophages expressed CD163 (%WT 14.4±4.1, HOM 5.6±2.8, $p<0.001$) and CD209b (%WT 67.7±3.8, HOM 58.8±8.1, $p=0.003$). Serum CD5L levels were lower in mutant mice (ug/ml WT 5.8±1.6, HOM 1.5±0.8, $p<0.001$). At peak EAE, mutant mice did not have increased disease severity or spinal cord histopathology. However, in the cerebellar white matter, HET mice had increased leukocyte infiltrates ($p=0.02$), activated macrophages/microglia ($p=0.009$), demyelination ($p=0.004$), and axonal damage ($p=0.003$). In the CNS, WT and HET mice had similar composition of lymphocytes, monocytes/macrophages, and granulocytes, but brain myeloid cells in HET mice had a less reparative phenotype shown by reduced CD163 expression on macrophages ($p=0.03$) and microglia ($p=0.045$). At chronic EAE, HOM mice had greater cumulative disability (WT 79.7±10.8, HOM 157.5±14.9, $p<0.01$) and no recovery vs. WT mice. HOM mice had greater spinal cord pathology shown by increased activated macrophages/microglia ($p=0.007$), demyelination ($p<0.001$), and axonal damage ($p<0.001$). HOM mice also had increased Oil Red O+ lipid accumulations in white matter lesions ($p<0.001$), pointing to the failure of macrophages to clear lipid debris and resolve lesions.

Conclusions: The Nr1h3 mouse model shows increased disability, neurodegeneration, and reduced ability to repair, elucidating key myeloid reparatory mechanisms underlying aggressive and progressive disease.

2

Ali Khajegili Mirabadi

SUPERVISOR(s): DR. ALI BASHASHATI

GRAPH-STRUCTURED PYRAMIDAL WHOLE SLIDE IMAGE REPRESENTATION

Ali Khajegili Mirabadi
(grad student)

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ABSTRACT

Background/objectives: Cancer subtyping is one of the most challenging tasks in digital pathology, where recent deep learning models only focus on one magnification or use a fixed hierarchy to use different magnifications in Whole Slide Images (WSIs). Here, our objective is to introduce the first dynamic deep learning model, namely GRASP, that can flexibly zoom in and out between different magnifications in WSIs to emulate pathologists' behavior for identifying tumors in WSIs.

Methods: A graph-based structure is designed to represent the multi-magnification structure of WSIs and then graph convolutional networks are used in this research to design the model. Two datasets of Ovarian Carcinoma and Bladder Cancer are used to evaluate the model: the Ovarian dataset consists of 948 WSIs (484 patients) and the Bladder dataset consists of 262 WSIs (86 patients). To show that the model is reliable, it is compared with eight of the state-of-the-art deep learning models in terms of Balanced Accuracy, F1 Score, Inference Time, and Number of Parameters. Moreover, two pathologists interpret the model's decision-making process.

Results: Our model, GRASP, can dynamically switch across different magnifications in WSIs to look for tumor areas to predict subtypes for WSIs. GRASP outperforms state-of-the-art methods over two distinct cancer datasets by a margin of up to 10% balanced accuracy, while being 7 times smaller than the closest-performing state-of-the-art model in terms of the number of parameters. Furthermore, we show that GRASP understands that depending on patients or subtypes, tumor information is potentially contained in certain magnifications, so it can find informative magnifications for each WSI.

To interpret the result, GRASP's annotations of tumors on small regions of WSIs were confirmed by pathologists. Also, the dynamic of the model for selecting the important magnifications has been confirmed by two pathologists over the two datasets.

Conclusions: GRASP is a dynamic model that can learn multi-magnification interactions in the data, and it has comparably fewer parameters than other state-of-the-art multi-magnification models in the field. GRASP outperforms all of the competing models in terms of Balanced Accuracy over two complex cancer datasets. For the first time in the field, confirmed by two expert genitourinary pathologists, we showed that our model is dynamic in finding and consulting with different magnifications for subtyping two challenging cancers. We hope that the strong characteristics of GRASP and its straightforward structure, along with the theoretical basis, will encourage the modeling of structure-based design in the field of digital pathology for WSI representation.

3

Forouh Kalantari

SUPERVISOR(s): DR. DAVID HUNTSMAN

MODELING THE DEVELOPMENT OF CLEAR CELL OVARIAN CARCINOMA (CCOC) USING ORGANOID CULTURE

Forouh Kalantari
(grad student)

AFFILIATIONS

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ABSTRACT

Background/objectives: Clear Cell Ovarian Carcinoma (CCOC) is an uncommon ovarian carcinoma which accounts for about 12% of all ovarian cancers in North America. CCOC is inherently resistant to chemotherapy. ARID1A, a subunit of SWI/SNF chromatin remodeling complex, is frequently mutated in CCOC. Our lab has discovered inactivating mutations of ARID1A in about 50% of CCOC, often together with a PIK3CA activating mutation. CCOC is strongly associated with endometriosis. ARID1A and PIK3CA mutation occur with high prevalence in precursor lesions adjacent to CCOC suggesting this mutation to be an early event in tumor development. In this project I will use organoid culture of human endometrium to model early initiation events of cancer progression. Organoid culture is a specialized 3D cell culture system that favours the stimulation of progenitor/stem cells to differentiate. We have hypothesized that ARID1A deficiency in CCOC results in temporal epigenetic and transcriptomic alterations along the transformation continuum which may provide insights on the biology of CCOC and suggest potential therapeutic options.

Methods: Organoid cultures derived from dissociated primary normal human endometrium tissue in which ARID1A is knocked down using CRISPR-Cas9, and mutant PIK3CA is introduced using expression lentiviral transductions. For the histology study, organoids were fixed and hematoxylin and eosin (H&E) staining and IHC experiments was performed. We dissociated the organoids and performed single cell RNA and ATAC sequencing using the 10X genomics platform on the organoids. The transcriptomes and chromatin accessibility compared between uninfected, single or double mutant conditions to elucidate the mechanisms underlying oncogenic transformation.

Results: ARID1A/PIK3CA double mutant human organoids demonstrate phenotypic differences compared to the non transduced ones. The mutant organoids are 3 times larger than the uninfected organoids and at later passage, the organoids manifest CCOC histopathology, including hobnail cells in H&E staining. Single cell gene expression profiles from passage1(LogFC 0.84 P= 1.028e-58) and passage6 (logFC 2.23/P=7.29E-33) experiments showed upregulation of S100A4, a known metastasis gene, in the ARID1A knockout cells. These data correlated with the increased accessibility of the chromatin of the S100A4 gene in passage6 of the mutant organoids in single cell ATAC-seq analysis. This was validated in wild type and mutant CCOC cell lines doing western blot analysis. Additionally, the IHC results from CCOC (p value=0.022) and endometrial cancer (p value= 1.8e-06) from tissue microarrays (TMAs) showed significant association between S100A4 and ARID1A, means higher s100a4 expression observed in ARID1A loss in patient derived tissues.

Conclusions: S100A4, a known cancer metastasis gene was one of the genes to be upregulated in transcriptomic data, which has been validated in CCOC cell lines and TMAs. Understanding the biological function of S100A4 protein may lay the groundwork for the development of new therapeutics for CCOC patients.

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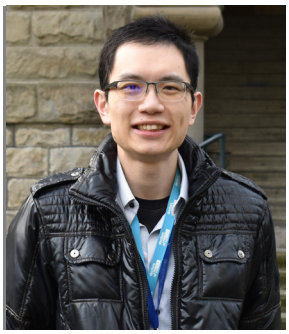

Calvin Ka-Fung Lo

SUPERVISOR(s): DR. CHRISTOPHER LOWE

EVALUATING THE ACCURACY OF THE MBT LIPID XTRACT KIT FOR ASSESSING COLISTIN RESISTANCE IN COMPARISON TO BROTH MICRODILUTION

Calvin Ka-Fung Lo
(resident)

AFFILIATIONS

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ABSTRACT

Background/objectives: Colistin is a bactericidal antibiotic that destabilizes lipopolysaccharide-rich outer membranes of Gram-negative organisms via displacement of calcium and magnesium ions, leading to bacterial death. Current existing susceptibility testing practices for colistin are labour-intensive and time consuming (e.g., broth microdilution (BMD)). MBT Lipid XtractTM Kit on MALDI Biotyper Sirius system can extend application of proteomic analysis to identify specific cell wall modifications associated with colistin resistance. However, this rapid diagnostic modality has been validated on E. coli isolates only.

As the first Canadian pilot study for this rapid resistance detection kit, our purpose is to investigate performance of MBT Lipid XtractTM Kit versus reference standards (i.e., BMD; ComASPTM Colistin, Liofilchem) in detecting colistin resistance. Another objective was to extend validation of rapid colistin resistance testing for various Gram-negative organisms.

Methods: Each isolate was tested twice on MBT Lipid XtractTM Kit for initial run, as per manufacturer recommendation. Isolates were repeated on subsequent run if any discrepancy occurred between MBT and BMD minimum inhibitory concentration (MIC) breakpoint interpretation. Categorical agreement was computed between MBT and BMD MIC interpretation values. Separate analyses were also conducted for non-E. coli isolates, including manual analysis of MALDI-ToF (matrix-assisted laser desorption ionization time-of-flight) spectra. Gram-negative organisms with reported BMD values were included (colistin non-resistance if MIC ≤2, resistant if MIC ≥4 defined by BMD results; CLSI M100 Ed 32, 2022). Organisms intrinsically resistant to colistin (e.g., Morganella spp., Proteus spp., Providencia spp., Serratia spp.) were excluded.

Results: Amidst 36 Gram-negative isolates in database, 78% were either E. coli or K. pneumoniae. MBT Lipid XtractTM Kit testing had 80.6% total agreement (29/36 isolates) with BMD. No identified discrepancies were identified for E. coli (16 isolates) along with E. cloacae complex (3 isolates), C. freundii complex (2 isolates) and P. aeruginosa (1 isolate). Amidst 7 discordant isolates, 6 were from K. pneumoniae. Repeat runs corrected on 5 following isolates (one remained falsely resistant, one had persistently inconsistent readings).

Conclusions: MBT Lipid XtractTM Kit demonstrated 80.6% agreement with BMD and no identified discrepancies for E. coli isolates, which provides promising modality for rapid colistin resistance screening. 85.7% of non-E. coli discrepancies were noted with K. pneumoniae isolates. Limitations included sample size across specific organism species given low frequency of colistin susceptibility testing within routine clinical lab contexts. Larger sample size including real-time clinical specimens is required to assess colistin resistance in non-E. coli isolates, before widespread clinical application.

5

Conor Broderick

SUPERVISOR(s): DR. CLAUDINE DESRUISSEAUX, DR. MARTHE CHARLES

HUMAN ENHANCING TECHNOLOGY IN THE MICROBIOLOGY LABORATORY: ANALYTICAL PERFORMANCE AND VALIDATION OF METASYSTEMS FOR DETECTION OF RESPIRATORY MYCOBACTERIAL INFECTIONS AND IMPLEMENTATION CONSIDERATIONS

Conor Broderick
(resident)

Claudine Desruisseaux[1,2], Conor Broderick[2], Kim Sy[1], Gaurav Barot[1], Duang-Jai Garcia[1], Kerstin Locher[1,2], Charlene Porter[1], Mélissa Caza[2], Valery Lavergne[1,2], Marthe K. Charles[1,2]

AFFILIATIONS

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ABSTRACT

Background/objectives: The rate of active tuberculosis (TB) infection in Canada is amongst the lowest in the world. In 2020, the rate of active TB was 4.7 per 10,000 population. The reference method for screening and diagnosis of TB remains by microscopy and culture. Thus, a significant amount of laboratory resources are deployed daily for diagnostic and for infection control in healthcare settings purposes. Nationally and internationally, laboratories are facing workforce challenges. Innovative solutions are required to continue to support increasing testing volumes in the face of decreasing skilled laboratory technologists.

The primary objective of this study is to establish the analytical performance and to validate the Metafer software from Metasystem (Altlussheim, Germany) on respiratory samples submitted for mycobacteriology screening. The secondary objective was to measure the impact of the level of expertise of the reviewer required to operate the instrument. Lastly, this study sought to establish a framework and considerations checklist for implementation.

Methods: Microscopy slides of respiratory samples: Sputum and tracheal aspirate (n= 104), bronchoalveolar lavage and bronchial wash BAL/BW (n= 145) and pleural fluid (n=37) were archived between August 31st, 2021 and May 25th, 2022. The slides were chosen to represent an array of grading (1+, 2+, 3+, 4+, and negative). A total of 320 slides were scanned using the MetaSystem platform and Neon Metafer AFB Module (version 4.3.130). The images were reviewed by 3 different levels of expert reviewers. The probability threshold (PT) on the instrument was set at 96% and tiles with inferior PT were rejected.

Results: A total of 286 slides were fit for interpretation by Neon Metafer AFB Module for a total failure rate of the instrument of 10.6%. The limit of detection of digital microscopy was similar to manual microscopy for both TB and MAC. The overall agreement between digital microscopy and manual microscopy was 94.4% after discrepant analysis. The positive percentage agreement and negative percentage agreement between digital microscopy and culture was 89.3% (95 CI 80.1-95.3%) and 91.9% (95CI 87.4-95.2%). The two most experienced reviewers had an interrater agreement of 0.838.

Conclusions: Metasystem Mycobacteria Scanner provides automation of reading and classification of AFB smear. In the context of an incidence-low, resource rich setting, this instrument has an acceptable performance for testing of respiratory samples at a PT of 96% combined with an expert reviewer. Further testing will be required to establish the clinical performance of this instrument within a prospective study.

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Karina Chornenka

SUPERVISOR(s): DR. CHRISTOPHER DUNHAM

INFERRING BRAIN MATURITY IN INFANTS BORN WITH CARDIOTHORACIC DEFECTS USING NEURODEVELOPMENTAL TIME WINDOWS OF HYPOXIC-ISCHEMIC PATHOLOGY

Karina Chornenka
(resident)

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ABSTRACT

Background/objectives: Neuroradiologic investigations have demonstrated that cerebral development is delayed by 2-4 weeks in infants suffering from congenital heart defects and congenital diaphragmatic hernia (CHD/CDH). These estimates are based upon application of the "total maturation score" (TMS) system that evaluates brain development using magnetic resonance imaging (MRI) by assessing myelination, cortical gyration, insular development, T1 white matter signal intensity and the involution of the germinal matrix (N Engl J Med 2007;357: 1928-1938, J Pediatr Surg 2012;47:453-461). These infants often require surgical correction of their malformations shortly after birth, but unfortunately some do not survive. Of those coming to autopsy, it is not uncommon to encounter acute hypoxic ischemic injury (HII). Periventricular leukomalacia (PVL) and pontosubicular necrosis (PSN) are manifestations of HII, which are typically encountered in the context of prematurity and occur during specific developmental time windows (i.e., PVL: 24-32 weeks gestational age; PSN: 20 weeks gestational age – 2 months postnatal) (Acta Neuropathol 1995;90:7-10, Acta Neuropathol 2005;110:563-578).

Methods: We examined three individuals born at term with CHD/CDH, ranging in age from 3 to 5 months, who died shortly after surgery.

Results: Neuropathological examination showed evidence of pathology that included acute HII characterized by periventricular leukomalacia (PVL) and pontosubicular necrosis (PSN).

Conclusions: Given the ages of the affected individuals herein, we suggest that the presence of premature-type neuropathology in the form of acute HII could be used to support the hypothesis that infants with CHD/CDH incur delayed brain development. Moreover, based on our observations we propose that the delay in cerebral development could be longer than previous estimates using MRI.

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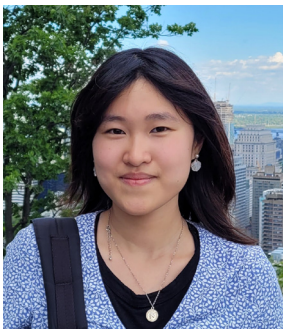

Honor Cheung

SUPERVISOR(s): Dr. CHERYL WELLINGTON

USING LIGHT SHEET MICROSCOPY TO INVESTIGATE THE ROLE OF APOLIPOPROTEIN E4 IN TRAUMATIC VASCULAR INJURY

Honor Cheung
(grad student)

AFFILIATIONS

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ABSTRACT

Background/objectives: Traumatic Brain Injury (TBI) is the leading cause of disability worldwide. In Canada, over 1.5 million people are living with acquired brain injury. Blood brain barrier (BBB) damage is common in moderate to severe TBI, leading to infiltration of neurotoxic blood borne molecules and inflammatory cells, which may increase the risk of cognitive impairment. To better understand traumatic vascular injury, we investigated Apolipoprotein E (ApoE), which exists in three common isoforms: ApoE2, ApoE3 and ApoE4. Compared to ApoE3, apoE4 increases risk of cerebrovascular disease by exacerbating inflammation. However, whether ApoE4 renders TBI patients more susceptible to vascular injury is not known. This project compares the extent of vascular injury after TBI in mice that express either human ApoE3 or ApoE4. We are using cutting-edge tissue clearing technology to render brain samples transparent to enable high-resolution visualisation of 3D blood vessels with less bias than traditional 2D histopathology.

Methods: Male and female mice expressing human ApoE3 or ApoE4 (Cure Alzheimer Fund) were bred in-house and randomized to TBI or sham groups at 4-5 months of age (N=14/group). Animals then underwent TBI procedures using the closed head impact model of engineered rotational acceleration (CHIMERA) model, which delivers a closed head, surgery free injury with unrestricted head rotation. TBI animals received a single 2.6J impact while sham controls received no impact. To enable 3D mapping of vascular damage after TBI, mice were perfused with Alexa Fluor 594 conjugated wheat germ agglutin (WGA) upon 1 day after injury, whole brain were bisected to enable both 2D sectioning and 3D tissue clearing in one animal. Hemibrain tissue were subsequently cleared using the SHIELD passive clearing protocol per commercial instructions and imaged using light sheet microscopy at 5X magnification.

Results: CHIMERA TBI resulted in significant increase ($p<0.0001$) in loss of righting reflex duration, a measurement of loss of consciousness among TBI animals compared to sham groups. We established a labeling and imaging pipeline for the 3D vasculature. Pilot analysis of ApoE3 animals revealed dramatically altered vascular network after TBI compared to sham controls. Further, we established an analytical pipeline for 3D quantification of vascular density and branching. 2D immunofluorescent assessment of the vascular basement membrane component collagen IV revealed an overall increase of branching among ApoE4 animals compared to APOE3 animals regardless of TBI status ($p=0.025$), which suggests aberrant angiogenesis.

Conclusions: Our preliminary findings establish tissue clearing as an efficient tool for the assessment of vascular injury sustained after TBI. We are currently generating more 3D imaging results to test for ApoE3 and ApoE4 isoform differences in vascular injury after TBI.

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Jennifer Cooper

SUPERVISOR(s): DR. CHERYL WELLINGTON

APOE4 CARRIER STATUS MODIFIES ALZHEIMER'S DISEASE PLASMA BIOMARKER CONCENTRATIONS IN HEALTHY ADULTS OVER 85 YEARS OLD

Jennifer Cooper
(grad student)

Jennifer Cooper[1], Mohammad Ghodsi[1], Sophie Stukas[1], Stephen Leach[2], Angela Brooks-Wilson[2], Cheryl Wellington[1,3,4]

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ABSTRACT

Background/objectives: Many blood-based biomarkers have been thoroughly investigated in the context of Alzheimer's disease (AD) and other dementias for the purposes of screening, diagnosis and prognosis. However, few studies have examined these biomarkers in healthy seniors in the absence of dementia or other age associated morbidities. Investigating biomarkers in a healthy geriatric population allows for the identification of novel biological associations that might otherwise be masked by presence of disease. This study will investigate the effect of APOE genotype on plasma biomarkers that are established markers of AD pathologies. We aim to determine whether carrying the APOE4 allele, which increases risk of AD, modifies biomarker concentrations in healthy seniors. Plasma biomarkers analyzed include: amyloid beta 42/40 (Aβ42/40), reflecting the pathology of amyloid plaques; phosphorylated tau-181 (p-tau-181), reflecting the formation of neurofibrillary tangles; neurofilament light (NF-L), reflecting axonal damage observed in neurodegeneration; and glial fibrillary acidic protein (GFAP) reflecting neuroinflammation through astrocyte activation.

Methods: 370 plasma specimens were obtained from the Super Seniors study, which enrolled participants ≥85 years old who have never been diagnosed dementia, cancer, diabetes, cardiovascular or major pulmonary disease. Plasma biomarkers were analysed on the Quanterix Simoa HD-X analyzer using commercial Neurology 4-plex E and p-tau-181 assays. Group comparisons were performed using a Mann-Whitney test for continuous variables and a Fisher's exact test for categorical variables. The association between APOE genotype and biomarker concentration was analyzed using multivariable linear regression.

Results: 80 (22%) participants were APOE4 carriers (E3E4 N=71, E4E4 N=3, E2E4 N=6) and 290 (73%) were non-carriers (E2E2 N=3, E2E3 N=58, E3E3 N=229). No significant differences were found between APOE4 carriers and non-carriers in age (p=0.3215), sex (p>0.9999), or mini mental state exam scores (p=0.1327). In APOE4 carriers, Aβ42/40 is lower (0.0596 vs 0.0618 pg/ml, p=0.0462), and p-tau-181 (3.24 vs 2.78 pg/ml, p=0.0069) and GFAP (196 vs 172 pg/ml, p=0.0474) are higher than in non-APOE4 carriers. NF-L had no significant difference between carrier status (p=0.1188). After adjusting for age and sex, p-tau-181 remains significantly associated with APOE4 carrier status (β=0.066, p=0.0112), while Aβ42/40 and GFAP no longer have significant associations.

Conclusions: Plasma p-tau-181 concentration is associated with APOE4 genotype in healthy seniors, making it an important variable to account for in all future analysis of this biomarker.

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Katlyn Richardson

SUPERVISOR(s): Dr. DAVID GRANVILLE

GRANZYME K IN PSORIASIS: NOVEL MECHANISMS UNDERLYING EPIDERMAL HYPERPLASIA AND CUTANEOUS INFLAMMATION

Katlyn Richardson
(grad student)

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ABSTRACT

Background/objectives: Psoriasis is a chronic inflammatory disease that currently affects over one million Canadians. It is characterized by increased epidermal hyperplasia (thickening) and skin inflammation forming red, scaly skin plaques. Activation of the IL-23/IL-17 pro-inflammatory signaling pathway is a hallmark of psoriasis that is key to its clinical management. This inflammatory axis is maintained by keratinocytes and a complex web of immune cells. During active disease, stimulation of keratinocytes promotes increased cellular proliferation, leading to epidermal hyperplasia. In the dermis, predominant infiltrating immune cell populations include IL-23-producing macrophages. Still, knowledge regarding the mechanisms underlying the activation of keratinocytes and immune cells remains incomplete, averting potential for affordable, efficacious therapeutic measures. Previously, we demonstrated that serine protease Granzyme K (GzmK) is elevated in inflammatory skin conditions, as well as exacerbates epidermal hyperplasia and cutaneous inflammation in corresponding murine models of disease. We hypothesized that increased GzmK levels contributes to psoriatic disease severity through augmenting epidermal hyperplasia and cutaneous inflammation.

Methods: GzmK levels were assessed in human skin with and without psoriasis. The functional roles of GzmK were evaluated in the imiquimod-induced murine model of psoriasis, comparing GzmK knockout (GzmK KO) to wild-type (WT) mice. Psoriasis-like skin severity was scored using a modified Psoriasis Area and Severity Index. Pro-proliferative and pro-inflammatory markers were examined in murine skin sections and findings were validated using human keratinocytes and macrophages in vitro.

Results: GzmK was elevated 856% in human psoriasis skin compared to unaffected controls ($p \leq 0.05$), primarily within the immune cell infiltrate. In vivo, GzmK KO mice exhibited a 26% (auc $p \leq 0.05$) decrease in psoriasis severity compared to WT mice throughout the experiment. GzmK KO mice exhibited a 25% reduction in epidermal thickness compared with WT mice at day 7 ($p \leq 0.01$). Within the epidermis of GzmK KO mice, keratinocytes displayed a 63% reduction in staining for pro-proliferative marker Ki67 compared with WT mice ($p \leq 0.01$). In the dermis, GzmK KO mice exhibited a 37% reduction in inflammatory cell infiltrate (total number of leukocytes) compared to WT mice at day 7 ($p \leq 0.01$). More specifically, there was a 74% decrease in the number of macrophages in GzmK KO mice compared to WT mice ($p \leq 0.05$). Accordingly, levels of pro-inflammatory cytokines (IL-1, IL-6, IL-17, IL-23) which are key to IL-23/IL-17 axis induction were also decreased in GzmK KO mice compared to WT mice. In vitro, GzmK induced keratinocyte proliferation and macrophage secretion of IL-23, implicating roles for GzmK in augmenting epidermal hyperplasia and sustaining a pro-inflammatory phenotype.

Conclusions: GzmK is elevated in human and murine psoriasis and contributes to disease onset and progression by promoting epidermal hyperplasia and cutaneous inflammation. Inhibition of GzmK may represent a novel therapeutic approach for treating psoriasis.

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Reed Huber

SUPERVISOR(s):

TERT PROMOTER MUTATIONS IN ATYPICAL MELANOCYTIC LESIONS: A SERIES OF SEVEN CASES WITH ADVERSE MELANOMA-SPECIFIC OUTCOME

Reed Huber
(resident)

Reed Huber[1], Jonathan Lee[1], Lisa Borretta[1], Basile Tessier-Cloutier[2], Amy Lum[3], Stephen Yip[1], Basil A Horst[1]

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ABSTRACT

Background/objectives: The majority of melanocytic proliferations can be readily categorized as benign or malignant based on histologic assessment under the microscope by a trained dermatopathologist. However, a subset of lesions, termed Atypical Melanocytic Proliferations (AMPs), are histologically ambiguous, leading to possible diagnostic error and suboptimal treatment. Mutations in the promoter region of the catalytic subunit of telomerase, telomerase reverse transcriptase (TERT), are commonly found in melanomas but are rare in melanocytic nevi. In this study, we aimed to determine the prevalence of hot spot TERT promoter (TERT-p) mutations in AMPs with adverse melanoma-specific outcome.

Methods: We performed a search of our regional laboratory database in Greater Vancouver between January 2003 and December 2018 to allow for at least 2 years of clinical follow-up. Cases with adverse outcome were then manually identified by chart review, defined as new melanoma diagnosis at initial biopsy site or metastatic melanoma from an unknown primary lesion. Corresponding H&E stained slides were reviewed by a dermatopathologist to confirm original diagnoses and suitable residual tissue for mutational analysis. DNA was isolated from FFPE tissue and using allele specific PCR for TERT-p hotspot mutations, we investigated promoter regions of initial AMP lesions.

Results: Our database search identified over 330 cases diagnosed as AMPs for subsequent chart review. Eight cases were manually identified which resulted in adverse outcome as defined above, seven of which included sufficient residual material for analysis, and three of seven cases (43%) showed hotspot TERT-p mutations.

Conclusions: The prevalence of TERT-p hotspot mutations in ambiguous lesions remains understudied due to limited case numbers, but may be estimated at approximately 5%. Our results suggest that histologically ambiguous lesions with adverse outcome are enriched for TERT-p mutant tumors. The rate of TERT-p hotspot mutations in our study of AMPs with adverse outcome of 43% is comparable to the mutation frequency reported in melanomas, and is significantly higher than that reported in nevi. Limitations of the present study include retrospective design and relatively small case numbers, a major factor being the infrequency of events in question.

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Spencer Martin

SUPERVISOR(s): Dr. ANDREW CHURG

DIFFERENTIATING MALIGNANT PLEURAL MESOTHELIOMAS FROM BENIGN MESOTHELIAL HYPERPLASIA USING BAP1, MTAP, AND MERLIN (NF2) IMMUNOHISTOCHEMISTRY

Spencer Martin
(resident)

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AFFILIATIONS

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ABSTRACT

Background/objectives: Malignant mesothelioma is a rare but deadly disease with patient median survival of about 12 months. Histologically differentiating malignant mesotheliomas versus benign mesothelial proliferations is challenging but carries profound clinical ramifications. Since identification of mesothelioma can be difficult using morphology alone, reliable immunohistochemical (IHC) markers that provide high specificity and sensitivity for making this distinction are needed. Currently, loss of BAP1 and/or MTAP by IHC are specific markers of mesothelioma, but they fail to identify many cases. NF2 (which encodes Merlin) mutations or deletion are common in mesotheliomas, and one recent study detected lost IHC staining of NF2/Merlin in mesothelioma. We hypothesized that IHC for Merlin would increase sensitivity for identifying mesothelioma compared to using IHC for BAP1 and MTAP alone.

Methods: Previously, we had performed IHC for BAP1 and MTAP on a tissue microarray (TMA) containing epithelioid and sarcomatoid mesothelioma cases and reactive mesothelial proliferations. Here, we additionally performed IHC using two separate Merlin antibodies, and we assessed for loss of Merlin alone, BAP1 alone, MTAP alone, and combinations of these markers.

Results: Analyzed as single stains, BAP1 loss was observed in 47% of epithelioid and 11% of sarcomatoid tumors, and MTAP loss was observed in 22% and 33%, respectively. Merlin loss was identified in 33% of epithelioid mesotheliomas and 39% of sarcomatoid tumors. Of all two-stain combinations, loss of BAP1 and/or Merlin had the highest sensitivity in epithelioid mesotheliomas at 69%, and MTAP and Merlin had the highest sensitivity in sarcomatoid mesothelioma at 56%. By using all three IHC stains, 75% of epithelioid and 61% of sarcomatoid mesotheliomas were correctly detected. All reactive mesothelial proliferations showed positive staining for all three markers.

Conclusions: Our results demonstrate that adding IHC for Merlin to IHC for BAP1 and MTAP increases sensitivity for differentiating malignant and reactive mesothelial proliferations without compromising specificity. Merlin IHC has promise for improving diagnostic accuracy for these challenging cases.

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Toby Schmitt

SUPERVISOR(s):

HEMATOXYLIN AND EOSIN-LIKE COLOURIZATION OF BLACK AND WHITE HISTOLOGY IMAGES USING DEEP LEARNING

Toby Schmitt
(resident)

AFFILIATIONS

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[1]University of British Columbia, Vancouver, British Columbia, Canada

ABSTRACT

Background/objectives: Black and white micrographs are frequently found in older scientific papers and are occasionally present in online pathology learning resources. These are more difficult to interpret than colour images and are of more limited educational value. Machine learning methods have been applied to black and white photographs to produce realistic-appearing colour images, but these models perform poorly on black and white micrographs.

Methods: A deep learning model was trained to generate a colour image resembling hematoxylin and eosin (H&E) staining based on a black and white image. Forty-five low-power H&E images from publicly-available virtual slides (University of Michigan Histology and Virtual Microscopy) were segmented into a total of 975 tiles of 256x256 pixels each. Corresponding greyscale images were generated for each tile using the OpenCV imaging library. This data was used to train a generational adversarial network (GAN) for 100 epochs based on the pix2pix model (https://github.com/junyanz/pytorch-CycleGAN-and-pix2pix). After training completion, ten 256x256 pixel black and white images were colourized using the model. These artificially colourized images, along with ten genuine images of H&E-stained slides of the same size, were shown to two senior anatomic pathology residents who were asked to classify the images as genuine or artificially-generated.

Results: The first anatomic pathology resident flagged six of the ten artificial images and three of the ten genuine images as artificial. The second resident flagged four of the ten artificial images and six of the ten genuine images as artificial.

Conclusions: This model produces artificially-colourized images that cannot be reliably distinguished from genuine H&E-stained images. In addition to colourization of black and white images, the model may be used to restore older slides with faded staining.

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Lauren Forgrave

SUPERVISOR(s): Dr. MARI DEMARCO

PROTEOLYTIC FRAGMENTS OF TDP-43 ARE DIAGNOSTIC BIOMARKERS FOR FRONTOTEMPORAL DEMENTIA

Lauren Forgrave
(grad student)

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ABSTRACT

Background/objectives: The ability to diagnosis TDP-43 pathology prior to autopsy is highly desired given the challenge to differentiate frontotemporal dementia with TDP-43 pathology (FTD-TDP) from phenotypically related disorders like Alzheimer's disease. Unfortunately, the concentration of TDP-43 is not a useful biomarker as previous studies found TDP-43 concentration had no discriminatory power for FTLD-TDP. Post-translational modifications of TDP-43 have been associated with TDP-43 pathology, however, this has been done via low resolution techniques (i.e., Western blot). Studies aimed at targeting these disease-specific TDP-43 proteoforms report inconsistent findings, which may be attributed to the low sample size, lower-resolution instrumentation, and lack of appropriate controls. To advance biomarker efforts and improve characterization of disease-specific TDP-43 proteoforms, we performed mass spectrometry analysis of brain tissue in the largest cohort to date.

Methods: High resolution mass spectrometry was used to determine TDP-43 proteoform composition in frontal lobe brain tissue from immunohistochemically confirmed FTLD-TDP (n= 13), related dementias (i.e., Alzheimer's disease and FTLD-tau without TDP-43 deposits; n=10) and neuropathologically unaffected controls (n=3). Brain tissue was homogenized and subjected to gel electrophoresis, where molecular weight regions were excised to tie molecular weight back to the identified TDP-43 proteoforms. Each fraction was subjected to HRMS to identify the TDP-43 proteoforms that had the best discriminatory power for FTLD-TDP. Biomarker findings were verified in a blinded study, using an independent mass spectrometry method in a larger cohort of FTLD-TDP (n=24), non-TDP dementias (i.e., Alzheimer's disease, FTLD-tau, and dementia with Lewy bodies; n=24), and unaffected cases (n=3).

Results: Quantitative analysis revealed the concentration of TDP-43 proteoforms with truncated primary sequences relative to physiological TDP-43 was significantly increased in FTLD-TDP cases compared to controls ($p < 0.0001$). In the discovery phase, truncated TDP-43 separated FTLD-TDP cases from related dementias and unaffected controls with 85% sensitivity and 100% specificity. The verification phase revealed similar findings, with truncated TDP-43 differentiating FTLD-TDP cases from controls with 83% sensitivity and 89% specificity.

Conclusions: This is the first study to perform both biomarker discovery and verification for a TDP-43 pathology specific marker in FTLD-TDP. Herein, we identify as subset of truncated TDP-43 proteoforms with a molecular weight <28 kDa as the most promising biomarker of TDP-43 pathology. Next, TDP-43 proteoforms <28 kDa should be explored in other biospecimens, with the aim of translating this assay into an ante-mortem biomarker test for TDP-43 pathology.

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Michael Lane

SUPERVISOR(s): Dr. David Granville

GRANZYME B MEDIATES DEGRADATION OF DERMAL-EPIDERMAL JUNCTION PROTEINS IN STEVENS-JOHNSON SYNDROME AND TOXIC EPIDERMAL NECROLYSIS

Michael Lane
(grad student)

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ABSTRACT

Background/objectives: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening, immune-mediated, cutaneous adverse drug reactions with a paucity of effective treatments. Pathology is characterized by the separation of the epidermis from the dermal layer of the skin resulting in severe blistering and peeling. Due to the high mortality rate and susceptibility to infection and moisture loss, patients are often admitted to the Burn Clinic to manage their symptoms. Through degradomic and other approaches, we have recently identified that serine protease, extracellular Granzyme B (GzmB) proteolytic activity contributes to the onset and progression of bullous pemphigoid, the most common autoimmune blistering condition in the elderly. Dermal-epidermal junction proteins Alpha6/Beta4 integrin, Collagen VII, Collagen XVII, and Laminin-alpha5 were previously identified as GzmB substrates. In the present study, we hypothesize that GzmB degrades key proteins that anchor the epidermis to the dermis in SJS and TEN and an extracellular GzmB inhibitor can inhibit this degradation.

Methods: Skin biopsies (n=8) collected from SJS/TEN patients were analyzed by immunohistochemistry (IHC) to detect protein levels of GzmB and its substrates, Collagen XVII, Alpha6/Beta4 integrin, Collagen VII, and Laminin-Alpha5, compared to healthy skin (n=8).

Results: IHC analysis of skin biopsies from SJS and TEN patients revealed significantly elevated levels of GzmB in the epidermis and dermis compared to healthy skin. IHC analysis of SJS/TEN sections exhibited reduced Alpha6/Beta4 integrin, Collagen VII, and Collagen XVII at the dermal-epidermal junction compared to normal skin. IHC staining of Laminin-Alpha5 is in progress. Studies investigating cell sources of GzmB in patient SJS/TEN samples, GzmB levels and activity in patient blister fluids, and inhibition of blister fluid GzmB activity with an extracellular GzmB inhibitor are ongoing.

Conclusions: SJS and TEN are life-threatening conditions that affect individuals of all ages, and there is a lack of effective therapies available. The present study will provide new insights into the pathological mechanisms of SJS and TEN by investigating the role of extracellular GzmB in the degradation of dermal-epidermal junction proteins. The identification of GzmB as a potential therapeutic target and the evaluation of VTI-1002 as a potential therapeutic option could lead to the development of novel and more effective treatments for SJS and TEN.

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Ryan Chan

SUPERVISOR(s): Dr. BRIAN KWON

HISTOPATHOLOGICAL ANALYSIS OF CAVITY FORMATION AND EARLY SYRINGOMYELIA IN A PORCINE MODEL OF SPINAL CORD INJURY

Ryan Chan
(grad student)

Ryan Chan[1,2], Sigrún Jarlsdóttir[2], Jing Wang[2], Kitty So[2], Neda Manoucheri[2], Femke Streijger[2], Brian Kwon[1,2]

AFFILIATIONS

[1] UBC Department of Pathology and Laboratory Medicine, [2]ICORD (International Collaboration on Repair Discoveries)

ABSTRACT

Background/objectives: Spinal cord injury (SCI) affects over 85000 Canadians, with 1800 cases reported annually. Along with characteristic motor and sensory deficits, there are many debilitating chronic effects of SCI, like respiratory illness and urinary dysfunction. One poorly understood symptom is posttraumatic syringomyelia (PTS), the chronic formation of longitudinal cystic cavities in the spinal cord, called syrinxes, causing pain, weakness, and sensory deficits. PTS pathophysiology is unknown, with leading theories from histopathological analysis of small animal models pointing to irregularities in cerebrospinal fluid (CSF) flow. The porcine model is a closer approximation of the human spinal cord, used to analyze surgical interventions currently employed in PTS treatment, such as expansile duraplasty and direct cavity drainage. Therefore, studying PTS pathogenesis in a porcine model may reveal translationally-relevant results. We hypothesize that the porcine model is an accurate histopathological model for posttraumatic syringomyelia.

Aims: Characterize histopathological syrinx presentation in the porcine spinal cord and evaluate similarity to human data. Determine how injurious factors and current treatments for posttraumatic syringomyelia in humans influence syrinx development in the porcine model.

Methods: Laminectomy at the T10 vertebral level was performed on female Yucatan minipigs (n = 36), followed by a contusion injury by falling impactor, with females used to accommodate a simultaneous urodynamics study. Dura mater in certain animals (n=18) was removed and an expansile duraplasty was performed. Cords were harvested 12 weeks post-injury and sectioned axially at 20 µm. Eriochrome cyanide and hematoxylin and eosin staining was used to assess demyelination and tissue damage. Images were captured by semi-automated Brightfield microscopy with the Zeiss Axio imager M2 of axial sections spaced 200 µm apart across the cord. Syrinx and damaged tissue area were quantified using Zeiss ZEN 3.4.

Results: Preliminary data from non-duraplasty cords show similarities between porcine cord cavities and early human syrinx presentation, in that cavity volume was higher caudal to the epicenter of impact than rostral. Likewise, multiple cavities are distributed at the same axial level outside of the central canal in the porcine cord, as seen in early human PTS. Unlike in small PTS animal models, the porcine model readily develops syrinxes post-injury without optimization required.

Conclusions: Volume and distribution of cavitation in the porcine spinal cord is comparable to human PTS, supporting its use as a model for PTS. By providing an early-stage model of PTS, the porcine model reveals a previously unexplored stage in PTS pathology prior to human symptom presentation. Further analysis of this stage may inform theories of syrinx formation mechanisms. The next step of this study is to use this model to assess factors that influence PTS progression, such as CSF flow variation and duraplasty.

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John Perrier

SUPERVISOR(s): Dr. Ed Pryzdial

ROLE OF COAGULATION INHIBITORS IN INFECTION AND PATHOLOGY OF THE FAMILIES CORONAVIRIDAE AND RETROVIRIDAE

John Perrier
(grad student)

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ABSTRACT

Background/objectives: Aberrant blood clots are the leading cause of death world-wide and are observed in the final stages of most diseases. Viruses affect the clotting pathway, many enhancing clot formation. In order to carry their life cycle they must enter a host cell. When they replicate and exit the cell, many virus types coat themselves with the host cell membrane forming an envelope. Furthering our understanding of how viral infection of the cell and blood clotting are connected, the Pryzdial lab discovered that tissue factor (TF), an integral host membrane protein that initiates blood coagulation, is integrated into the envelope of oral herpes and is required for infection in vivo. Can both viral infection and a pathological hyper-coagulant state be simultaneously targeted? To address this question, the importance of envelope TF across two diverse enveloped virus families will be investigated, Coronaviridae and Retroviridae. In the current project, effects of coagulation inhibitors on Human Coronavirus 229E (HCoV-229E)- and HIV-mediated clotting and cell infection will be assessed with a final goal of using gene editing tools and intracellular inhibitors to demonstrate that envelope TF is pivotal in viral infection and pathology. The hypothesis will be addressed that envelope TF confers broad-spectrum viral coagulation activity and enhances infection in vitro. These data will further support the concept that any virus with an envelope can acquire TF, providing novel antiviral therapeutic opportunity.

Methods: HCoV-229E was propagated in human hepatoma-derived Huh7 cells. HIV was propagated in human embryonic kidney 293T cells (HEK293T). Sucrose gradient ultracentrifugation was used to purify virus. Immunogold electron microscopy and/or immune blot were used to characterize TF antigen associated with the virus and host cells. The enzymatic cofactor function of TF on the purified virus surface will be evaluated by chromogenic and plasma clotting assays, and the effect of coagulation proteases produced along the TF-axis will be studied for effects on infectivity bioassays in vitro. Mechanism will be dissected using specific therapeutics and gene editing tools.

Results: Recent unpublished data from our lab shows that lab strain-purified HIV elicited TF cofactor activity, accelerating purified clotting factor VIIa-mediated factor X activation and shortened concentration-dependant plasma clotting time. Under conditions enabling generation of factors VIIa, Xa and thrombin, TF on HCoV-229E and HIV is expected to enhance cell infection. I demonstrated that these purified viruses and the cells from which they emerge indeed have TF antigen associated with them to explain the functional data.

Conclusions: To identify why viral infection may affect the blood clotting system we have identified TF associated with HCoV-229E and HIV. Further studies will define the effect of TF-axis clotting proteases on HCoV-229E and HIV serving as the framework for future broad-spectrum antiviral therapy.

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Yu-Yu (Tami) Lin

SUPERVISOR(s): Dr. ANDREW SEAL

THEODOR BILLROTH: SURGEON, MUSICIAN, AND COMPOSER

Yu-Yu (Tami) Lin
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ABSTRACT

Background/objectives: Music and surgery have a long and shared history, and the existence of musician-surgeons has been well documented. Best known for his two gastric reconstruction operations, Theodor Billroth was also a skilled musician and made valuable contributions to the field of music theory and composition. While he was a prolific composer during his lifetime, most of his works have unfortunately been lost. His one surviving composition ***Todessehnsucht*** offers insight into his composition process and serves as a testament to the amalgamation of his personal life, his career as a surgeon, and his lifelong love of music.

This presentation seeks to analyze Billroth's only musical composition from a historical, musical, and sociopolitical perspective and to illustrate the close interplay between science and art in one of the most quintessential musician-surgeons in history.

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Sanaz Ashraf Nouhegar

SUPERVISOR(s): Dr. Honglin Luo

INVESTIGATING THE ROLE OF EXOSOMES EXTRACTED FROM COXSACKIEVIRUS B3-TREATED BREAST CANCER CELLS AND THEIR CONTENT IN CANCER IMMUNOTHERAPY: AN ANALYSIS OF THEIR IMMUNOMODULATORY EFFECTS

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(undergraduate)

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ABSTRACT

Background/objectives: Increasing evidence suggests that exosomes originating from cancer cells have the ability to transport immunomodulatory cargo, which can change the behavior of immune cells in the microenvironment of the tumor. Exosomes are small vesicles that cells release into the extracellular space and can be taken up by neighboring cells. They contain various bioactive molecules that can affect cell behavior and signaling patterns. Furthermore, the CVB3 virus has previously been studied for its oncolytic properties in cancer immunotherapy and has shown promise as a potential treatment option. The purpose of this study is to investigate the potential impact of exosomes obtained from CVB3-treated breast cancer cells and their contents on immunomodulatory agents. Specifically, we examine how the contents of these exosomes affect the expression of some of the key immunomodulatory molecules.

Methods: The study uses using various in vitro assays such as western blots, immunohistochemistry (IHC), and flow cytometry on breast cancer cell lines and their exosomes. To culture the cells, we will use standard cell culture protocols, and we will follow established procedures for infecting the cells with the CVB3 virus and exosome extraction. Exosomes extracted from CVB3-treated breast cancer cells and their protein contents are analyzed to determine the expression of immune-infiltrating proteins and immune-activating molecules in exosomes.

Results: Our study successfully confirmed the effects of CVB3 infection on 4T1 cell exosomes, and we plan to expand this to include at least two additional triple-negative breast cancer cell lines. So far, we observed significant differences in the expression of immunomodulatory proteins in exosomes following infection with CVB3. Specifically, we found that CVB3 caused a decrease in the expression of proteins that play a significant role in immune suppression in cancer. Additionally, we observed an increase in the expression of certain proteins with immune-activating roles in the exosomes following infection. These findings suggest that CVB3 virus infection may have an impact on the immunomodulatory properties of exosomes derived from breast cancer cells, which could potentially enhance the immune response against the tumor. Therefore, we aim to extend this study to different human breast cancer cell lines to investigate the generalizability of these findings and further elucidate the underlying mechanisms. The findings of this study will contribute to a better understanding of the complex interplay between cancer cells and the immune system. By understanding the role of exosomes and their cargo in this dynamic, this research may have implications for the development of novel immunotherapy strategies targeting triple-negative breast cancer. Therefore, this study has the potential to impact the field of cancer immunotherapy and advance our understanding of the mechanisms underlying cancer immunity.

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Tyrone Borja

SUPERVISOR(s): Dr. SAKARA HUTSPARDOL

COMPARISON OF ROTATIONAL THROMBOELASTOMETRY AND CONVENTIONAL COAGULATION TESTS IN IDENTIFYING TRAUMA-INDUCED COAGULOPATHY DURING MASSIVE HEMORRHAGE PROTOCOL

Tyrone Borja
(undergraduate)

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ABSTRACT

Background/objectives: Trauma-induced coagulopathy (TIC) resulting from massive hemorrhage is potentially fatal but preventable if recognized early. Evidence has shown that viscoelastic hemostatic assays such as rotational thromboelastometry (ROTEM) are sensitive and guide goal-directed transfusion but comparatively expensive. We believe ROTEM can identify TIC more rapidly than conventional coagulation tests (CCTs). We therefore evaluated TIC-defining parameters using ROTEM and CCT results at Vancouver General Hospital (VGH), the primary trauma centre in British Columbia, Canada, to determine their correlation with blood component utilization and clinical outcomes during trauma-based massive hemorrhage protocol (MHP) activations.

Methods: This was a retrospective observational study of trauma patients who received transfusion as per 1:1:1 ratio-based MHP at VGH from June 1, 2020, to May 31, 2022. Patient characteristics, CCT and ROTEM results, transfusion data and mortalities at 24 hours and 28 days were collected from patient and blood bank disposition records. We defined TIC based on institutional algorithms using ROTEM and CCT transfusion triggers in the MHP, including: (a) ROTEM results of Extem A10 < 40 mm, Extem CT > 100 sec, Extem maximum lysis (ML) > 10%, Fibtem A10 < 10 mm; and (b) CCT results of INR > 1.8, PTT > 1.5 times of upper normal limit, platelet count < 50 x 109/L, and Clauss Fibrinogen level < 1.5 g/L. Lab findings of TIC were correlated with blood component utilization and mortality using univariate analysis. Continuous variables were compared using an independent t-test.

Results: Sixty-eight patients underwent CCT and ROTEM testing during a trauma MHP. Thirty-one patients (46%) did not have TIC defined by initial CCT and/or ROTEM results. Twenty-four patients (35%) presented with abnormal ROTEM alone, and 13 patients (19%) had both abnormal CCTs and ROTEM. Of 55 patients with no additional blood components suggested by CCTs, the median number of red blood cells (RBC), frozen plasma (FP), platelet units and grams of fibrinogen concentrate (FC) transfused within the first 4 hours of MHP was significantly higher in patients who had abnormal initial ROTEM (median RBCs: 7.8 vs 4.3, FP: 5.5 vs 2.6, platelets: 1.3 vs 0.4, FC: 4.2 vs 1.4; $p<0.05$). Comparing fibrinogen results, 20 of 68 patients (29%) had hypofibrinogenemia based on fibrinogen < 1.5 g/L by CCTs vs 30 of 68 patients (49%) by ROTEM within the first 24 hours of MHP. Patients with hypofibrinogenemia per CCT had significantly higher 24-hour and 28-day mortalities compared to those with fibrinogen > 1.5 g/L [24h: 6/20 (30%) vs 4/48 (8%), $p = 0.028$; 28d: 11/20 (55%) vs 8/48 (17%), $p = 0.001$]. However, patients with hypofibrinogenemia based on Fibtem A10 (ROTEM) had no significant difference in 24-hour and 28-day mortalities [24h: 6/33 (18%) vs 4/35 (11%), $p = 0.507$; 28d: 10/33 (30%) vs 9/35 (26%), $p = 0.673$], but received significantly higher transfusion requirements within the first 4 and 24 hours of MHP compared to the normal Fibtem A10 group. Finally, all blood component requirements within the first 4 hours of MHP were significantly higher in patients with a CCT fibrinogen level of 1.5-1.9 compared to those greater than 1.9 g/L (all p-values<0.05).

Conclusions: ROTEM, particularly FIBTEM A10, may be more sensitive in identifying hypofibrinogenemia and TIC than CCTs. There was a significant association between increased blood component usage in patients with TIC defined by ROTEM but not by CCTs, and Clauss fibrinogens lower than 1.9 g/L. Thus, TIC identified by ROTEM and higher cut-offs for Clauss fibrinogen may be more sensitive indicators for transfusion, thereby benefitting patient outcomes, but this requires further study.

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Fares Burwag

SUPERVISOR(s): Dr. Alberto Delaidelli

ELUCIDATING MECHANISMS OF ENHANCED OXIDATIVE PHOSPHORYLATION IN MYC-AMPLIFIED MEDULLOBLASTOMA

Fares Burwag
(undergraduate)

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ABSTRACT

Background/objectives: Brain tumours are the deadliest type of childhood cancer, and medulloblastoma (MB) accounts for almost two thirds of pediatric brain tumors. Even with current standards of treatment, the 10-year survival rate of Group 3 MBs (the subtype we are currently studying) is around 50% for cases that occur in children. Additionally, treatment tends to leave children with long-lasting developmental impacts including difficulties in speech, learning, hearing, and hormone functions. This underscores a need to identify new therapies that improve both short-term and long-term patient outcome. Due to their rapid growth, tumours need high quantities of nutrients; however, increased nutrient supply to cancer cells is not always possible for the organism. Our laboratory team has identified that eukaryotic elongation factor-2 kinase (eEF2K) helps brain cancer cells survive in the absence of nutrients. Interestingly, high expression of eEF2K is associated with poor patient survival in Group 3 medulloblastoma. Based on in vitro studies, our team has identified that eEF2K also plays a substantial role in the expression of mitochondrial proteins involved in oxidative phosphorylation. Our team is currently trying to tease out how this function may play a role in mediating tumour resistance to nutrient deprivation.

Methods: D425 eEF2K knockouts were generated using CRISPR-Cas9. Protein expression data is based on pulseSILAC DIA proteomics and RNA-seq. To study the impact of OXPHOS protein expression on mitochondrial complex assembly, we utilized Blue Native PAGE. Gels were followed up with gel-derived mass spectrometry to analyze proteins at high molecular weight bands. Colorimetric assays were used to probe mitochondrial complex activity in addition to common dyes such as JC-1 (membrane potential) and mitoSOX (mitochondrial ROS production). siRNA experiments were used to determine the impacts of knocking down OXPHOS proteins to induce eEF2K -/- phenotype in control cell lines.

Results: eEF2K knockdown strongly impacts the expression of mitochondrial proteins in Group3 medulloblastoma cells when exposed to nutrient deprivation compared eEF2K expressing cells. Based on blue native gels, knockdown cells showed a significant decrease in mitochondrial supercomplex assembly, an effect that was absent in nonMYC-amplified MB cells. This was reflected in gel-derived proteomics when applied to gel bands containing supercomplexes. These knockdown effects were reversed in rescue cells, but not eEF2K kinase dead mutants. Based on colorimetric assays, the knockdown of eEF2K functionally decreases mitochondrial complex activity (I, III, and IV) and this is reflected in the cell's ability to generate mitochondrial membrane potential. Upon knocking down COA7, which is one of the proteins that is differentially expressed in eEF2K-/- cell lines, we observed that supercomplex assembly was significantly impeded as observed in eEF2K -/- cells.

Conclusion: Based on the above results we conclude that eEF2K plays a critical role in mediating high efficiency oxidative phosphorylation in myc-amplified medulloblastoma through the formation of mitochondrial supercomplexes.

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Rachel Floyd

SUPERVISOR(s): Dr. NATALIE PRYSTAJECKY

SIMULTANEOUS DETECTION AND QUANTIFICATION OF SARS-COV-2, INFLUENZA A, INFLUENZA B, AND RESPIRATORY SYNCYTIAL VIRUS FROM WASTEWATER AS A POPULATION-LEVEL SURVEILLANCE TOOL

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(undergraduate)

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ABSTRACT

Background/objectives: Wastewater testing has been demonstrated to be an important surveillance tool during the COVID-19 pandemic. This study aims to evaluate the feasibility of monitoring wastewater for multiple respiratory viruses using a single assay. We adapted and optimized a clinical assay for the detection of SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV) for use in wastewater testing. After validation, the assay was then applied retrospectively to archived wastewater samples from the Metro Vancouver area.

Methods: To adapt the clinical assay for use in wastewater testing, two targets were removed, probe dyes were reassigned, a passive reference dye was added, and external standard curves were generated. Primer and probe concentrations were optimized and assay suitability was examined by: (1) determining the lower limits of quantification, (2) assessing potential competition between multiplexed targets, and (3) evaluating trends between wastewater results and clinical data from September 2021 to June 2022.

Results: (1) SARS-CoV-2, influenza A, and RSV were all consistently detected (≥90%) at 20 copies/reaction. Influenza B was consistently detected (100%) at 5 copies/reaction. (2) The presence of a highly concentrated target (2000x-4000x) did not significantly affect the Ct values of weaker targets. The coefficients of variation of Ct values between weak targets with and without the presence of a highly concentrated competing target were, on average, below 3.5%, and were considered negligible. Importantly, (3) preliminary analyses indicated that wastewater quantifications of influenza A, influenza B, and RSV from September 2021 to June 2022 trended with clinical data.

Conclusions: The molecular multiplex assay developed in this study was able to reliably detect and quantify SARS-CoV-2, influenza A, influenza B, and RSV in wastewater. Monitoring multiple respiratory viruses within a single assay maximizes wastewater testing capacity and provides public health officials with unbiased and consistent population-level data on the incidence of SARS-CoV-2, influenza, and RSV within local communities.

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Vivian Gusmao

SUPERVISOR(s): Dr. Tracy Tucker

PATTERNS OF GENETIC TESTING AND DETECTION OF PATHOGENIC VARIANTS IN OVARIAN CANCER PATIENTS IN BRITISH COLUMBIA

Vivian Gusmao
(undergraduate)

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ABSTRACT

Background/objectives: Ovarian cancer (OVCA) is a major contributor to cancer-related deaths in women. Approximately 15% to 20% of OVCA cases are caused by germline changes in BRCA 1/2 genes. Poly (ADP-ribose) polymerase inhibitor (PARPi) treatment has been found to improve the survival rate of advanced OVCA patients with BRCA pathogenic variants, either somatic or germline. The purpose of this study was to analyze the genetic testing patterns in OVCA patients and the rate of pathogenic variant (PV) detection in OVCA susceptibility genes, both in germline and tumour testing. The study also assessed the time interval between both tests and the mean age of patients with a BRCA PV, based on the testing strategy employed. Results of the study will help clinicians make informed decisions regarding genetic testing for patients with advanced OVCA.

Methods: This was a retrospective chart review for 920 OVCA patients with tumour and/or germline report dates between January 1, 2018, and December 31, 2021. The study analyzed next-generation sequencing data and identified PVs associated with OVCA risk in genes including ***BRCA1, BRCA2, BRIP1, CHEK2, PALB2, ATM, MLH1, MSH2, MSH6, PMS2, RAD50, RAD51D, RAD51C,*** and ***TP53***.

Results: The overall detection rate of PVs in high-risk genes was 20.1% in 920 patients. Of the 855 patients who underwent germline testing, 18.4% had a pathogenic germline variant (PGV). For the 338 patients who received both tumour and germline testing, the overall detection rate of PVs was 21.9%, with 16.3% (55/338) having a PGV and 5.6% (19/338) having a pathogenic somatic variant (PSV) in a high-risk gene. Out of the 55 PGVs detected, 39 (70.9%) were identified by tumour testing. All PGVs in BRCA genes were also identified by tumour testing, except for a deletion of exons 1-6 in BRCA1, as the tumour test assay is unable to identify copy number variants. PGVs in BRCA1/2 genes were detected in 10.7% (36/338) of patients, while 5.3% (18/338) had PSVs in BRCA genes that would have been missed by germline testing alone. Thus, these 18 patients would not have been eligible for PARPi treatment if a germline-only testing strategy was followed.Germline testing was typically ordered first, with an average interval of 41 ± 161 days between tests. There was no significant age difference between patients who received both tests or germline testing alone, and tested positive for a BRCA PV.

Conclusions: Based on these results, 5.3% of patients with a BRCA PV who received both tests would not have been eligible for PARPi if a germline-only testing strategy was followed. Overall, the findings emphasize the significance of comprehensive genetic testing strategies in OVCA patients, as a way to improve management and guide personalized treatment decisions.

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Kate Halverson-Kolkind

SUPERVISOR(s): Dr. JAYACHANDRAN KIZHAKKEDATHU

POLYMER CONJUGATES FOR TARGETING AND TREATING GLYCOCALYX DYSFUNCTION IN INFLAMMATORY CONDITIONS

Kate Halverson-Kolkind
(undergraduate)

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ABSTRACT

Background/objectives: Endothelial cells are coated in a delicate layer of sugar-protein polymers, collectively known as the glycocalyx, which provides a protective interface between tissue and blood. Although the glycocalyx functions in maintaining normal vascular physiology, it is fragile and sheds in response to inflammation, high blood pressure, hyperglycemia, and ischemia. Glycocalyx shedding contributes to many inflammatory and immune-mediated diseases, including diabetes, stroke, sepsis, and transplant rejection. With their prevalence anticipated to increase, glycocalyx dysfunction and treatment have become topics of interest. We aim to apply cell surface engineering techniques to target and rebuild depleted glycocalyx, thereby restoring normal vascular physiology. This will be achieved through the systemic administration of highly biocompatible polymer conjugates that bind to and mimic the natural glycocalyx. Here, we explore the feasibility of antibody-mediated binding of therapeutic polymer conjugates to the endothelial cell surface.

Methods: Antibodies directed towards perlecan (aPLC), syndecan-1 (aSYN), and intracellular adhesion molecule-1 (aICAM) were evaluated as possible targeting units. As a proof of concept, antibodies were functionalized with mPEG-maleimide (mPEG-mal) as a model polymer. Antibodies were reduced with dithiothreitol (DTT) to expose free thiols for the subsequent modification with mPEG-mal. The antibody-polymer conjugates were assessed by SDS-PAGE. Cell assays were performed on EA.hy962 human umbilical vein endothelial cells to determine the binding efficacy of functionalized antibodies. Cells were incubated with the antibody-polymer conjugate, which was detected by an anti-PEG antibody. A final incubation with fluorescently labeled antibody allowed for signal detection by both flow cytometry and confocal microscopy. To evaluate the efficacy of functionalized antibodies to treat glycocalyx dysfunction, inflammatory conditions were modeled in vitro. Cells were stimulated with lipopolysaccharide or tumor necrosis factor alpha (TNFa) to induce glycocalyx shedding prior to performing the cell assays.

Results: Optimal parameters for antibody functionalization and in vitro detection were established. Successful functionalization of aPCL and aICAM was confirmed by SDS-PAGE. The detection of functionalized aICAM and aPCL was confirmed by both flow cytometry and confocal microscopy, with aPCL providing the highest detection signal. On endothelial cells activated by TNFa, the detection of PEG-functionalized anti-ICAM was significantly higher compared to non-inflammatory conditions.

Conclusions: Our findings show the successful modification of glycocalyx-targeting antibodies with a PEG- polymer and the subsequent conjugate-mediated delivery to the endothelial glycocalyx. The data generated will further help to develop the cell surface engineering approach for the treatment of inflammatory and immune-mediated diseases.

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Johnny Huang

SUPERVISOR(s): Dr. Cheryl Wellington

THE EFFECT OF APOLIPOPROTEIN E GENOTYPE ON INTENSIVE CARE UNIT INTERVENTIONS AND OUTCOMES IN CRITICALLY ILL PATIENTS WITH COVID-19

Johnny Huang
(undergraduate)

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ABSTRACT

Background/objectives: *APOLIPOPROTEIN E (APOE)* is a multifunctional lipoprotein with key roles in the brain like development, maintenance, and injury repair. It has 3 major alleles, APOE2, APOE3, and APOE4 which have different structures and functions. Existing literature suggests that having the APOE4 allele instead of APOE2 or APOE3 may be a significant risk factor for Sars-CoV-2 infection and resultant COVID-19 severity. However, the findings are mixed and there is limited data on critically ill patients requiring admission to the intensive care unit (ICU). Concurrently, emerging evidence suggests that COVID-19 is associated with acute and chronic neurological injuries which requires more research to understand. This study aims to determine if the APOE4 allele impacts the frequency of life saving measures, neurological complications, and mortality in COVID-19 patients in the ICU.

Methods: COVID-19 patients were prospectively enrolled from the Vancouver General Hospital ICU. Data including demographics, co-morbidities, and ICU interventions applied such as mechanical ventilation, venovenous extracorporeal membrane oxygenation, and continuous renal replacement therapy were collected. Neurological complications were identified based on signs of brain ischemia or hemorrhage from head computed tomography (CT) scans. APOE genotype was determined using qRT-PCR on extracted genomic DNA from buffy coat samples. The outcomes were separated by ***APOE4*** carriership and compared with the Fisher's exact test or the Mann-Whitney U test.

Results: Out of 237 ICU COVID-19 patients enrolled, 57 (24%) were APOE4 carriers and 180 (76%) were non-carriers. There is no significant difference in the age (56y vs 59y; p = 0.23) or sex (74% male vs 62% male; p = 0.57) between the carrier and non-carrier groups. Similarly, there is no significant difference between co-morbidities, presenting symptoms, ICU interventions, ICU stay length, 28d mortality, or total mortality. However, ***APOE4*** carriers had a significantly higher prevalence of neurological complications compared to the non-carriers (30% vs 17%; OR = 2.18, CI = 1.07 – 4.43, p = 0.047).

Conclusions: Although ***APOE*** genetic status did not affect demographic, interventions required, or mortality of critically ill COVID-19 patients, APOE4 carriers are more susceptible to neurological complications like ischemia and hemorrhage by almost two-fold based on CT scan findings. These observations can help us understand the underlying risk factors for neurological complications due to COVID-19. Replicate studies with larger sample sizes to confirm these findings would be valuable, particularly ones that assess the long-term impact of neurological complications on ICU patients.

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Ava Keshavarzsafiei

SUPERVISOR(s): Dr. AUDI SETIADI

DATABASE-GUIDED ANALYSIS FOR IMMUNOPHENOTYPIC SCREENING OF INBORN ERRORS OF IMMUNITY AT BC CHILDREN'S HOSPITAL

Ava Keshavarzsafiei (undergraduate)

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ABSTRACT

Background/objectives: Inborn Errors of Immunity (IEI) are heterogeneous group of disorders resulting from genetic defects that impair one or more components of the immune system. Flow cytometry is a useful technique to screen for IEI but can be challenging due to a lack of age-specific reference ranges for lymphocyte subsets, proper standardization, and individual variations. Difference in sample processing, antibody panels and gating strategies can greatly impact the quantitation of lymphocyte subsets. The objective of this study is to explore new tools for multiparametric data analysis to allow for the recognition of immunophenotypic patterns of various lymphocyte subsets.

Methods: Blood samples from 20 adults and 41 children without immune-related disorders were immunophenotyped according to the standardized clinical protocol at BC Children's Hospital. The subjects were separated into 5 age groups: newborn, 1-5 years old (YO), 6-10 YO, 11-16 YO, and adults (>16 YO). Proportions of the lymphocyte subsets and automatic population separator (APS) plots were analyzed using Infinicyt 2.0 software for the following T-cell subsets: CD4+ and CD8+ Naïve, Central Memory (TCM), Effector Memory (TEM), Terminal Effector Memory Cells with CD45RA (TEMRA), CD57+, PD1+ T-cells, and B-cell subsets (Naïve, Unswitched Memory, Switched Memory, Plasmablasts, Transitional, and CD21low38low B-cells). Patient samples with known genetic diagnosis of IEI were analyzed and classified into the following phenotypic patterns in a software-generated database: 1) Abnormal T cell distribution, 2) B cell maturation defect, 3) Immune dysregulation, and the accuracy of the database will be evaluated in a prospective validation study.

Results: 95% confidence intervals were obtained for the 18 lymphocyte subsets. CD4+ and CD8+ TEM, TCM, and TEMRA populations increased in proportion from birth to adulthood. CD4+ and CD8+ PD1+ and CD57+ cell proportions also showed an increasing trend with age, compatible with increased T-cell exhaustion/senescence. In contrast, naïve CD4+ and CD8+ T-cells and B-cells decreased from birth to adulthood. CD21low38low, switched, and unswitched memory B-cells increased from birth, peaked at 1-5 YO, and plateaued in adulthood. Transitional B-cells are most abundant at birth, decreased with age, and comprised a minority of B-cells in adulthood. IEI with abnormal T cell distribution pattern was generally characterized by reduced naïve T-cells, increased PD1+ T-cells, and/or increased CD57+ T-cells. IEI with B-cell maturation defect was associated with reduced switched memory with or without increased transitional B-cells, and immune dysregulation disorders often showed increased CD21lowCD38low B cells and/or increased PD1+ T cells.

Conclusions: This study provides essential data to interpret lymphocyte subset immunophenotyping profiles at different age groups. We showed that reference images, APS plots, complemented with local database-guided analysis are useful tools for IEI screening in our center, especially those with subtle immunological abnormalities.

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Sebastian Kondratowski

SUPERVISOR(s): Dr. Jonathan Bush

IDENTIFICATION OF H3K27ME3 AND H3S10T11PHOS AS POTENTIAL BIOMARKERS IN PEDIATRIC OSTEOSARCOMA

Sebastian Kondratowski (undergraduate)

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ABSTRACT

Background/objectives: Osteosarcoma (OS), the most common primary bone tumour, is often defined as having a non-recurrent and heterogeneous genetic profile. Most OS patients are treated with conventional chemotherapy and subsequent resection, allowing for the evaluation of the neoadjuvant response, which may facilitate ongoing therapeutic choices. Although 5-year overall survival rates have been static for those without metastatic disease, those with or who subsequently develop metastatic disease have markedly worse outcomes. Identifying potential biomarkers that may impact event-free and overall survival could better support future clinical trials attempting to improve the outcomes in OS. One potential area that may be explored is epigenetic modifications in OS, such as the methylation pattern at various histone residues. Particular methylation studies using immunohistochemistry (IHC) have found both diagnostic and prognostic significance. Tri-methylation at lysine 27 of histone H3 (H3K27me3) has been associated with gene repression, and loss of tri-methylation may allow for tumour growth. H3K27me3 can be used in the diagnosis of malignant peripheral nerve sheath tumours and may be prognostic. Limited studies have investigated the role of histone modifications in OS. We aim to characterize the methylation and phosphorylation status in OS using cited histone markers found in primary diagnostic biopsies and compare them to relevant clinical outcomes. We also aim to capture any changes to the epigenetic landscape of OS by comparing the primary tumour histone marker patterns to their paired metastases or recurrences.

Methods: We constructed four tissue microarrays from 58 primary cases, and 54 related metastatic neoplasms, with tissue blocks available from 2002-2022. The clinical charts were reviewed for post-therapy response, development of metastasis, and overall survival. Non-OS samples were also included in the TMAs, ranging from normal bone and bone marrow, cartilage, and other tumours within the spectrum of OS differential diagnoses. We evaluated 6 histone H3 residues using IHC, including H3K4me3, H3K9me3, H3K27me2, H3K27me3, H3S10T11phos, and H3S28phos. Tumour cores were scored on a dichotomous scale of low (<25%) and high (≥25%) tumour nuclei staining using QuPath cell detection software.

Results: H3K27me3 immunoexpression was associated with post-therapy tumour necrosis response. Diagnostic biopsies that showed low H3K27me3 nuclear staining were associated with poor treatment response (<90% necrosis) at the time of definitive excision ($P<0.05$). Loss of H3S10T11phos expression at the first event tumour compared to the primary tumour was also observed ($P<0.05$). Survival trends were also revealed with loss of H3S10T11phos in the primary tumour.

Conclusions: This pilot study identified H3K27me3 and H3S10T11phos as potential biomarkers for OS which may predict a poor neoadjuvant response and signify epigenetic changes to gene expression in metastases and recurrences, respectively. Although studies with a larger cohort are needed for substantiation, these results support the expanded evaluation for risk stratification of other histone markers.

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Cecilia Lee

SUPERVISOR(s): Dr. LEANDRO VENTURUTTI

CHARACTERIZING TOLL-LIKE RECEPTOR 7 AND TOLL-LIKE RECEPTOR 9 EXPRESSION IN DIFFUSE LARGE B CELL LYMPHOMA

Cecilia Lee
(undergraduate)

Cecilia Lee[1,3] , Abhimanyu Minhas[2,3], Melissa Ferrad[3], Leandro Venturutti[3,4]

AFFILIATIONS

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ABSTRACT

Background/objectives: Diffuse large B cell lymphoma (DLBCL), the most common lymphoid malignancy among adults, is stratified into molecular subtypes, defined by recurring mutations. One particular subtype, known as "MCD" DLBCL, has a 5-year survival rate of just ~25%, compared to ~65% for all DLBCL cases. Currently, all patients receive the same chemoimmunotherapy regime, independent of subtype. The disparity in outcomes highlights a critical need for studying subtype-specific mechanisms of DLBCL pathogenesis and progression, to guide therapeutics. MCDs are characterised by a gain of function mutation (L265P) in the Toll-like receptor (TLR) signalling adaptor MYD88. MYD88.L265P constitutively activates NF-kB signalling, which is critical for tumour cell survival. TLRs are innate immune receptors that recognize conserved pathogenic antigens, but can also become aberrantly activated to drive B cell dysregulation. For example, TLR7 and TLR9 have been implicated in the pathogenesis of autoimmune disorders. However, it remains unclear whether these play a role in MCD transformation or progression. As a first step, here, we sought to characterise TLR7 and TLR9 expression in MCD precursor and fully transformed cells.

Methods: To characterise TLR expression in MCD precursor cells, mice expressing MYD88. L252P (murine L265P equivalent) in activated B cells or WT controls (n=4) were immunised with the T-cell dependent antigen sheep red blood cells, and sacrificed at the peak of the adaptive immune response. Spleens were harvested, and TLR7/TLR9 expression was analysed in activated B cells using flow cytometry. In order to study TLRs expression in fully transformed MCD cells, RT-qPCR was conducted on human DLBCL cell lines (n=10) and primary normal B cell samples (n=10). Flow cytometry was conducted on the same DLBCL lines to assess expression at the protein level. Lastly, TLR7/TLR9 expression was analysed by RNAseq in two independent primary DLBCL cohorts, BCCA (n=300) and NCI (n=481).

Results: TLR7 was found to be significantly upregulated in MCD precursor cells compared to wildtype activated B cells ($p<0.05$), while TLR9 expression was significantly downregulated ($p<0.01$). RT-qPCR of transformed DLBCL cell lines showed significantly higher TLR9 expression compared to normal B cells ($p<0.001$) or to other DLBCL subtypes ($p<0.01$, all groups). Comparatively, TLR7 expression was not upregulated in the MCD group compared to normal B cells or other DLBCL subtypes. Flow cytometry data showed similar relationships at the protein level. Lastly, RNAseq data analysis in clinical cohorts showed a trend of higher TLR9 expression in MCDs, while TLR7 was not upregulated.

Conclusions: Our work shows that TLR7 is upregulated in MCD precursor models, while TLR9 appears upregulated in fully transformed MCDs. This suggests a switch in expression, and potentially dependency, in TLR7/TLR9 at some point along MCD transformation. Future studies will explore the functional roles of these receptors through selective activation and inhibition experiments. These findings may provide insight into therapeutically targeting TLRs in MCD tumours.

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Phoebe Lu

SUPERVISOR(s): Dr. Mari DeMarco

STRUCTURAL CHARACTERIZATION OF NEURODEGENERATION-ASSOCIATED PROTEINS IN FRONTOTEMPORAL DEMENTIA AND ALZHEIMER'S DISEASE

Phoebe Lu
(undergraduate)

Phoebe Lu[1], Lauren M. Forgrave[1], Kyung-Mee Moon[2], Yun Li[1], Leonard J. Foster[2], Ian R. A. Mackenzie[1,3], Meng Wang[1], Mari L. DeMarco[1,4*]

AFFILIATIONS

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ABSTRACT

Background/objectives: The two most common forms of early onset dementia are Alzheimer's disease (AD) and frontotemporal dementia (FTD). Pathologically, AD is characterized by protein aggregates of amyloid-beta and tau, and FTD is characterized by transactive response DNA binding protein (FTD-TDP), tau (FTD-tau) or FET aggregates. To look for biomarkers for FTD-TDP we have previously analyzed insoluble protein fractions of FTD-TDP, FTD-tau, and AD pathological brain tissues. Since this data is rich in information, herein we present additional analyses of this data focusing on proteins associated with neuropathology to characterize their primary structure and solubility.

Methods: Data analyzed herein is from immunohistochemically confirmed brain tissue from cases of AD, FTD-TDP, and FTD-tau pathology that were homogenized and fractionated into soluble and insoluble protein fractions. Electrophoresis was used to isolate proteins by molecular weight to study intact, modified, and cleaved protein primary sequences via bottom-up high-resolution mass spectrometry. We focused on proteins involved in neurodegenerative disorders including alpha-synuclein, amyloid precursor protein (APP), tau, TDP-43, and transmembrane protein 106B (TMEM106B). Protein physicochemical properties and primary structure were characterized using UniProt. From the mass spectrometry analysis, protein solubility was assessed qualitatively. Proteoforms were assessed for concentration differences defined by a fold change greater than two between disease groups in the insoluble fraction. Quantification analyzes are ongoing for soluble tissue fractions.

Results: Intact alpha-synuclein was detected in both soluble and insoluble fractions, and no cleavage products were identified. Proteoforms consistent with intact and truncated APP were detected, only in the soluble fraction. Proteoforms consistent with intact and truncated tau were detected, with similar proteolytic profiles in soluble and insoluble fractions. Proteoforms consistent with aggregated and/or modified, intact, and truncated TDP-43 were detected; these proteoforms had similar proteolytic profiles in soluble and insoluble fractions. Proteoforms consistent with intact, truncated and aggregated and/or modified TMEM106B were identified; modified and cleaved proteoforms were predominantly in the insoluble fraction, and intact TMEM106B had similar profiles in soluble and insoluble fractions. Two proteins had differences between diseases. We found a 12-fold increase of cleaved TDP-43 in FTD-TDP compared to FTD-tau and AD; and a 7-fold increase of all tau proteoforms in AD and FTD-tau compared to FTD-TDP.

Conclusions: We have characterized the proteoform profiles of neurodegeneration-associated proteins via analysis of soluble and insoluble brain tissue fractions from FTD-TDP, FTD-tau and AD cases. Such proteoforms have been critical targets in the development of biomarkers that can differentiate forms of dementia with overlapping clinical presentations. Through further examination of disease-specific proteoform profiles we hope to further biomarker efforts for these proteins.

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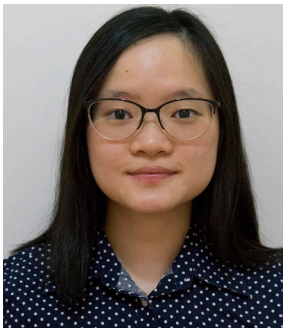

Crystal Ma

SUPERVISOR(s): Dr. STEPHEN YIP

SIMULTANEOUS DETECTION AND QUANTIFICATION OF SARS-COV-2, INFLUENZA A, INFLUENZA B, AND RESPIRATORY SYNCYTIAL VIRUS FROM WASTEWATER AS A POPULATION-LEVEL SURVEILLANCE TOOL

Crystal Ma
(undergraduate)

AFFILIATIONS

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ABSTRACT

Background/objectives: Neuropathology has become increasingly reliant on molecular features to differentiate between subtle brain tumor classifications. Since 2019, VGH's department of neuropathology has embarked on an ambitious initiative to digitize and store microscopic histopathology images of hematoxylin & eosin (H&E)- stained slides and developed nascent classifiers for brain cancers. These digitized images are annotated on a collaborative platform, and ultimately used as input for machine learning. The online classifier, when given a scanned histology slide, then produces a probability score of underlying genomic aberrations. This new technology will benefit hospitals and labs that do not have facile access to costly sequencing assays and democratize pathology in an era of precision medicine.

Methods: An assortment of H&E slides was curated for cases of digital scanning from a cohort of low- and high-grade glioma cases that had undergone molecular profiling with a custom NGS panel (PMID 35526077). A robust database was then developed to systemize pathology diagnosis, grade, and genomic features, and the slides were digitized to create a pipeline. Using the Pathportal platform, web-based annotation was then performed on scanned images to annotate specific regions of brain tumor for machine learning training.

Results: 79 H&E slides selected and digitized and imported into PathPortal platform. A database was created with a pathology diagnosis of oligodendroglioma, astrocytoma, or glioblastoma, and they were matched with their corresponding molecular features. These include, but are not limited to IDH, CIC, ATRX, TP53, PIK3CA, PIK3R1, NOTCH1, FUBP1, and CDKN2A/B status. The digitized slides were annotated, differentiating between regions of tumor, stroma, immune cells, and necrosis.

Conclusions: Our established approach presents as an efficient and effective method in refining a machine learning pipeline that is trained to accurately estimate the probability of clinically relevant genomic mutations given a digitized neuropathology specimen. By training the algorithm against a known set of molecular "ground truth," it can reliably identify subtle yet potentially important pathological elements in brain tumor slides.

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Abhimanyu Minhas

SUPERVISOR(s): Dr. Leandro Venturutti

THE CHEMOKINE RECEPTOR CXCR3 AS A CANDIDATE DRIVER OF AGGRESSIVE B CELL LYMPHOMA DISSEMINATION

Abhimanyu Minhas
(undergraduate)

AFFILIATIONS

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ABSTRACT

Background/objectives: Diffuse large B cell lymphoma (DLBCL), the most common lymphoid malignancy in adults, encompasses a group of clinically and biologically heterogeneous diseases derived from mature activated B cells. DLBCLs typically localize to lymph nodes, but can also develop in, or disseminate to, other organs, resulting in challenging clinical management and poor patient outcomes. Among DLBCL, the "MCD" molecular subtype shows the highest incidence of extranodal presentation, which could explain its 5-year survival rate of just ~25%. Still, there are no predictive biomarkers for dissemination, or strategies to prevent/delay these events, and little is known about the mechanisms involved. Our group recently conducted transcriptional profiling of MCD early precursor cells derived from murine models, and found consistent differential upregulation of the chemokine receptor Cxcr3, triggered by different MCD "founder" mutations. Under physiological conditions, this G-Protein coupled receptor is primarily expressed in activated T cells, and mediates cell trafficking and chemotaxis in response to its ligands (CXCL9/10/11). Interestingly, CXCR3 has been shown to be expressed in other blood cancer types, suggesting a broad involvement in tumor cell circulation. The primary objective of this study was to identify expression levels of CXCR3 in MCD precursor and tumor cells. Furthermore, we aimed to elucidate functional roles this receptor may play in DLBCL dissemination.

Methods: To characterize ***CXCR3*** expression in different DLBCL subtypes at the transcript level, we used RNA-Seq patient data from a BC Cancer clinical cohort (n=264), and performed qPCR analysis on DLBCL human cell lines (n=10). To analyze CXCR3 expression at the protein level, we employed flow cytometry on murine B cells collected from MCD mouse models or wild type controls, and on human DLBCL cell lines. CXCR3 function was investigated using transwell assays to measure the migratory capacity of murine MCD precursor cells towards CXCL9/10/11.

Results: Primary patient data analysis suggested that CXCR3 is upregulated in MCD cases vs non-MCD cases. In line with these findings, we found differential upregulation of CXCR3 mRNA (62.10 vs 0.74 mean fold change in expression) and protein (21.66 vs 4.25 average MFI value) in human MCD cell lines (n=4), as compared to non-MCD ones (n=6). CXCR3 protein upregulation was similarly detected in MCD precursor cells from our murine models, as compared to wild type B cells ($p<0.01$). Finally, our preliminary findings show that CXCL11 could induce migration of MCD precursor cells in a transwell assay setting.

Conclusions: Our results suggest that CXCR3 is upregulated both in MCD precursor and tumor cells, at the transcript and protein level. Our preliminary functional experiments show that CXCR3 may play a role in MCD migratory capacity. Future steps include validating this data, and characterizing CXCR3 function and downstream signaling cascades in human MCD models. Our work could provide a rationale for targeting CXCR3-driven DLBCL dissemination using existing pharmacological inhibitors.

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Chanhyeok Park

SUPERVISOR(s): Dr. ANNA F LEE

IN LUNGS FROM FETUSES WITH CONGENITAL DIAPHRAGMATIC HERNIA, BETA-CATENIN MRNA EXPRESSION IS NOT DIFFERENT FROM CONTROL LUNGS, BUT TISSUE SECTIONS SHOW DIFFERENT DISTRIBUTIONS OF BETA-CATENIN PROTEIN

Chanhyeok Park
(undergraduate)

AFFILIATIONS

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[1]Department of Pathology and Laboratory Medicine, [2]Pediatrics, [3]Pediatric Surgery, and [4]BC Children's Hospital Research Institute, BC Children's Hospital; University of British Columbia; Vancouver, Canada

ABSTRACT

Background/objectives: Congenital diaphragmatic hernia (CDH) is a developmental abnormality characterized by a defect in the diaphragm, with abdominal organs herniating through the defect into the chest cavity. CDH is associated with lung hypoplasia, a life-threatening complication. The dual-hit hypothesis of lung hypoplasia in CDH states that gene expression changes in developing lungs plus mechanical factors are responsible for the pathogenesis of lung hypoplasia in CDH. Animal model studies of CDH show alterations of the Wnt/beta-catenin signaling pathway in lungs. We hypothesized that similar alterations will be evident in human cases of CDH.

Methods: The pathology archive was searched for autopsies (2017-2021) of stillbirths with CDH from terminations of pregnancy (TOP) or intrauterine fetal demise, or livebirths with CDH expiring less than 24 hours of age and no surgical correction (n=20). Controls were randomly selected autopsies (2018-2021) of TOP for brain anomaly or neural tube defect and no CDH (n=9). Clinical data including sex, gestational age, and type of CDH were collected.

For mRNA quantification by qPCR, mRNA was extracted from formalin-fixed paraffin-embedded (FFPE) lung tissues. A stable gene expression library was established by reverse transcription. Gene expression was assessed by qPCR using primers for beta-catenin (normalized to beta-actin).

A subset of cases and controls with highest bulk mRNA were subjected to beta-catenin immunohistochemistry (IHC) on FFPE lung sections. From images collected from scanned stained slides, non-epithelial (interstitial) cells with nuclear, cytoplasmic, and/or membranous beta-catenin positivity, and negative interstitial cells, were counted..

Results: Beta-catenin mRNA Quantification: For cases and controls that had detectable beta-catenin mRNA, there was no significant difference (p=0.331) in beta-catenin mRNA levels between cases (RQ: 2.17; RQmin=0.74, RQmax=6.38, n=6) and controls (RQ: 1.00; RQmin=0.38, RQmax=2.64, n=3). Beta-catenin mRNA in cases and controls both had slight positive correlations with gestational age, but neither were significant (cases r2=0.3194, p=0.2425; controls r2=0.040, p=0.8717).

Beta-catenin Protein in Lung Tissue Sections: In cases (n=6; 18-28 weeks of gestation) and controls (n=7; 18-36 weeks of gestation), beta-catenin protein was consistently strongly and uniformly expressed in epithelial cells, and variably expressed in the interstitial (non-epithelial) cells, as follows: on average and across the tested gestational ages, the percentage of interstitial cells expressing beta-catenin was lower in cases (range: 1.8–27.9%; average 13.2%) than in controls (range: 7.5–64.4%; average 42.3%) (p=0.006).

Conclusions: Our findings may suggest that human CDH is associated with Wnt/beta-catenin pathway alterations, as reported in animal model studies. Our study found alterations at the protein level, with a lower percentage of beta-catenin protein expressed in interstitial lung cells from fetuses with CDH compared to non-CDH control lungs.

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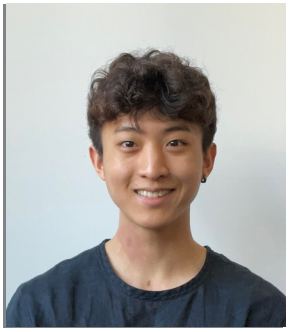

Sangwook Michael Woo

SUPERVISOR(s): Dr. Natalie Prystajecky, Dr. Linda Hoang

VALIDATION OF A MULTIPLEX qPCR ASSAY TO QUANTIFY ANTIBIOTIC RESISTANCE GENES IN WASTEWATER

Sangwook Michael Woo (undergraduate) **(CANCELLED)**

Sangwook Michael Woo[1], Jennifer Kopetzky[2], Sarah Mansour[2], Ben Hon[2], Tracy Lee[2], Shannon Russell[2,3], Liam Byrne[2], Natalie Prystajecky[2,3], Linda Hoang[2,3]

AFFILIATIONS

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ABSTRACT

Background/objectives: Antimicrobial resistance (AMR) is a pressing public health issue worldwide. Since 2015 when mobile-colistin resistance gene (MCR-1) was first isolated from a pig, MCR-1 has been described in 57 countries including Canada. In addition, blaNDM, blaKPC, blaOXA-48 are among the three most clinically relevant, plasmid-mediated antibiotic-resistant genes (ARG) associated with carbapenamase-producing organisms (CPO). As the risk of CPOs continues to increase in Canada, there is an urgent need for robust, sensitive, and specific tools to carry out CPO surveillance in communities. We present the validation, optimization, and longitudinal data of a multiplex qPCR assay for detecting and quantifying OXA-48, NDM, KPC, and MCR-1 in wastewater (WW).

Methods: Control organisms harbouring OXA-48, NDM, and KPC were cultured. WW from five wastewater treatment plants was spiked with the isolates, concentrated, then spiked with MCR-1 oligonucleotides; MCR-1 synthetic oligonucleotide was used due to the biocontainment considerations. WW concentrates containing OXA-48, NDM, KPC, and MCR-1 were extracted, and the resulting eluates were tested with singleplex and multiplex assays in parallel. Singleplex and multiplex cycle thresholds (Ct) for each target were analyzed using ANOVA, Coefficient of Variation (CoV), and standard deviation (SD). Further work was done to assess the assay's ability to quantify ARGs at significantly different concentrations in a single reaction well. Cts generated from ARGs at mixed concentrations (one high, three low) were compared to Cts of ARGs at low, equimolar concentrations. External standard curves were generated for every ARG to determine the limits of quantification (LOQ). We analyzed the assay's performance with WW samples that were collected from January 2022 to December 2022. Samples were tested in triplicate, plotted, and compared to CPO clinical data from the Provincial Infection Control Network of British Columbia (PICNet).

Results: Spiked WW yielded similar Cts in singleplex and multiplex for each ARG. Weak pooled samples containing ARGs at equimolar, low concentrations produced Cts that were similar to mixed pooled samples that contained three low-concentrations ARGs with one high-concentration ARG. The efficiency of the multiplex assay for each target ranged between 89.988% to 96.06%. The limit of quantification was 10 copies/µl for OXA-48, NDM, MCR-1, and 5 copies/µl for KPC. WW collected between January 2022 to December 2022 tested positive for all four ARGs. OXA-48, KPC, and NDM, were consistently detected at 10^6 to 10^{10} copies per day, whereas MCR-1 detection was low and sporadic.

Conclusions: The multiplex CPO assay demonstrated robustness and sensitivity at detecting the four ARGs in WW. There was no major correlation in ARG trends between WW data to lab-verified CPO cases. Future work using the solid fraction of WW can enhance the recovery of ARGs during WW processing, which can improve the assay's ability to detect ARGs like MCR-1 which are less abundant in WW. This assay can be used to generate valuable WW data to support current CPO surveillance strategies in BC and across Canada.

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Derek Wu

SUPERVISOR(s): Dr. DAVID J. GRANVILLE

PROFILING GRANZYMES IN IDIOPATHIC INFLAMMATORY MYOPATHIES

Derek Wu
(undergraduate)

AFFILIATIONS

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ABSTRACT

Background/objectives: Idiopathic inflammatory myopathies are conditions best characterized by muscle weakness and atrophy, leading to substantial disability. Although the etiology is still under study, autoimmune mechanisms are hypothesized to underlie the pathogenesis of inflammatory myopathies. Research in the past decade has established key roles for granzymes — a family of serine proteases classically known to induce targeted cell death as a part of the immune response — in cleaving targets in the extracellular space, contributing to both autoimmunity and inflammation. Granzyme levels are typically low or undetectable in healthy tissues, but upregulated in autoimmune and inflammatory conditions. Notably, granzyme B substrates include FHL-1 and Mi-2, two major autoantigens of inflammatory myopathy. As such, we hypothesize that granzymes are elevated in patients with inflammatory myopathies and their levels are correlated to disease severity.

Methods: Muscle biopsies obtained from clinical collaborators at Vancouver General Hospital and St. Paul's Hospital, Vancouver, Canada were analyzed using established IHC protocols and digital pathology software for granzymes A, B, H, K, and M. To date, we have acquired 24 muscle biopsies from patients with inflammatory myopathies. The granzyme levels were then correlated with patient disease severity measurements (i.e., creatine kinase levels and Medical Research Council muscle strength score).

Results: All 5 granzymes (A, B, H, K, and M) have been detected in the collected muscle biopsy samples. Preliminary analyses suggest that granzymes A, B, H, and M are elevated in inflammatory myositis samples relative to the healthy tissue. Moreover, there may be evidence that subtypes of idiopathic inflammatory myopathy (e.g., inclusion body myositis, dermatomyositis, polymyositis) have differing granzyme level profiles. Analyses are ongoing.

Conclusions: This is the largest study of its kind evaluating protein levels of the granzyme family in idiopathic inflammatory myopathies. Early analyses show that granzymes A, B, H, and M are elevated in inflammatory myositis samples, potentially supporting their utility as biomarkers or indicators of disease.

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Jennifer Wu

SUPERVISOR(s): Dr. Lucy Perrone

INVESTIGATING THE IMPACT OF MALDI-TOF MS AS A DIAGNOSTIC TECHNIQUE ON PROFICIENCY TESTING: A META-ANALYSIS OF 10 LABORATORIES ACROSS CANADA

Jennifer Wu
(undergraduate)

Jennifer Wu, Selvarani Vimalanathan, Veronica Restelli, Lucy A. Perrone

AFFILIATIONS

[1] Dept of Pathology and Laboratory Medicine, UBC

ABSTRACT

Background/objectives: Participation in external quality assessment (EQA) programs is a requirement of accredited health and environmental testing laboratories. One core application of EQA includes proficiency testing (PT) and participation in an objective PT scheme for every test a laboratory offers on its testing menu is a quality practice that helps laboratories ensure they are consistently producing accurate test results for their customers. These customers include the public, patients, clinicians and public health policy makers. The Canadian Microbiology Laboratory Proficiency Testing program is a not-for-profit, academically rooted clinical service located at UBC that for over 40 years has provided EQA services and PT challenges for microbiology testing laboratories. CMPT formulates PT samples which mimic clinical and environmental samples and include a variety of clinically relevant microorganisms such as bacteria, viruses, parasites and fungi. CMPT produces these challenges and distributes them to laboratory subscribers as blinded challenges. Laboratories are evaluated on tier performance based on correctly identifying and characterizing microorganisms (including their antibiotic susceptibility patterns) given a specific clinical context.

Methods: This study aimed to investigate factors that contribute to laboratory performance by comparing the performance of laboratories in CMPT's clinical bacteriology EQA program. Aggregate performance grades from 10 laboratory subscribers located across Canada during the period from 2019-2023 was evaluated for patterns and correlations with respect to sample type, location, and diagnostic technique among other factors. A subgroup analysis of the effect size of said factors on performance was first assessed by polychoric correlation followed by meta-analytic structural equation modeling. Further qualitative analysis investigating reasons for subpar performance and the range of diagnostic techniques was executed to contextualize lab performance.

Results: Laboratories serving rural populations that rely on conventional biochemical methods for organism identification were often unable to process specific sample types or report organisms to the species level, and were consequently outperformed by larger urban facilities using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for organism identification. These laboratories were able to consistently and correctly identify organisms to the species level with supporting antimicrobial susceptibility results.

Conclusions: As MALDI-TOF MS becomes the technique of choice for microbial identification and diagnosis, it is worth investigating its impact on laboratory proficiency. The findings of this study will help support labs in improving laboratory performance, and ultimately clinicians in their diagnoses.

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Angel Yao

SUPERVISOR(s): Dr. CATHERINE HOGAN

PRE-ANALYTICAL OPTIMIZATION OF DERMACENTOR TICK SPECIES FOR MOLECULAR IDENTIFICATION IN BRITISH COLUMBIA

Angel Yao
(undergraduate)

Angel Y. Yao [1,2], Martin Cheung [2], Min-Kuang Lee [2], John Tyson [1,2], Tracy Lee [2], Muhammad G. Morshed [1,2], Catherine A. Hogan [1,2]

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ABSTRACT

Background/objectives: In British Columbia, *Dermacentor andersoni* ticks are historically a major microscopically identified population. In contrast, a recent report in household pets utilizing ITS-2 targeted sequencing displayed a greater prevalence of ***D. variabilis*** (a vector for Lyme disease). Molecular testing could provide clinical value for ticks of challenging morphology. This study aimed to investigate the pre-analytical optimization of tick processing and provide preliminary downstream polymerase chain reaction (PCR) and whole genome sequencing (WGS) results for ITS-2 targeted *Dermacentor* species identification.

Methods: Methods: 36 ***D. andersoni*** ticks submitted in 2022 underwent DNA extraction utilizing the Qiagen Blood and Tissue kit. Tick body content (one leg, three legs, half body, whole body), tissue preparation (cutting versus grinding) and tick gut content were predominately examined. Additional variables of micropestle components and proteinase K digestion time were investigated. Reproducibility of the proposed method was completed in duplex over a range of tick weights. All DNA concentration was quantified by Qubit and Nanodrop. PCR amplicons of extracted DNA were created with ITS-2 primers and ran on TapeStation.

Results: DNA extraction of half a tick body yielded the appropriate DNA concentration range for molecular applications. Grinding tissues with matched micropestle components yielded higher DNA concentrations. For ticks of average weight (5.5 mg), both overnight and 10-minute Proteinase K digestion yielded similar DNA concentrations. Longer digestion proved beneficial in larger ticks. On average, a 16.95 ng/uL (Qubit quantified) DNA concentration difference was identified in weight and sex matched ticks over a wide weight range (2 mg-11 mg). PCR and TapeStation revealed a thick ITS-2 band of ~450bp from extracted D. andersoni DNA.

Conclusions: With consideration to cost and time, DNA extraction of half a tick body grinded with matching micropestle components can provide sufficient primary material for downstream molecular identification. PCR of ITS-2 and TapeStation can provide ***Dermacentor*** species identification.

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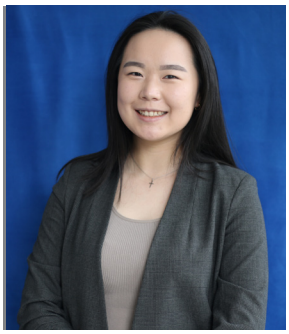

Serena Yeung

SUPERVISOR(s): Dr. Mari L. DeMarco

ALZHEIMER'S DISEASE BIOMARKER TESTING EDUCATIONAL TOOLKIT FOR PHYSICIANS

Serena Yeung
(undergraduate)

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ABSTRACT

Background/objectives: St. Paul's Hospital in Vancouver performs Alzheimer's disease cerebrospinal fluid (CSF) biomarker testing for all of Canada. Testing includes the quantification of amyloid-beta and tau proteoforms, which when used in combination have a 91% sensitivity & 90% specificity for the detection of Alzheimer's disease pathology. Via this service, the laboratory has accumulated common queries from healthcare professionals. To provide additional educational resources for physicians, we aimed to expand our educational toolkit to include a series of short educational videos addressing commonly asked questions.

Methods: Topics for the videos were chosen based on physicians' inquiries about Alzheimer's disease CSF biomarkers testing with initial topics selected including: (1) interpretation of the amyloid-beta 42/40 ratio, and (2) the mass spectrometry method for quantification of amyloid-beta 1-42 and 1-40 proteoforms. A set of learning objectives were developed to guide the design of each video. To further inform content for the videos, a background literature search on these topics was performed. With the Microsoft PowerPoint program, we used simple animated graphics to make the video, coupled with plain scientific language to facilitate knowledge translation to a broad clinical audience. Videos were subjected to an iterative process of review and revision prior to finalizing.

Results: For the first video, the learning objectives were to: 1) describe how Aβ42 and Aβ40 peptides are produced, 2) explain how Aβ42 is the biomarker for AD pathology, and 3) describe the diagnostic value of using CSF Aβ42/40 ratio to detect Alzheimer's disease pathology. Learning objectives for the second video were to: 1) describe how mass spectrometry performs multiplex quantification, 2) identify the benefit of direct detection by mass spectrometry, and 3) describe why it is beneficial to use a method whose calibration is traceable to the international reference material. Each finalized video had a runtime of less than 5 minutes.

Conclusions: The developed videos serve to provide a free and on-demand access to concise summaries of key topics related to the Alzheimer's disease biomarker testing program at St. Paul's Hospital. The value of this knowledge translation activity will be evaluated via several metrics including access metrics (e.g., number of views), and feedback from health care providers utilizing these resources.

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Raneen Abdul-Rahman

SUPERVISOR(s): Dr. JACQUELINE QUANDT

CHARACTERIZING NEURONAL PAS-DOMAIN CONTAINING PROTEIN 4 IN INFLAMMATORY AND NEURODEGENERATIVE SETTINGS RELEVANT TO MULTIPLE SCLEROSIS

Raneen Abdul-Rahman
(graduate student)

Raneen Abdul-Rahman[1], Jacqueline Quandt[1]

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ABSTRACT

Background/objectives: Multiple sclerosis (MS) is a chronic, inflammatory, and neurodegenerative disease of the central nervous system (CNS) that affects over 100,000 Canadians. It is characterized by inflammatory demyelination, which drives oxidative stress and glutamate excitotoxicity that increase neuronal calcium levels and contribute to disease progression associated with irreversible cell loss. While current treatments target the inflammatory phase of MS, they afford little benefit in the later degenerative phases, highlighting the need for approaches that promote neuroprotection. Neuronal PAS-domain containing protein 4 (NPAS4) is a neuronal transcription factor activated by shifts in intracellular calcium. It regulates over 200 genes, with some playing key roles in neuronal health, stability, and synaptic plasticity. We have shown neuronal NPAS4 expression increases in the presence of activated immune cells, an effect prevented by glutamate inhibitors. To further assess the regulation of NPAS4 expression by inflammatory mediators, we examined the direct effects of glutamate excitotoxicity and oxidative stress (H2O2) over time in an in vitro cortical cell model, where we hypothesize that these inflammatory mediators will increase NPAS4 protein expression to affect cell viability/function.

Methods: NPAS4 protein expression was assessed immunocytochemically using 10 days in vitro mouse primary neuron-enriched cortical cultures treated with increasing concentrations of glutamate (1, 3, 10 & 30 microM) and H2O2 (12.5, 25, 50 & 100 microM) for 1, 3, 5, 7 and 9 hours. Within each concentration, the neuronal nuclear mean fluorescent intensity (MFI) values were normalized to the phosphate buffered saline (PBS) treated control MFI at each time point to enable comparisons across three or four biological replicates. The MFI and percent positive cells were compared across time/concentration and to the PBS control, respectively, using a two-way analysis of variance with a Dunnet's or Tukey's adjustment for multiple comparisons.

Results: The addition of 10 and 30 microM glutamate rapidly increased nuclear NPAS4 protein expression at 3 hours ($p<0.05$), which returned to baseline levels within 7-9 hours. Compared to PBS, increases in the percentage of NPAS4+ cells were observed at 1 hour from 3-30 microM and up to 7 hours from 10-30 microM ($p<0.05$). Higher concentrations of H2O2 suggest a trend for increasing late NPAS4 expression, however, only 50 microM significantly increased expression, for both the MFI and the percentage of NPAS4+ cells, above baseline at 5 hours compared to lower doses and shorter time points ($p<0.05$). A decrease in cell viability was observed at the highest concentrations for both treatments, and a qualitative assessment suggests that the remaining cells are largely NPAS4+.

Conclusions: MS-related inflammatory mediators alter cortical neuron NPAS4 protein expression ***in vitro,*** with glutamate playing a direct role. Understanding how neuroinflammation affects NPAS4 expression and mechanisms associated with neuroprotection relevant to MS may identify new targets with the potential to limit neuronal damage in inflammatory settings.

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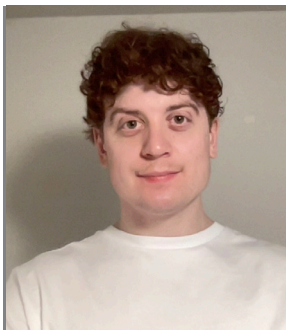

Graham Archibald

SUPERVISOR(s): Dr. Ali Bashashati

CLASSIFICATION OF MICROPAPILLARY AND UROTHELIAL CARCINOMA USING ARTIFICIAL INTELLIGENCE-BASED HISTOPATHOLOGY IMAGE ANALYSIS

Graham Archibald
(graduate student)

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ABSTRACT

Background/objectives: Micropapillary urothelial carcinoma (MPC) is a rare, aggressive histologic subtype that frequently co-occurs with conventional urothelial carcinoma (UC) and comprises 2-5% of all bladder cancers. Proper identification and reporting of the proportion of MPC subtype are crucial for optimal risk stratification and treatment selection. Given the complex histological features of MPC and moderate (κ:0.54) interobserver agreement among genitourinary (GU) pathologists, an artificial intelligence (AI) tool that can not only reliably identify this subtype but also report the proportion of it within a sample would be a valuable resource for pathologists and clinicians.

Methods: For the training dataset, we identified 29 MPC patients (with 128 whole slide images, or WSIs) and 57 UC patients (with 134 WSIs). For the validation dataset, we obtained 88 MPC cases (with 88 WSIs) and 72 UC (with 72 WSIs) from across British Columbia. One GU pathologist annotated the MPC and UC-containing regions in all WSIs. Due to differences in scanners and staining conditions, the validation dataset occupies a slightly different color space than the original dataset. We fed the data into a ResNet18 model using three-fold cross validation which trained to classify these subtypes based on slide-level labels, with each WSI labelled as either MPC or UC. To improve model generalization to the validation dataset, we normalized the external color space based on reference images from the training dataset.

Results: Our classification of UC vs MPC cases using AI attained 91.0% slide level accuracy on the training dataset and 85.6% ($p<0.032$) slide level accuracy on our validation dataset.

Conclusions: Utilizing an AI-based approach for histopathology image classification, we have successfully classified MPC and UC without the use of manual pathologist annotations for identifying tumor regions. These findings demonstrate AI as a promising tool for the diagnosis of this rare and aggressive subtype of urothelial carcinoma. Future work will seek improvement of our AI algorithm to attain higher accuracy, and further validation in an external dataset. Furthermore, we will include additional histological subtypes.

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Puria Azadi Moghadam

SUPERVISOR(s): Dr. ALI BASHASHATI

A LOCAL GLOBAL GRAPH-BASED DISTILLATION MODEL FOR REPRESENTATION LEARNING OF GIGAPIXEL HISTOPATHOLOGY IMAGES WITH APPLICATION IN CANCER RISK ASSESSMENT

Puria Azadi Moghadam
(graduate student)

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ABSTRACT

Background/objectives: The great rise of deep learning in the past decade and our ability to digitize histopathology slides using high-throughput slide scanners have fueled interests in the applications of deep learninn histopathology image analysis. The majority of the efforts, so far, focus on the deployment of these models for diagnosis and classification. As such, there is a paucity of efforts that embark on utilizing machine learning (ML) models for patient prognostication and survival analysis.

In the ML domain, patient prognostication can be treated as a weakly supervised problem, which a model would predict the outcome (e.g., time to cancer recurrence) based on the histopathology images. Their majority have utilized Multiple Instance Learning (MIL) that have shown superior performances in grading or subtype classification in comparison to outcome prediction. This is perhaps due to the fact that MIL-based techniques do not incorporate patch locations and interactions as well as tissue heterogeneity which can potentially have a vital role in defining clinical outcomes. To address this issue, graph neural networks (GNN) have recently received more attention in histology. They can model patch relations by utilizing message passing mechanism via edges connecting the nodes (i.e., small patches in our case). However, most GNN-based models suffer from nodes' small receptive fields. While local contexts mainly capture cell-cell interactions, global patterns such as immune cell infiltration patterns and tumor invasion in normal tissue structures (e.g., depth of invasion through myometrium in endometrial cancer) could capture critical information about outcome. Hence, locally focused methods are unable to benefit from the coarse properties of slides due to their high dimensions which may lead to poor performance.

Methods: This work aims to investigate the potential of extracting fine and coarse features from histopathology slides and integrating them for risk stratification in cancer patients. Therefore, we propose a novel graph-based model for risk assessment that extracts both local and global morphological properties and uses a fine-coarse feature distillation module to mutually aggregate interactions at different scales. We also utilize two prostate cancer datasets to evaluate the performance of our method.

Results: Our model outperforms state-of-the-art (SOTA) for Hazard Prediction and the results indicate that the proposed method is capable of stratifying patients into statistically significant risk groups ($p < 0.01$ across both datasets) with clinical utility, suggesting that global histo-morphological properties improve patient stratification performance.

Conclusions: While risk assessment is relatively under-explored, most existing methods are focused only on small fields of view. In this work, we introduce a graph-based model for integrating global and local features, which utilizes interactions at a larger scale for improved risk stratification. Our results suggest that the proposed model outperforms SOTA. The full capacity of this work can be demonstrated by extending it to other cancer types or tasks.

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Vriti Bhagat

SUPERVISOR(s): Dr. Bruce Verchere

CHARACTERIZING PROHORMONE PROCESSING DEFICIENCIES IN NON-OBESE DIABETIC MICE USING ADENO-ASSOCIATED VIRUS AAV8-INS1-CRE MEDIATED GENE DELETION

Vriti Bhagat
(graduate student)

Vriti Bhagat[1,2,4], Yi-Chun Chen[2,3,4], Mitsuhiro Komba[2,3,4], Paul C. Orban[2,3,4], Galina Soukhatcheva[2,3,4], Melanie Lopes[2,4,7], Natalie Nahirney[5,6], Peter Overby[5,6], James D. Johnson[5,6], C. Bruce Verchere[1,2,3,4]

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ABSTRACT

Background/objectives: Pancreatic beta cells produce the prohormone, proinsulin, which is processed to its mature biologically active form, insulin, in the beta-cell secretory pathway. This process involves the prohormone convertases PC1/3 and PC2, and carboxypeptidase E (CPE). Individuals living with type 1 diabetes exhibit persistent secretion of proinsulin, indicating that processing of beta-cell prohormones is impaired in type 1 diabetes. Indeed, non-obese diabetic (NOD) mice, a model of autoimmune diabetes, display elevated levels of proinsulin early in life, mirroring impaired beta-cell prohormone processing observed in humans. However, the contribution of processing enzyme loss to diabetes onset remains unknown. We hypothesize that knockout of the processing enzymes Pc1/3 and Cpe will exacerbate impaired prohormone processing in NOD mice, and lead to earlier and/or increased diabetes incidence.

Methods: NOD mice with floxed genes for Pc1/3 and Cpe, ***Pcsk1*** and Cpe respectively are being generated. To induce beta-cell specific knockout of ***Pcsk1*** and Cpe ***in vivo***, an adeno-associated virus expressing Cre recombinase under the control of the insulin 1 promoter, AAV8-Ins1-Cre, will be introduced surgically into the pancreatic ducts of prediabetic female and male NOD mice. Diabetes progression will be measured by recording blood glucose levels and body weight. Proinsulin and C-peptide will be detected by ELISA.

Results: Preliminary data demonstrate efficient beta-cell recombination at low ($1x10^{11}$ vgp/mouse) and high ($5x10^{11}$ vgp/mouse) doses of AAV8-Ins1-Cre administered intra-ductally (88.7% and 93.8%, respectively) and intraperitoneally ($1x10^{12}$ vgp/mouse; 93.76%).

Conclusions: This study will enable better understanding of the contribution of loss of prohormone processing enzymes to the pathogenesis of type 1 diabetes. The results from this study may have implications for identifying new targets for therapeutics for the treatment of type 1 diabetes.

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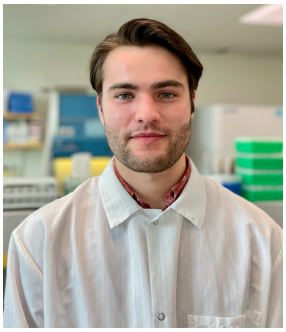

Liam Byrne

SUPERVISOR(s): Dr. NATALIE PRYSTAJECKY

A MULTI-TARGET MOLECULAR ASSAY FOR THE SURVEILLANCE OF ENTERIC PATHOGENS IN METRO VANCOUVER WASTEWATER

Liam Byrne
(graduate student)

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ABSTRACT

Background/objectives: Hospitalization and clinical diagnosis rates are traditionally used to estimate the prevalence of diseases in the broader population, but for gastrointestinal illnesses caused by enteric pathogens, this methodology is likely ineffective. The majority of cases are mild and therefore go undiagnosed and unreported; as such, clinically derived statistics underrepresent the true burden of enteric disease in Canada. In fact, the Public Health Agency of Canada estimates that for every reported clinical case of ***Salmonella*** and ***Escherichia coli***, there are at least 20 unreported cases. One way to address the underreporting of gastrointestinal illness in Canada is to integrate new surveillance methods. Wastewater testing has been increasingly utilized in public health because it is not susceptible to the same sampling biases that affect clinical data. Leveraging methods for wastewater collection and processing developed during the SARS-COV-2 pandemic, this study aimed to develop a multiplex quantitative PCR (qPCR) assay for the molecular detection and quantification of ***Salmonella spp***, and Shiga toxin producing E. coli (STEC) from wastewater samples.

Methods: A literature review was performed and primers and probes specific for STEC and Salmonella spp. were compiled. Primers and probes were tested in-silico by aligning them against the consensus sequences of their respective targets from the Pathogen Detection Microbial Browser for Identification of Genetic and Genomic Elements database. Primers and probes that matched target consensus sequences and had minimal nonspecific interactions were chosen. Probe fluorophores were modified so that the probes were compatible for multiplexing and primer/probe concentrations were optimized. Master mixes were tested in singleplex and multiplex to assess performance and comparability. External standard curves were generated for each target. A panel of control organisms was used to test the specificity of the multiplex assay. Wastewater samples from the five metro Vancouver wastewater treatment plants were collected and processed according to previously validated methods. The resulting nucleic acid samples were tested for ***Salmonella*** and STEC via the multiplex qPCR assay.

Results: The developed multiplex qPCR was 100% specific for STEC and ***Salmonella*** when tested against a panel of 17 control organisms. ***Salmonella*** and STEC were detected in wastewater from all five wastewater treatment plants surveilled, with 75% of wastewater samples tested containing both.

Conclusions: This study successfully developed a specific multiplex assay for the simultaneous detection of STEC and ***Salmonella***. Preliminary surveillance data from the five Metro Vancouver wastewater treatment plants demonstrates the suitability of these methods to monitor enteric pathogens in wastewater.

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Loulou Cai

SUPERVISOR(s): Dr. Helene Cote

BIVARIATE CORRELATION OF SEX, HIV STATUS AND NUMBER OF CHRONIC/ LATENT VIRAL INFECTIONS WITH MARKERS OF IMMUNE AGING

Loulou Cai
(graduate student)

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ABSTRACT

Background/objectives: People living with HIV (PLWH) experience accelerated cellular and immunological aging relative to their HIV-negative peers. This may be influenced by co-infection with other chronic/latent viruses (including CMV, EBV, HHV-8, HSV-1, HSV-2, HCV, and HBV) that are known to be associated with markers of aging or age-associated diseases. The objective is to determine associations between sex, HIV status, number of chronic/latent viral infections, and markers of immune aging, in a cohort of PLWH versus controls.

Methods: Fifty-one CARMA cohort participants, evenly distributed for sex, HIV status, and age [20-76y] with a broad range of viral coinfections were selected for this analysis. Infection status for CMV, EBV, HHV-8, HSV-1, and HSV-2 was determined serologically; HIV, HCV, and HBV were self-reported. Stored live PBMCs were used to assess the CD4:CD8 and proliferation-competent:senescent CD8+T-cell (CD8+,CD28+:CD28-) ratios via flow cytometry. Participants were dichotomized by sex, HIV status, and above and below median number of non-HIV chronic/latent viral infections.

Results: Female participants exhibited higher CD4:CD8 ($p=0.019$) and CD8+,CD28+:CD28- ratios ($p=0.012$) than males. These ratios were also lower in PLWH vs. controls CD4:CD8 ($p<0.001$) and CD8+,CD28+:CD28- ($p=0.0198$). Individuals with above-median chronic/latent viral infections exhibited lower CD4:CD8 ($p=0.021$), but no difference in CD8+,CD28+:CD28- ratio. No correlation was observed with age.

Conclusions: These data suggest male sex and HIV status may be associated with unfavourable immune cell ratios. Increasing our sample size to >300 CARMA participants with well-balanced age, sex, and HIV status will allow multivariable analysis and identification of independent associations; thus increasing our understanding of immune aging.

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Taylor Da Silva

SUPERVISOR(s): Dr. DANA DEVINE AND DR. HUGH KIM

IDENTIFYING BIOMARKERS OF PLATELET TRANSFUSION OUTCOMES IN PLATELETS OF DIFFERENT STORAGE QUALITY

Taylor Da Silva
(graduate student)

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[1]Centre for Blood Research, UBC, Vancouver, BC; [2]Department of Pathology and Laboratory Medicine, UBC; [3]Canadian Blood Services, Toronto, Ontario; [4]Department of Biochemistry and Molecular Biology, UBC; [5]Faculty of Dentistry, UBC

ABSTRACT

Background/objectives: Over 4.5 million people in Canada and the U.S. need blood transfusions each year. Platelet concentrate (PC) is one of 3 blood products produced by Canadian Blood Services. The 2 types of PC used for transfusions are buffy coat (BCPC) and apheresis (APPC). BCPC is made by collecting blood from 4 donors, separating the platelets from the blood, and pooling them into one unit. APPC is made using a machine that collects whole blood from one donor, separates the platelets from the blood, and returns the rest of the blood to the donor. APPCs will be studied because individual donor characteristics will have a greater effect on the units, and APPCs from unique donors will be used as biological replicates. PC is stored for 7 days at room temperature, and storage quality is limited by bacterial contamination and changes in platelet structure and function. PCs from unique donors have differences in storage quality. Platelet activation and low pH are indicators of poor storage quality. There are currently no biomarkers for the prediction of platelet transfusion outcomes. Previously, the Devine lab used proteomics to identify 8 candidate protein biomarkers in platelets. In vivo clinical trial data was used to correlate expression of these proteins with platelet transfusion outcomes (how effectively the transfusion increased the patient's platelet count). We hypothesize that if expression of these proteins doesn't change over time, they could be used as biomarkers to predict platelet transfusion outcomes.

Methods: The 8 proteins to be studied are: cortactin, ILK, talin, JAM-A, catalase, TPM4, myosin IIA, and myosin 14. The 3 donor groups are normal storing, sometimes low pH storing, and always low pH storing. Low pH is defined as pH < 6.7 on expiry. APPCs will be sampled at the following 3 time points: days 4-5, 7-8, and 10-11. Platelets will be washed, pelleted, lysed, and frozen until running polyacrylamide gel electrophoresis. Western blotting will be performed and normalized to actin. Two-factor ANOVA with repeated measures will be used to compare protein expression at the 3 storage times between the 3 donor groups. The following assays will be used to assess storage quality: swirling of the bag, platelet activation scoring (phase contrast microscopy), platelet count and mean platelet volume (hematology analyzer), glucose, lactate, and pH (blood gas analyzer), clotting time and clot formation rate (rotational thromboelastometry), CD62P expression +/- adenosine diphosphate, and binding of annexin V to phosphatidylserine (flow cytometry).

Results: The low pH storing APPCs are expected to have lower pH and greater activation (worse storage quality) than normal storing APPCs.

Conclusions: If protein expression doesn't change during APPC storage, then these proteins will be validated as good candidates for biomarkers because they could be sampled at any time during storage. They could also identify which APPCs will store poorly if expression differs between donor groups. Identifying biomarkers of platelet transfusion outcomes could help to ensure that patients receive the best quality blood products.

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Lauren Deneault

SUPERVISOR(s): Dr. Marianne Sadar

THE ROLE OF CYSTEINE MUTATIONS IN THE BINDING OF NOVEL THERAPEUTICS FOR CASTRATION RESISTANT PROSTATE CANCER

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(graduate student)

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ABSTRACT

Background/objectives: Therapies for prostate cancer (PC) involve pharmaceutical castration by targeting the androgen receptor (AR) ligand-binding domain (LBD). These therapies are not curative with the malignancy progressing to castration-resistant PC (CRPC). Most CRPC remains driven by AR through multiple resistance mechanisms, e.g., constitutively active AR splice variants that lack LBD (AR-Vs), or gain-of-function mutations in LBD. This has led to clinical development of EPI analogues ("EPI") which target the AR N-terminal domain (NTD) to block the transcriptional activities of AR-Vs and mutated ARs. The EPI-binding pocket involves region 341-446 amino acid residues of the NTD. AR-NTD is enriched in cysteine residues with C404 suggested to be important in the binding mechanism of EPI. We hypothesize that free cysteine residues, which are able to interact to form disulfide bonds, are also important in intra- and intermolecular protein-protein interactions. Here we begin to elucidate the role of cysteines in the inhibitory mechanism of EPI on AR activity.

Methods: The role of cysteines in the covalent binding mechanism of EPI to AR used a fluorescein-labelled EPI with fragments of recombinant AR-NTD (rNTD) with or without the addition of iodoacetamide. Iodoacetamide is an alkylating agent that caps reduced cysteines thereby eliminating potential covalent binding of EPI to these cysteines. Protein-protein interactions requiring disulfide bonds with AR was examined by non-reducing SDS-PAGE and diagonal SDS-PAGE of protein lysates from cells treated with inhibitors that target the AR-NTD compared to the LBD. A role of specific cysteines in the activity of EPI to block AR transcriptional activity was assessed by cysteine mutants using a reporter gene construct driven by AR.

Results: Capping cysteine residues on rNTD with iodoacetamide reduced the amount of covalent binding of EPI. This data suggests that cysteines are essential for covalent binding of EPI to AR-NTD. EPI bound preferentially to the oxidized form of rNTD suggesting the importance of disulfide bridges. Diagonal SDS-PAGE showed differential alterations in intramolecular and intermolecular disulfide bonds in response to EPI compared to AR-LBD inhibitors. These gels exposed unique differences in protein-protein interactions with AR depending on the inhibitor. AR-driven reporter gene assays revealed mutations of cysteine residues in the AR- NTD did not adversely affect AR transcriptional activity in response to androgen.

Conclusions: Cysteine residues in the AR-NTD were required for covalent binding of EPI, but mutation of C404 in the EPI-binding site was not essential for EPI's efficacy to block AR transcriptional activity thereby supporting the importance of reversible binding of EPI within its binding site. There were differential patterns in protein-protein interactions with AR depending upon which domain the inhibitor targeted. Our future goal is to identify these unique proteins to provide insight into the mechanisms and develop more potent and specific therapeutics for the treatment of CRPC.

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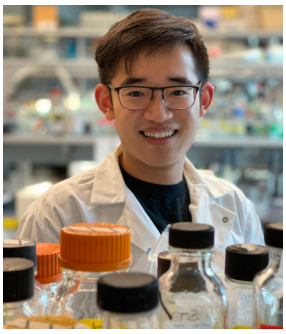

Yuchen Ding

SUPERVISOR(s): Dr. DAVID HUNTSMAN

DEVELOPMENT OF AN EX VIVO MODEL IN THE STUDY OF CLEAR CELL OVARIAN CANCER (CCOC) AND ENDOMETROID OVARIAN CARCINOMA (ENOC)

Yuchen Ding
(graduate student)

Yuchen Ding, Shary Chen, Michelle Woo, Amal El-Naggar, and David Huntsman

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ABSTRACT

Background/objectives: Clear Cell Ovarian Cancer (CCOC) and Endometroid Ovarian Carcinoma (ENOC) are two subtypes of ovarian cancer, which account for about 20% of all ovarian carcinomas. Accumulating evidence has suggested a more important role of hematogenous spread in ovarian cancer metastasis. However, this role has been overlooked due to the lack of models addressing vascular ovarian cancer metastasis. This underscores the importance of tackling research questions with novel approaches and exploring new models that enable new discoveries, and help in designing novel treatment strategies. Lung metastasis is relatively common for CCOC and ENOC, as the lungs receive a large amount of blood flow from the heart, making them a prime location for cancer cells that have broken away from the primary tumor to settle and grow. Additionally, the lungs have a high number of small blood vessels called capillaries, which can provide cancer cells with the nutrients and oxygen necessary for their growth and survival. In this study, we used a newly established in vivo approach, namely the Pulmonary Metastasis Assay (PuMA), to study CCOC and ENOC cancer progression.

Methods: The Pulmonary Metastasis Assay (PuMA) has been developed for many types of solid tumors including Osteosarcoma (OS) and Ewing Sarcoma (EwS), for studying lung metastasis as well as drug tests, as it provides a better microenvironment compared to other 2D and 3D models. Cancer cells with fluorescent tags were injected into mice by tail vein injection and allowed to settle for hours to days. Fluorescent metastatic cells were then able to interact with this framework and subsequently develop into metastatic colonies. The lung was then inflated with agarose gel dissolved in media, solidified, and sectioned into 2-3 * 2-3 mm pieces. The lung sections were then incubated with special media and/or drugs for up to 28 days, and the growth of cancer cells was visualized using epifluorescence or confocal microscopy.

Results: In this study, we optimized the methods and conditions of PuMA for CCOC and ENOC cell lines. The assay conditions described in the study allowed for the preservation of the lung structure for more than 21 days and the proliferation of cancer cells for more than 14 days. This method provided a 3D collagen framework with lung epithelial cells, inflammatory cells, and other connective tissues, allowing us to test drugs on CCOC and ENOC cell lines in a model with a more complex microenvironment.

Conclusions: Several CCOC and ENOC metastatic cell lines have been identified and tested in PuMA in this study, and conditions for those cell lines to grow have been optimized. However, further optimizations are needed for the study of cellular stresses and inducible systems.

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Ramin Ebrahim Nakhli

SUPERVISOR(s): Dr. Ali Bashashati

MULTI-STAIN GRAPH TRANSFORMER FOR REPRESENTATION LEARNING OF GIGA-PIXEL HISTOPATHOLOGY IMAGES

Ramin Ebrahim Nakhli / Puria Azadi Moghadam
(graduate student)

Ramin Nakhli[1], Puria Azadi Moghadam[1], Haoyang Mi[2], Hossein Farahani[1], Alexander Baras[2], Blake Gilks[1], Ali Bashashati[1]

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ABSTRACT

Background/objectives: Histopathology images are critical for cancer diagnosis and prognosis, but the complex tissue microenvironment makes it challenging to extract meaningful insights from these images. Most machine learning studies in this context focus on small image patches and do not consider the role of individual cells. In this study, we aim to develop a machine learning model that focuses on cellular interactions within the tissue to improve the prediction of patients' survival outcomes. Our objective is to demonstrate the applicability of cell-based processing in survival outcome prediction and stratification of patients, separating them into low- and high-risk groups based on their predicted survival outcomes for clinical management.

Methods: We proposed a multi-stain graph neural network (GNN) to process immunohistochemistry (IHC) images by focusing on cells and their interactions. We aimed to develop an end-to-end trainable model that can efficiently encode complex interactions between cells, tissues, and microenvironments, which play a crucial role in cancer development and progression. Using IHC images from different biomarkers, we extracted cellular graphs by identifying individual cells within each image, using each cell as a node, and connecting them based on proximity to build graphs from cells in the tissue. We processed and combined the cellular graphs of a patient from different stains using novel techniques, including Shared-Context Processing and Batch Censor Portion, for survival prediction. We tested our model on two high-grade serous ovarian and muscle-invasive bladder cancer datasets, including 188 and 58 patients, respectively, in a 3-fold cross-validation setting.

Results: Our proposed model outperformed all other methods in both datasets, achieving a higher concordance index (c-index) than patch-based models. Our model was able to extract the microenvironment structure from cellular graphs, highlighting the importance of cellular interactions. Our model remained highly robust to data sparsity, achieving similar performance with as low as 20% of the data during training, making it computationally tractable. Our model also successfully stratified patients into low- and high-risk groups based on their predicted survival outcomes using only the IHC images (as the first study to do so on high-grade ovarian cancer cases), highlighting its potential clinical utility.

Conclusions: Our study demonstrates the importance of considering cellular interactions for image representation in histopathology analysis. Our multi-stain GNN, which focuses on cells and their interactions, outperforms state-of-the-art methods in predicting survival outcomes in high-grade serous ovarian and bladder cancer patients. Our findings suggest that this approach can be used for patient stratification, offering a new tool for facilitating patient management. Future work could explore the use of our model for linking histopathology images to genomic information, which could lead to new biological insights and potential biomarkers for cancer diagnosis and prognosis.

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Maria Elishaev

SUPERVISOR(s): Dr. YING WANG

USING A NEW MULTIPLEX IMAGING PLATFORM TO VISUALIZE INCREASED INFLAMMATION ASSOCIATED WITH CHOLESTEROL OVERLOAD IN HUMAN ATHEROSCLEROTIC LESIONS

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(graduate student)

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ABSTRACT

Background/objectives: Cardiovascular events are a leading cause of death, taking an estimated 17.9 million lives globally every year. Most cardiovascular events are the result of advanced atherosclerotic lesions that start with the formation of lipid-laden foam cells derived from smooth muscle cells (SMCs) and macrophages. Macrophages have been considered a primary source of foam cells and have always been the target of therapeutic development, but recent studies demonstrated that at least 50% of all foam cells in atherosclerotic lesions come from SMCs. There are multiple indications that SMC foam cells are less efficient in removing cholesterol than macrophage foam cells. However, it is not clear whether these defects in SMC foam cells will stimulate cell death and account for inflammation that leads to disease progression and cardiovascular event onset. Conventional tissue staining methods used in previous studies can only visualize three protein markers per tissue section, which is not adequate to characterize the features of foam cells and inflammation in human atherosclerotic lesions. In this project, I will apply a novel multiplex imaging technology that visualizes multiple protein markers using barcoded antibodies and repeated sequential imaging cycles to move beyond these limitations. The objective of this study is to characterize the capability of cholesterol removal of SMC and macrophage-derived foam cells and determine whether cholesterol overload in SMCs is associated with increased cell death and inflammation, which need to be therapeutically targeted to prevent cardiovascular events.

Methods: 60 coronary artery samples from the Bruce McManus Cardiovascular Biobank were sectioned and stained to categorize histological stages of atherosclerosis. Based on single-cell RNA-sequencing data, we designed a unique 12-plex antibody panel to characterize cell phenotypes and inflammation markers using the PhenoCycler imaging platform. To generate this panel, selected antibody clones were conjugated to oligonucleotide barcodes and validated on adjacent tissue sections by immunostaining to confirm their antigen recognition after conjugation. After PhenoCycler imaging, we performed HALO cell segmentation analysis to characterize the foam cells.

Results: 14 early-stage, foam cell-rich lesion samples have been selected for the PhenoCycler imaging. We identified macrophage and SMC foam cells using multiple cell lineage markers and lipid staining. Next, we will measure the expression of proteins related to cholesterol removal, cell death, and inflammation. We expect to see that defects in cholesterol removal in SMC foam cells are associated with cell death and increased inflammation.

Conclusions: This is the first time the relationship between cholesterol overload and inflammation in human atherosclerotic lesions is defined, enabled by a multiplex imaging platform, which is superior to conventional imaging technologies. The results of this study can reveal that in addition to macrophage foam cells, cholesterol-overloaded SMCs are another major source of inflammation, which should be studied further for therapeutic development.

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Mohammad Ghodsi

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EXPLORING UTILITY OF NEUROLOGICAL BLOOD-BASED BIOMARKERS TO IMPROVE DIAGNOSIS OF ACUTE BRAIN INJURY DUE TO INTIMATE PARTNER VIOLENCE

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ABSTRACT

Background/objectives: It is estimated that over 200,000 Canadian women experience intimate partner violence (IPV)-caused brain injury (BI) every year. BI caused by IPV (IPV-BI) can occur from non-fatal strangulation, head impact, or a combination of both. It is likely that the prevalence of BI amongst IPV survivors is underestimated due to the lack of sensitive and objective diagnostic tools, as well as hesitancy to seek medical treatment after IPV. This lack of appropriate diagnosis can negatively affect survivors' ability to seek treatment, recover, and thrive in their personal, professional, and social lives. These observations highlight the immediate need for objective, feasible and IPV-sensitive diagnostic tools to identify and characterize BI amongst survivors of IPV. Blood-based neurological biomarkers are emerging as a revolutionary new tool for objective examination of BI and its severity in many neurological indications such as sport related concussions, spinal cord injuries, and hypoxic-ischemic brain injuries. However, despite their prominent research use, we do not yet know the extent to which the levels of biomarkers of IPV-BI may resemble the levels of biomarkers of BI due to sport injuries, motor vehicle accidents (in the case of head impact due to IPV), hypoxic-ischemic injuries (in the case of strangulation due to IPV), or whether having both injuries would uniquely alter the biomarker signals. We also do not yet know how IPV-associated psychological stressors may modify biomarker response. In this study, we will explore the utility of blood-based neurological biomarkers to improve the diagnosis of acute IPV-BI.

Methods: We will conduct a prospective observational study of individuals who have experienced IPV within the previous 30 days. Based on previous reports, we anticipate enrolling 240 individuals within the first two years of the study, expecting approximately 75% of whom will have symptoms consistent with BI. Clinical assessment and a questionnaire will be used to identify participants with suspicion of IPV-BI, as well as whether the suspected IPV-BI is inflicted through head impact, strangulation, or a combination of both. Plasma collected from participants will be analysed using ultrasensitive digital immunoassay technology for neurofilament-light (marker of neuroaxonal damage), glial fibrillary acidic protein (marker of astrocyte activation and inflammation), phosphorylated tau, and brain-derived tau (markers of neurofibrillary tangle formation and chronic traumatic encephalopathy). Biomarker concentrations will be compared between survivors of IPV with suspected BI to those without, as well as between the survivors of IPV-BI with both head impact and strangulation to those with head impact or strangulation alone. Plasma biomarker concentrations will also be compared to valid Canadian normative data.

Conclusions: This study will be an important step in the implementation of blood-based biomarkers as an accessible and objective tool to improve diagnosis of acute IPV-BI. This tool will have the potential to readily inform and help survivors of IPV-BI seek appropriate treatment and recovery supports they need.

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Amir Hadjifaradji

SUPERVISOR(s): Dr. ALI BASHASHATI AND DR. JONATHAN LOREE

DEEP LEARNING FRAMEWORK FOR CLASSIFICATION OF NEUROENDOCRINE TUMOUR WHOLE SLIDE IMAGES

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ABSTRACT

Background/objectives: Neuroendocrine tumours (NETs) are rare neoplasms which can arise in cells across the body. The last few decades have seen NET incidences increase by 7-folds, putting a larger demand on patient care. NETs are categorized by their site of origin, differentiation, and grade. Grades are morphologically indistinguishable for well-differentiated tumours and are determined from the tumour proliferation, which is measured by mitotic count and Ki-67 Index. Unfortunately, these measures suffer inter- and intra-observer variability, and are cumbersome tasks for pathologists. To mitigate these challenges, we developed a novel machine learning framework to identify candidate mitotic figures, calculate the Ki-67 index, and build features from the proliferation to grade NETs.

Methods: Our study included 186 cases with 385 samples of multi-centre, multi-organ NET tissues with two different stains (247 H&E and 138 Ki-67 images) and patient-level labels for grade. A pathologist annotated 80 slides for tumour areas, which helped train a tumour-normal classifier and was applied to generate pseudo-labels for the remainder of our dataset. An object detection model was trained on a publicly available external dataset (MIDOG2022) with 354 cases from 5 different cancers annotated for mitotic figures and applied to our data. Based on the mitotic figures, we generated density maps of mitotic activity in 2mm2 areas and created a histogram from these maps for each slide. A similar approach for aggregating slide information was used with Ki-67 slides, whereby an image-processing approach was used for each tumour patch to calculate the Ki-67 index, and a histogram of indices was generated for each slide. Both histograms were fed into a feedforward network to assess the grade of the tumour.

Results: The H&E portion of the framework achieved a balance accuracy of 72.1% across 6-folds for three-class classification (G1, G2, G3). Stratifying patients based on our model's predictions, we demonstrated that the survival outcomes for patients are comparable to the pathologist's assessment, with c-index values of 0.63 and 0.64, respectively. Analysis of the survival outcome for pathologist assessed H&E G1s demonstrated significant (p-value=0.01) separation amongst the misclassified G1s (n=28; median survival 4.88 years) and correctly classified G1s (n=62; median survival 7.78 years). When compared to pathologist's true grade assessment (both stains), the separation exists but is no longer significant (p-value=0.05). Our Ki-67 algorithm achieves a balance accuracy of 83.9% on Ki-67 slides, with Ki-67 added to our pipeline, grading improves the balance accuracy to 78.5%.

Conclusions: By identifying both mitotic figures and Ki-67 positive cells, our method may provide value as a tool for pathologists to verify hot-spots in both H&E and Ki-67 slides for further analysis of grades. Our framework was able to grade H&E NET slides with a balance accuracy of 72.1%, and was able to further improve grading with Ki-67 by 6.5%. Misclassified G1 patients have lower median survival, and further analysis could help determine new treatment options for better prognosis.

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Cyril Helbling

SUPERVISOR(s): Dr. MARI DEMARCO

DIAGNOSTIC PERFORMANCE OF ALPHA-SYNUCLEIN SEED AMPLIFICATION ASSAYS: A META-ANALYSIS

Cyril Helbling
(graduate student)

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ABSTRACT

Background/objectives: Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA)—collectively termed synucleinopathies—are defined pathologically by the aggregation of alpha-synuclein in the central and peripheral nervous systems. Misdiagnosis is common in these disorders, particularly in the early stages of disease, given the syndromic focus of current clinical diagnostic criteria and given that early symptoms overlap with other disorders. Thus, a biomarker diagnostic tool that enables early identification of alpha-synuclein pathology is highly desired. Seed amplification assays (SAA) are ultrasensitive methods that detect protein aggregates in human specimens and have shown great promise in the detection of synucleinopathies. Most of the current literature that utilizes SAA, differentiates synucleinopathies from a single cohort including both healthy controls and disease mimics (DM), which is not representative of a clinically relevant situation. Thus, we performed a meta-analysis to assess the diagnostic performance of these assays for synucleinopathies and reanalysed literature data by grouping into clinically relevant cohorts.

Methods: A database search was conducted for alpha-synuclein SAA studies on PD, DLB, or MSA using cerebrospinal fluid (CSF), skin, or olfactory mucosa specimens. Diagnostic accuracy, which combines the sensitivity and specificity in one metric ranging between 0 and 1, was calculated to enable comparison between studies, along with 95% confidence interval. The control group was divided into DM, which includes other neurodegenerative diseases (e.g., Alzheimer's disease), and non-neurological controls (NC; i.e., control cases without neurodegenerative disease) to assess diagnostic performance in clinically relevant cohorts.

Results: Of the 412 studies identified, 43 were included in the analysis based on our inclusion/exclusion criteria. SAA demonstrated the following average diagnostic accuracies by specimen types and control groups. Using CSF, SAA differentiated synucleinopathies from DM and NC with diagnostic accuracies of 0.87 [0.83-0.91] and 0.91 [0.88-0.94], respectively. Using skin, SAA differentiated synucleinopathies from DM and NC with diagnostic accuracies of 0.85 [0.81-0.90] and 0.91 [0.88-0.94], respectively. Using olfactory mucosa, SAA differentiated synucleinopathies from DM and NC with diagnostic accuracies of 0.83 [0.76-0.89] and 0.77 [0.67-0.86], respectively.

Conclusions: Alpha-synuclein SAA demonstrate encouraging diagnostic performance characteristics for the detection of synucleinopathies from other neurodegenerative disorders. For CSF and skin, the significant differences observed in diagnostic accuracy between DM and NC highlights the need to analyze SAA results by clinical-relevant cohorts. Notably, many of the skin and olfactory mucosa groups had an insufficient number of studies to draw strong conclusions. Overall, more studies that closely matching the clinical scenarios and situations in which testing would be deployed in medical care are needed; for instance, examining patients in the early symptomatic phase and utilizing prospective, longitudinal study designs.

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Rebecca Ho

SUPERVISOR(s): Dr. DAVID HUNTSMAN

TARGETING METABOLIC REPROGRAMMING IN ARID1A AND ARID1B DUAL-DEFICIENT DEDIFFERENTIATED ENDOMETRIAL CARCINOMA

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ABSTRACT

Background/objectives: Dedifferentiated endometrial carcinoma (DDEC) is a rare endometrial cancer histologically characterized by an undifferentiated portion and a differentiated endometrioid carcinoma component. DDEC is an aggressive cancer with poor patient outcomes, which are further exacerbated if the tumor has switch/sucrose non-fermentable (SWI/SNF) complex mutations. The SWI/SNF complexes are a family of chromatin remodelers that regulate gene expression. They consist of three subfamilies: cBAF, pBAF, and ncBAF, each composed of shared and unique subunits that assemble to form one large protein complex. Mutations in these SWI/SNF subunits are implicated in the development and progression of a multitude of cancers.

Our lab has shown that the loss of all three SWI/SNF complexes alters metabolism in some ovarian and lung cancers. Instead of glucose metabolism, these cancers rely on mitochondrial and glutamine metabolism to fuel their growth. This shift in metabolic dependencies can be targeted by specific inhibitors as potential therapies for people with these cancers. In DDECs, however, rather than complete loss of the SWI/SNF complexes, a proportion of this cancer only has reduced cBAF function due to dual loss of ARID1A and ARID1B, two DNA binding subunits specific to this complex. As such, we want to establish the generalizability of our previous observation and extend our findings to DDECs to determine if these metabolic changes are mediated by cBAF. Thus, for this study, we hypothesize that ARID1A/B dual-deficient DDECs depend on mitochondrial metabolism to sustain their growth.

Methods: Endometrial cancer cell lines a) expressing ARID1A and ARID1B, b) expressing only ARID1B, and c) not expressing either ARID1A or ARID1B are treated with inhibitors that target mitochondria metabolism (IACS-010759). Cell viability is measured seven days (short-term) or one and a half weeks to three weeks (long-term) after drug treatment.

Results: Endometrial cell lines with ARID1A and ARID1B dual loss are more sensitive to the mitochondrial metabolism inhibitor compared to the other cell lines. This suggests that endometrial cell lines lacking ARID1A and ARID1B (or the cBAF complex as a whole) may have an increased reliance on mitochondrial metabolism.

Conclusions: This study will address how generalizable mitochondrial metabolism dependency is in cancers with SWI/SNF mutations and, as a result, identify additional individuals that can also benefit from similar therapies. Our preliminary data indicate that ARID1A/B dual-deficient DDECs may have similar metabolic dependencies as cancers lacking all three SWI/SNF complexes. Future work will involve functional validation by measuring the level of glucose and mitochondrial metabolism in our panel of endometrial cell lines. Furthermore, ARID1A and ARID1B will be re-expressed or knocked out in our cell lines to identify additional metabolic genes regulated by cBAF and validate if this metabolic shift is mediated by these proteins.

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Emma Kang

SUPERVISOR(s): Dr. Dana Devine and Dr. Christian Kastrup

ACHIEVING mRNA-LIPID NANOPARTICLE TRANSFECTION OF DONOR PLATELETS IN CLINICALLY RELEVANT SYSTEMS

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ABSTRACT

Background/objectives: Failure to control bleeding accounts for more than half of all operating room deaths and is a leading cause of preventable deaths from trauma. Platelet transfusions are an integral therapy for managing bleeding and hemostatic dysfunction. Given their role in hemostasis and disease, genetically enhanced platelets have a lot of potential in clinical settings, however, there currently are no methods to genetically engineer platelets. Recent advancements in lipid nanoparticle (LNP) technologies have improved delivery efficacy of RNA products into its target cells, and is being used in COVID-19 vaccines. Recently, we have demonstrated that optimized LNPs can be used to engineer platelets to express exogenous cargo. However, this platform was developed using Tyrode's buffer, a crystalloid solution that is not clinically used and causes unwanted platelet activation. Currently, platelets are either stored in plasma or platelet additive solution (PAS) with plasma. In both storage mediums, plasma proteins are present which have been reported to form a protein corona around LNPs, affecting nanoparticle uptake and their interactions with target cells. Preliminary data shows that our current platelet optimized LNPs are not capable of transfection when plasma proteins are present. Therefore, in this study we will 1) identify LNPs that are capable of transfection in the clinically relevant buffers such as PAS and a combinations of PAS and plasma (PAS:plasma), 2) characterize retained function of LNP modified platelets in vitro and 3) evaluate the storability of LNP modified platelets in plasma and PAS:plasma over the standard platelet storage period of 7 days.

Methods: Obj. 1) The ionizable and helper lipid components of an LNP have an important effect on the type of protein corona that is formed. Various combinations of lipids will be screened for platelet transfection in plasma and PAS:plasma using a NanoLuc luciferase reporter system. Obj. 2) Platelets engineered with candidate LNPs will be functionally characterized using common platelets tests. We will test for activation and apotosis that may be induced by LNP engineering and use rotational thromboelastometry to evaluate retained in vitro function. Obj. 3) The assays mentioned will then be used to characterize LNP engineered platelet functions over a storage period of 7 days..

Results: Preliminary data demonstrate that transfecting in a combination of PAS:plasma is possible. Several candidate ionizable lipids formulated with other LNP components to encapsulate NanoLuc mRNA resulted in variable exogenous protein expression when transfection occurred in PAS and plasma. Compared to Tyrode's transfection, LNP delivery in PAS:plasma protected from unwanted platelet activation. For future studies, we will screen for the lipids with different time points as well as characterize platelet function and storability.

Conclusions: Optimizing our current lipid nanoparticle transfection platform to engineer platelets directly in their clinical storage medium presents an opportunity for seamless clinical integration which will be important for expanding platelets as a cell therapy.

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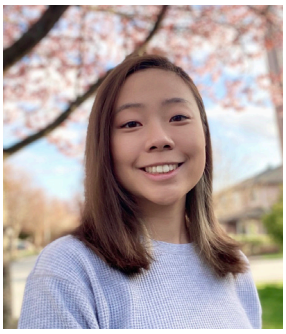

Fang Fang Li

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DETECTION OF ENTEROVIRUS D ANTIBODIES IN CEREBROSPINAL FLUID OF PATIENTS WITH NEUROLOGICAL SYMPTOMS

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ABSTRACT

Background/objectives: Acute flaccid paralysis (AFP) describes an acute onset of muscle weakness. While there are many potential causes of AFP, enterovirus (EV)-D infection is a cause observed in children that is often supported by molecular EV-D testing. However, viral detection by PCR in cerebrospinal fluid (CSF) is often negative, creating a need for antibody testing to supplement. Here, we sought to validate the ability of VirScan, a novel serological profiling method, to accurately identify anti-EV-D IgG in CSF and demonstrated its utility in the clinical environment to link neurological symptoms with viral infections.

Methods: We linked anti-EV-D IgG reactivity in CSF with neurological symptoms and assessed assay performance of EV-D detection by assembling a validation panel of clinical cases to compare antibody reactivities against EV-D and other respiratory viruses in CSF of PCR confirmed EV-D cases with neurological symptoms against CSF from individuals with neurological symptoms but no known EV-D infection. To detect antibody reactivity in CSF, we used VirScan, a phage-display library presenting viral epitopes of 206 viruses. Briefly, phages captured antibodies present in sample and epitope-specific reactivities of bound antibodies were determined by next-generation sequencing and bioinformatic analysis.

Results: We assembled a validation panel consisting of two EV-D cases with neurological symptoms that were PCR+ on nasopharyngeal swab and PCR- in CSF. One case had paired serum/CSF available. Negative controls consisted of CSF from patients 1) PCR+ for non-EV viruses in CSF (n=2); 2) PCR+ for non-D-group EV in CSF (n=2); and 3) PCR- for any virus in CSF collected during non-EV-D season (n=5). Preliminary results in the EV-D+ case with paired serum/CSF found highest antibody reactivity with respect to all EV-D epitopes in the following: capsid protein VP4 (serum: 106.77; CSF: 89.99), and non-structural proteins 3A (serum: 59.60; CSF: 76.31) and 3D (serum: 62.06; CSF: 77.47). The ratio of averaged normalized EV-D enriched epitope hits between serum and CSF was 11:1 — lower than typically observed — suggesting local central nervous system antibody production.

Conclusions: We have demonstrated the utility of VirScan in the clinical setting to link neurological symptoms with viral exposures to support diagnosis. Our work so far suggests that VirScan can be used to detect anti-EV-D IgG in CSF when viral diagnostic yield is low. Remaining work will focus on VirScan specificity in antibody reactivity detection and expanding our validation panel.

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Yi Fei Liu

SUPERVISOR(s): Dr. Samuel Aparicio

MUTATIONAL SIGNATURE ANALYSIS STRATIFIES PATIENT-DERIVED XENOGRAFT DRUG RESPONSE TO G-QUADRUPLEX STABILIZERS

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ABSTRACT

Background/objectives: Triple negative breast cancer (TNBC) and high grade serous ovarian cancer (HGSOC) have poor prognosis and limited treatment options, in part due to the inter- and intra-patient heterogeneity. Standard care is still chemotherapy, with the use of PARP inhibitor targeted therapy limited to the subset of patients who carry a predictive biomarker, such as BRCA inactivation. Identification of common genetic vulnerabilities shared by patient subgroups will be crucial for developing novel treatment options in these diseases. Patients are stratified by DNA repair mutational signature backgrounds, notably homologous recombination deficient (HRD) and enriched for foldback inversion (FBI) subtypes. Previous work has shown the sensitivity of the HRD subtype to a G-quadruplex (G4) DNA stabilizer which was recently pursued in a phase Ib trial in Canada. It has been proposed that the engagement of G4 sites in the genome further modulates DNA methylation, which is a repressive mark of transcription in promoter regions. Histone modifications also contribute to chromatin accessibility, such as acetylation at lysine 27 of histone 3 (H3K27ac) which is a marker of transcriptional activation. However, the relationship between G4 sites, DNA methylation, and chromatin accessibility is not well understood. We hypothesize that unique G4 ligands have different sensitivity in distinct repair backgrounds. We further hypothesize that repair backgrounds will be associated with distinct patterns of DNA methylation and H3K27ac.

Methods: Patient-derived xenograft (PDX) lines are established by serial transplantation of tumour tissue into immune deficient mice. PDX lines are stratified by mutational subtypes using a correlated topic model on SNV and SV counts derived from whole genome sequencing. Short term in vitro drug studies are carried out for organoids established from PDX lines of known repair backgrounds using an inhouse panel of DNA binding agents. Nanopore sequencing is used to assess DNA methylation. Cleavage under targets and tagmentation is used to determine H3K27ac.

Results: We demonstrate that G4 ligands have different patterns of sensitivity in vitro across PDX lines, with either similar concentration-response between HRD or FBI backgrounds, or increase sensitivity in FBI across 4 compounds of this class. We detected and co-mapped DNA methylation and H3K27ac to their genomic sites in these PDX lines, as markers of transcriptional activity. In normal, wild-type epithelial cells, DNA methylation and H3K27ac peaks are strongly associated across genomic sites. In HRD deficient backgrounds, this association is no longer observed.

Conclusions: We show there is epigenetic rewiring between DNA methylation and histone modifications in HRD backgrounds, pointing to a role in disease pathogenesis. We also show the sensitivity to G4 stabilizers across HRD backgrounds and FBI backgrounds, with modulation of sensitivity by the repair background. Further characterization of the key targets involved in the response to G4 stabilizers will contribute to elucidating the mechanism of action in TNBC and HGSOC.

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Peyman Malek mohammadi

SUPERVISOR(s): Dr. JAYACHANDRAN KIZHAKKEDATHU

NOVEL IMMUNOMODULATORY BLADDER CELL SURFACE ENGINEERING APPROACH FOR THE TREATMENT OF INTERSTITIAL CYSTITIS/ BLADDER PAIN SYNDROME

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ABSTRACT

Background/objectives: Interstitial Cystitis/ Bladder Pain Syndrome (IC/BPS) is a poorly understood condition, which affects nearly 470 per 100,000 population in US, mostly women. Major symptoms of IC/BPS include bladder pain, frequency, urgency, and incontinence. Moreover, its etiology is still a matter of debate. That is why the current treatments of IC/BPS only target the symptoms and not the underlying cause, leading to only temporary symptom relief and subsequent relapse.

The damage associated with bladder cell wall glycosaminoglycan (GAG) protective layer, comprised mainly of proteoglycans like mucins, is commonly mentioned as one of the main causes. Epithelial GAG layer shedding can lead to diffusion of solutes, urea, and bacteria into the underlying bladder tissue, causing inflammation, and stimulating the afferent nerves, leading to pain, frequency, urgency, and other symptoms. Thus, rescuing or rebuilding the bladder epithelial GAG layer can be an effective approach to treat IC/BPS in long-term. In this study, we hypothesize that cell surface engineering (CSE) of bladder epithelial cells and the bladder using mucin-like polymers can recover epithelial GAG layer function upon injury, reduce inflammation and treat IC/BPS over long-term.

Methods: To synthesize mucin-like immunomodulatory polymers, α-azido linear polyglycerol (LPG-N3) will be synthesized as the backbone. Next, several hydroxyl groups on the backbone will be propargylated, followed by the reduction of the azide end group to an amine group. Subsequently, a glutamine-containing peptide will be conjugated to the end of the polymer chain for cell surface engineering. Finally, sialic acid-containing sugars will be conjugated via click-chemistry to the propargylated side groups, to induce mucin-like as well as immunomodulatory properties. Human urinary bladder epithelial cells (HTB4) will be used as the in vitro cell model in this study. HTB4 cells will be engineered by mucin-like immunomodulatory polymers via an enzymatic approach using guinea pig liver tissue transglutaminase (gtTGase). The ligation can be done by simply incubating the cells with Q-peptide containing polymer, gtTGase, glutathione, and calcium chloride for 30 minutes at 37 °C.

Results: An enzymatic cell surface engineering approach using immunomodulatory glycopolymers has already been implemented to prevent immune-mediated rejection of organ transplants in our group recently. Additionally, preliminary studies on human urinary bladder cells have shown that using low molecular weight sulfated LPG, this CSE approach can reduce inflammation caused by an in vitro insult model. These findings will help with developing novel immunomodulatory mucin-like polymers for epithelial surface engineering approaches to treat IC/BPS.

Conclusions: The CSE approach using novel mucin-like immunomodulatory polymers is the first method of its kind to treat IC/BPS, targeting its underlying cause, potentially leading to long-term effect. This approach can increase the quality of life of people living with IC/BPS and eliminate the need for uncomfortable and frequent instillations of therapeutics, or use of oral treatments.

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Jessica Oliveira de Santis

SUPERVISOR(s):

eEF2K INHIBITION PROMOTES CYTOTOXICITY AND SUPPRESSES MALIGNANT PHENOTYPES OF HIGH-RISK PEDIATRIC MEDULLOBLASTOMA CELLS

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ABSTRACT

Background/objectives: Medulloblastoma (MB) is the most common pediatric cancer of the central nervous system. Molecular classification defines Group 3 and TP53-mutated SHH as high-risk tumors, with a 40-50% five-year overall survival. Cells of high-risk tumors frequently regulate protein translation through eEF2K, an essential gene activated to hold energy intake and survive under stressful conditions. Thus, we investigated the association of eEF2K expression with clinical features of worse prognosis and the effects of eEF2K modulation as a therapeutic approach in pediatric MBs.

Methods: We evaluated associations between clinical features and eEF2K expression using public datasets of pediatric MBs (GSE85217, n=628). Genes coregulated with eEF2K and enriched pathways were considered when $p<0.01$. We tested a compound able to module eEF2K expression (NH125) in combination with Cisplatin in MB cells (USP-13 and UW228).

Results: We identified that eEF2K is upregulated in G3 MB and that its overexpression is associated with lower overall survival and metastasis ($p<0.001$). Among genes positively correlated with eEF2K in MB, we found USP2 ($r=0.8$) and OTX2 ($r=0.72$), associated with poor prognosis in G3 MB; DOCK9 ($r=0.58$) and EPHA8 ($r=0.55$), involved with invasion in MB. We found an enrichment of neurodevelopment and neuronal metabolic pathways. In vitro assays showed that NH125 inhibited eEF2K activity in MB cells through decreased phosphorylation of its substrate, eEF2. Treatment with NH125 also significantly reduced cell viability, colony formation, and invasion and altered apoptosis and autophagy. Combined therapy results with Cisplatin showed that adding NH125 increased the drug's cytotoxicity, lowering up to five times the chemotherapeutic dose to ensure cell death.

Conclusions: These results support eEF2K as associated with poor prognosis in high-risk MB and its modulation presenting with antitumor effects in MB cell lines, suggesting eEF2K as a promising therapeutic target for high-risk MB.

Keywords: Medulloblastoma, translational control, eEF2K, combined therapy.

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Khushbu Patel

SUPERVISOR(s): Dr. MARI DEMARCO

CANADIAN PATIENT AND CARE PARTNER PERSPECTIVES ON DIAGNOSTIC BIOMARKER TESTING FOR ALZHEIMER'S DISEASE

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ABSTRACT

Background/objectives: It is well understood that the use of core Alzheimer's disease biomarkers (amyloid-beta peptides and tau proteoforms) in diagnostically challenging cases can improve the accuracy of an Alzheimer's diagnosis. In the context of cerebrospinal fluid (CSF) testing for Alzheimer's biomarkers, we have a limited understanding of the impact and personal value of biomarker testing to patients and their family and friends ('care partners'). Therefore, our study aimed to describe patient and care partner experiences with Alzheimer's disease CSF biomarker testing in medical care.

Methods: 'Investigating the Impact of Alzheimer's Disease Diagnostics in British Columbia' (IMPACT-AD BC, www.impactAD.org; NCT05002699) is an observational, longitudinal study examining the impact of Alzheimer's disease CSF biomarker testing on medical decision-making, health economics, and patients and their families. Testing was ordered by physicians when the clinical scenario met the appropriate use criteria for CSF biomarker testing, and patients underwent testing as part of their medical care (n=142). Phone interviews were conducted with a subset of these patients (n=34) and separately with their nominated care partners (n=31). 'Initial' post-disclosure interviews were performed 1 month (median [range]: 1.2 [0.7-2.3]) after result disclosure, with 'follow-up' interviews 6 months (median [range]: 6.0 [5.2-10.3]) after disclosure. Thematic content analysis was used to identify recurring patterns (themes) and frequencies of specific responses to investigate the effects of biomarker testing on lifestyle, financial and care planning decisions, and the resource and support needs of patients and care partners.

Results: The majority of patients (82%) reported overall positive feelings post-disclosure. The most common reasons for feeling positive included gaining certainty and more information around their diagnosis and having the ability to plan ahead. A few patients (7%) reported negative feelings post-disclosure, stemming from concern about progression of their cognitive symptoms. Most patients utilized the biomarker information to make positive health behavior changes, including increased physical exercise or encouragement to continue exercise (84%), and starting or continuing healthy dietary practices (59%). Common themes in care partner responses included an increased awareness of their future caregiving responsibilities (37%) and relief in having added information on the patients' health to make decisions for the future (26%).

Conclusions: Alzheimer's disease CSF biomarker results are highly valued by individuals undergoing testing and their care partners, particularly in making positive lifestyle changes and planning for the future. Our findings highlight a post-result disclosure time interval when patients and families are highly receptive to health and wellbeing-related interventions. This study has also identified important considerations during pre- and post-biomarker counselling procedures, and in the development of new resources to support patients and caregivers navigate living with dementia.

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Tetiana Povshedna

SUPERVISOR(s): Dr. Helene Cote

LIVING WITH CHRONIC PAIN: EXPERIENCES OF WOMEN LIVING WITH HIV AND HIV-NEGATIVE WOMEN ENROLLED IN THE BRITISH COLUMBIA CARMA-CHIWOS COLLABORATION (BCC3) STUDY

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ABSTRACT

Background/objectives: Chronic pain (CP) is a major cause of disability worldwide and can be associated with biological and psychogenic factors. It is prevalent among people living with HIV and when underdiagnosed/undertreated, can affect adherence to care, wellbeing, and ultimately, aging. We aimed to compare CP-related experiences among women living with HIV (WLWH) and HIV-negative controls (≥16y) in the BCC3 study to address the existing gap in women-specific HIV research. We hypothesized that CP is more prevalent among WLWH compared to controls and is associated with social determinants of health.

Methods: We used the Brief Chronic Pain Questionnaire (BCPQ) to screen for CP, and validated surveys to describe CP, psychological distress, and social support (Table 1). Groups were compared using t-, Chi-Squared, Mann-Whitney tests and logistic regression. Associations with CP were investigated using Spearman's correlation.

Results: Age-adjusted prevalence of CP was not different between WLWH (58/151;38%) and controls (73/230;32%), p=0.4, nor was the age of WLWH and controls with CP in both groups (Table 1). While women in both groups shared many pain characteristics and experiences, including pain intensity, interference, ability to cope, and number of pain locations (Table 1), WLWH reported higher social support. In both groups, CP intensity was high but showed no association with psychological distress. However, higher social support was associated with lower CP intensity among controls (rho=-0.3, p=0.02) but not WLWH (p=0.9).

Conclusions: In this study of WLWH and well-matched controls, 1/3 of women reported chronic pain in both groups but few differences were observed between groups with respect to CP or pain-related experiences. However, CP intensity and psychological distress were high in both groups, warranting further research and clinical attention to chronic pain to support healthy aging in women.

Table 1. Chronic pain characteristics and associated experiences among women living with chronic pain

Parameter	WLWH n=58	HIV-negative controls n=73	p value
Age (years), median [IQR]	51 [46-59]	51 [37-60]	0.22
Pain, Enjoyment of Life, and General Activity (PEG) Scale			
Pain on average last week (0-10 scale), mean ± SD	6.3 ± 2.2	6.5 ± 2.0	0.74
Pain interference with enjoyment of life (0-10 scale), mean ± SD	6.0 ± 2.6	6.0 ± 2.5	0.94
Pain interference with general activity (0-10 scale), mean ± SD	6.1 ± 2.6	6.4 ± 2.6	0.56
Body manikin for pain localization			
Number of body regions affected, median [IQR]	7 [3-13]	7 [3-11]	0.99
Pain Self-Efficacy Questionnaire (PSEQ)			
"I can cope with my pain in most situations", (0-6 scale), mean ± SD	3.9 ± 1.6	4.3 ± 1.4	0.13
"I can still do many of the things I enjoy doing, such as hobbies or leisure activity, despite the pain", (0-6 scale)	3.4 ± 1.8	3.9 ± 1.4	0.09
"I can still accomplish most of my goals in life, despite the pain, (0-6 scale)	3.7 ± 1.8	3.7 ± 1.6	0.89
"I can live a normal lifestyle, despite the pain", (0-6 scale)	3.6 ± 2.0	3.6 ± 1.7	0.90
Selected social determinants of health			
The Kessler Psychological Distress Scale (K6), n (%)			
5 ≤ K6 < 13 (moderate mental distress)	22 (38)	33 (46)	0.33
K6 ≥ 13 (severe mental illness)	18 (31)	22 (31)	0.99
Medical Outcome Study Social Support Survey (MOS-SSS), 0-20 scale, median [IQR]	15 [12-18]	12 [9-17]	**0.01**

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Tali Romero

SUPERVISOR(s): Dr. Cheryl Wellington and Dr. Thalia Field

ASSOCIATION BETWEEN NEUROLOGICAL BLOOD BIOMARKERS WITH BASELINE NEUROIMAGING AND COGNITIVE ASSESSMENTS IN ADULTS WITH MODERATE-SEVERE CONGENITAL HEART DISEASE

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ABSTRACT

Background/objectives: Recent advances in medical and surgical practices have increased the median lifespan of adults with severe congenital heart disease (CHD) by almost 20 years. To date, the vast majority of research on people with CHD has focused on cardiac or neurodevelopmental outcomes of the disease, and there have not been any longitudinal studies that investigate the long-term effects of CHD on brain health in this growing cohort of adults. Recent epidemiological research suggests that adults with CHD are at increased risk of dementia, and cross-sectional studies of adolescents and young adults indicate increased risks of impaired neurocognition. These observations highlight the need for future research aimed at investigating the pathological effects of CHD on brain health to determine whether these deficits are driven by early-life insults, ongoing accumulating injury, or both. Studies in other neurological indications have demonstrated the potential of blood biomarkers as cost-effective, minimally invasive, and sensitive tools to detect and quantify brain injury. This study will measure neurofilament light (NF-L) as a marker of neuronal and axonal injury and glial fibrillary acidic protein (GFAP) as a marker of astrocytic activation and vascular damage. The objectives of this study are to determine whether neurological blood biomarker concentrations in adults with moderate-severe CHD are associated with other indices of brain injury, such as neuroimaging markers and cognitive assessments. We also seek to assess if neurological blood biomarker concentrations differ between individuals with moderate versus severe lesions and how these individuals with CHD compare to healthy age-matched controls.

Methods: Data and biospecimens will be collected through the StrokE and vAscular Risk factors contributing to neuroCognitive decline in adult congenital Heart disease (SEARCH) study, a 3-year longitudinal observational study enrolling 400 participants with moderate-severe CHD. Upon enrolment, these participants undergo baseline cognitive testing (Montreal Cognitive Assessment and NIH Toolbox Cognitive Battery), an MRI, and a blood draw. Serum NF-L and GFAP will be quantified using commercial assays on the Quanterix Simoa HD-X, a highly sensitive, semi-automated ELISA platform. Spearman's rank correlation will then be used to assess correlations among biomarker concentrations, neuroimaging, and cognitive assessments. Groupwise differences and comparisons with healthy controls will be analyzed using Mann-Whitney tests.

Results: Thus far, 87 participants have been enrolled in the study and have completed baseline assessments.

Conclusions: Baseline analysis of neurological blood biomarkers and comparisons with neuroimaging and cognitive data will help to assess patterns and risk factors associated with markers of active brain injury. This may identify individuals living with CHD at risk for longitudinal neuroimaging changes and cognitive decline and may identify therapeutic targets for future preventative strategies.

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Josie Setiawan

SUPERVISOR(s): Dr. Marianne Sadar

MUTATION OF TRYPTOPHAN RESIDUES IN THE BINDING SITE OF NOVEL THERAPEUTICS FOR PROSTATE CANCER

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ABSTRACT

Background/objectives: Androgen receptor (AR) is a steroid hormone receptor and a major therapeutic target for prostate cancer. Androgen deprivation and antiandrogen therapies directed at the AR C-terminal ligand-binding domain (LBD) provide initial benefit to patients with advanced prostate cancer, but they eventually progress to lethal metastatic castration-resistant prostate cancer (CRPC). AR N-terminal domain (NTD) inhibitors are a novel class of antagonists that were developed to overcome resistance mechanisms related to the AR-LBD, including splice variants lacking the LBD (AR-V7). While mutations in the AR-LBD have been well-characterized, the impact of NTD mutations on AR transcriptional activity and drug efficacy remains unclear. The mutation W435L was discovered from clinical samples of prostate cancer from patients failing antiandrogen treatment. This mutation stabilizes interaction between the AR's NTD and LBD (N/C interaction) to increase AR transcriptional activity. Tryptophan residues W435 and W397 in AR NTD are essential in the binding site for the AR-NTD inhibitor EPI-7386 that is currently in clinical trials. We hypothesize that tryptophan mutations within the EPI-7386 binding site of AR are a potential mechanism of resistance.

Methods: Site-directed mutagenesis was used to create expression vectors with W435 and W397 point mutations in AR. Plasmids were transfected into AR-negative CV-1 cells to compare the transcriptional activity of wild-type (WT) versus mutant ARs. IC50s for NTD inhibitors and antiandrogens were generated for a panel of androgen-induced reporter gene constructs. A mammalian two-hybrid system assay was used to assess the impact of inhibitors and AR mutations on N/C interaction.

Results: WAR-W397G/W435L mutations increased transactivation compared to AR-WT in reporter gene constructs containing androgen responsive elements. The mutations led to higher IC50s for EPI-7386 and a well-known antiandrogen, enzalutamide, to inhibit androgen-induced ARR3tk-luciferase activity. Differences in N/C interaction were also observed between WT and mutant ARs treated with EPI-7386. Importantly, the ability of another EPI analog, EPI-7170, to inhibit androgen-induced ARR3tk-luciferase activity and decrease AR-N/C interaction was not impacted by the mutations. These data support that loss of efficacy with EPI-7386 is not across the entire class of EPI analogs and may be specific to EPI-7386.

Conclusions: Tryptophan mutations in the EPI-7386 binding site are predictive of resistance to this specific antagonist and do not broadly apply to the entire class of EPI analogs. Key differences were revealed between AR inhibitors that suggest distinct mechanisms of drug action and the involvement of different regions within the AR transcriptional complex. Future work will investigate the recruitment of coregulatory proteins to the NTD region in which W397 and W435 reside and will assess the implications of these mutations on AR-V7 in the context of CRPC. Ultimately, this will provide valuable insight into the development of novel treatment options for the terminal stage of prostate cancer where existing therapies fail.

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Marie-Soleil Smith

SUPERVISOR(s): Dr. Hélène Côté

SECOND-GENERATION HIV INTEGRASE INHIBITORS IMPAIR DIFFERENTIATION TOWARD ECTODERM LINEAGE IN CULTURED HUMAN EMBRYONIC STEM CELLS

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ABSTRACT

Background/objectives: Each year, ~1.1 million children are exposed to antiretrovirals (ARVs) in utero to prevent vertical transmission of HIV. A recent study demonstrated that second-generation HIV integrase inhibitors (InSTIs) bictegravir (BIC), cabotegravir (CAB), and dolutegravir (DTG) exert toxic effects in cultured human embryonic stem cells (hESCs) and a pregnancy mouse model. As their safety is not fully elucidated during pregnancy, our objective was to characterize the effects of four InSTIs in two hESC lines, with respect to markers of early germ layer differentiation.

Methods: CA1S and H9 hESCs (n=4 independent experiments) were directed to differentiate towards ectoderm (7-days), endoderm (5-days), or mesoderm (5-days) lineages. During culture, cells were exposed to BIC, CAB, DTG or raltegravir (RAL) (all at 0.5X peak plasma drug concentration) or 0.1% DMSO diluent control. At harvest, hESCs were counted and assessed via flow cytometry for viability, and ectoderm (PAX6+/NESTIN+), endoderm (SOX17+/FOXA2+), or mesoderm (TBXT+/CXCR4+) lineages. InSTI-exposed hESCs were compared to DMSO controls by one-way ANOVA with Bonferroni correction.

Results: CA1S hESCs exposed to BIC, CAB, or DTG during differentiation exhibited decreased proliferation (all ≥2-fold, $p \leq 0.03$), and BIC exposure reduced viability (2.5-fold) during mesoderm differentiation ($p < 0.001$). CA1S hESCs directed to differentiate toward ectoderm with CAB or DTG exposure showed a ≥90% decrease in ectoderm marker expression ($p \leq 0.007$). No changes were detected for cells differentiated toward endoderm or mesoderm. In H9 hESCs, exposure to BIC, CAB, and DTG was so cytotoxic that too few cells remained for flow. In both hESC lines, RAL did not induce any cytotoxicity or differentiation dysregulation.

Conclusions: Second-generation InSTIs can dysregulate ectoderm differentiation, which gives rise to neural tube, neural crest cells, and epidermis. In contrast, RAL exhibited a profile similar to controls, a reassuring finding warranting further clinical investigation. Now that the use of antiretrovirals has virtually eliminated HIV vertical transmission, it is imperative to concentrate on safety in the context of pregnancy and embryonic development.

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Ramlogan Sowamber

SUPERVISOR(s): Dr. David Huntsman

UTILIZING GENOMIC AND MOLECULAR TOOLS TO EVALUATE THE EFFICACY OF OPPORTUNISTIC SALPINGECTOMY

Ramlogan Sowambe
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ABSTRACT

Background/objectives: There are five histotypes of ovarian cancer which includes: high grade serous carcinoma (HGSC), endometrioid, clear cell, low-grade serous and mucinous carcinoma. Although the ovary is the site of tumour growth, considerable evidence suggests that epithelial ovarian cancers initiate from the fallopian tube epithelium and endometrium. Current primary prevention methods for high-risk ovarian cancer patients with a BRCA mutation involves bilateral oophorectomy (removal of the fallopian tube and ovaries). However, this procedure is associated with adverse health impacts to the cardiovascular system and skeletal systems. To improve outcomes of patients with minimal adverse effects, an alternative primary prevention strategy was implemented starting in BC called opportunistic salpingectomy (OS). This prevention method calls for fallopian tube removal without the ovaries during other procedures, like a hysterectomy or during tubal ligation. Preliminary work indicates OS is safe, cost effective and has minimal hormonal implications. In our current work, we hypothesize that OS will decrease the risk of certain histotypes of ovarian cancer and that tumours arising post-OS will be genomically distinct from tumours arising in patients with fallopian tubes in-tact.

Methods: We utilized cox proportional hazards models on epithelial ovarian cancer histotypes from the time of surgery to date of diagnosis to determine the effectiveness of OS on reducing ovarian cancer. We will sequence tumours arising from patients of post-OS using shallow whole genome sequencing to identify copy number changes and homologous recombination deficiencies. This work will be performed using the Illumina Novaseq and Qiagen QIAseq platform. Additionally, we will perform artificial intelligence image-based tissue analysis of tumour sections to analyze the histomorphological differences between tumours.

Results: Of a cohort of 30000 individuals who have undergone OS, less than 5 epithelial ovarian cancers were identified. To date, one serous tumor presented in a patient with salpingectomy (expected number of serous cases would be 5); review of the fallopian tube indicates a pre-cancerous lesion was present prior to surgery. P53 analysis of the tumour shows a frameshift mutation (c229fs) with a variant allele frequency of 12%. Our preliminary results indicate OS is an effective strategy at minimizing the incidence of ovarian cancer.

Conclusions: Our preliminary work indicates OS is safe and effective in reducing ovarian cancer incidence. Further work will support the procedure and will present an alternative option to conventional surgical procedures for women.

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Jorge Vallejos

SUPERVISOR(s): Dr. David Huntsman

FAP MARKS INVASION DURING THE DEVELOPMENT OF LOW GRADE SEROUS OVARIAN CARCINOMA

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ABSTRACT

Background/objectives: Fibroblast Activation Protein (FAP) is a membrane-bound protease that has been identified in approximately 90% of epithelial cancers. FAP is expressed in cancer-associated fibroblasts (CAFs), which participate in shaping the tumour microenvironment. There have been previous efforts, with mixed results, to therapeutically target FAP using small molecular inhibitors, antibody-mediated inhibition, and chimeric antigen receptor T cell therapy in a variety of cancer types. FAP expression has been identified in fibroblasts of low-grade serous ovarian carcinoma (LGSC) and lacking in its precursor, serous borderline ovarian tumours. The role of FAP in LGSC pathogenesis has yet to be explored.

Methods: Whole proteome data was collected from a cohort of low-grade serous ovarian carcinoma (LGSC; n = 11) and serous borderline ovarian tumours (SBT; n = 19) formalin-fixed paraffin embedded (FFPE) tissue blocks. Differential protein abundance analysis was performed to detect proteins that distinguish between LGSC and SBT. Immunohistochemistry (IHC) on tissue microarrays (TMA) was then performed to validate the presence of differentially abundant proteins. The TMA was generated from duplicate 1mm cores of the proteomic cohort. An independent TMA was also used to confirm presence of FAP in LGSC and SBT. IHC also aids in determining the location of the differentially abundant proteins that are expressed in the tissue block. FAP is a membrane-bound protease, but a solubilized recombinant human FAP (rhFAP) is commercially available. LGSC cell line, VOA4627, has been treated with 10ng of rhFAP and had its confluency traced over 7 days to measure difference in proliferation when rhFAP is present.

Results: Differential abundance analysis between LGSC and SBT revealed FAP as the most abundant (log2[FC] = 1.37) and significantly ($p < 0.01$) associated protein with LGSC. IHC of FAP on a TMA of the proteomic FFPE cohort validated the presence of FAP in LGSC (n = 9 / 11), and lack thereof in SBT (n = 1 / 19). An independent TMA of ovarian cancers also demonstrated FAP staining in LGSC cores (n = 4 / 9) and lack thereof in SBT (n = 2 / 17). In all cases, FAP was present, exclusively, in the fibroblast compartment of the cores. To determine the effects of FAP on LGSC cells, the LGSC cell line VOA4627 was treated with rhFAP increasing their proliferation. The time for VOA4627 to reach 50% confluency was reduced by approximately 26%. Conclusions: FAP expression is a predominant feature of LGSC compared to its putative precursor, SBT. The presence of FAP in LGSC, but not in its precursor, suggests that FAP or the cell type it is expressed in has a function that aids in disease progression. The in vitro proliferation assay demonstrated the FAP itself influences the behaviour of, at least, one LGSC cell type. FAP presents a novel therapeutic target for LGSC tumours, with the intent to deplete the FAP-positive CAFs and redefining the LGSC tumour microenvironment.

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Alexandra Witt

SUPERVISOR(s): Dr. Ed Pryzdial

DEVELOPMENT OF DOUBLE MUTANT CLOTTING FACTOR X AS A NOVEL THROMBOLYTIC AGENT

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ABSTRACT

Background/objectives: The favoured clot-dissolving drug (i.e. thrombolytic agent) is a recombinant (r), or lab-made, version of tissue plasminogen activator (tPA). The high rtPA dose that is required for clot lysis causes clinical hemorrhage in 3-7% of patients, resulting in part from systemic, rather than clot-localized, enzyme activity. The Pryzdial lab is working to translate a thrombolytic function for the plasma protein clotting factor (F) X, to act as a non-enzymatic alternative to rtPA. Several key characteristics have been incorporated in the recombinant FX (rFX): 1) a blocked active site; 2) accessibility of sites integral to localization and tPA-accelerating function; and 3) stability and increased half-life of tPA-accelerating function in plasma. Based on pilot data, we hypothesize that a recombinant variant of FX is thrombolytic and has superior safety compared to rtPA.

Methods: In addition to wildtype rFX, the double mutant rFX-Lys330Gln/Ser379Ala (rFX QA) was produced in HEK 293 cells and purified via column chromatography. The Lys330Gln mutation was made to stabilize thrombolytic activity in plasma by removing a detrimental cleavage site, and the Ser379Ala (active site mutation) to prevent the proteolytic clotting activity of activated FX. Characterization of the protein was done using SDS-PAGE electrophoresis, with subsequent immunoblotting where necessary, as well as multiple assays. These assays include prothrombin time clotting assays, to measure the time it takes plasma to form a clot, and purified protein chromogenic assays, to assess plasmin generation as a measure of how fast our added protein will break down a clot.

Results: To characterize the Lys330Gln mutation, a time course of plasmin incubation was analyzed via immunoblot electrophoresis, and the mutation was deemed successful when rFX WT was cleaved into a secondary form, FX-gamma, but rFX QA was not. The active site mutation was also confirmed to be successfully incorporated into the protein via a prothrombin time clotting assay, which showed that rFX QA was unable to act in its primary role as a clotting enzyme and cause a clot. Calcium-dependent localization of the mutants to anionic phospholipids, which are present at the site of a clot, was confirmed by immunoblot electrophoresis. A chromogenic assay demonstrated that rFX QA generates plasmin, the prime clot-busting enzyme, 10-fold more than its wild-type comparator.

Conclusions: These preliminary data support the initial hypothesis that rFX QA has thrombolytic activity and may eventually be used to replace rtPA in clinical settings. rFX QA binds anionic phospholipids in a calcium-dependent manner to localize its thrombolytic activity to the site of a clot. It does not degrade to a known inactive species, FX-gamma, has no residual clotting activity, and helps to generate plasmin and accelerate fibrinolysis in plasma. Next, the Pryzdial lab will assess thrombolytic efficacy in vivo and therapeutic safety ex vivo, and anticipate highlighting rFX QA as both an effective and safer alternative to rtPA.

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Nancy Yang

SUPERVISOR(s): Dr. Helene Cote

SEX DIFFERENCES AMONG INDIVIDUALS LIVING WITH OR WITHOUT HIV IN RELATION TO PERSISTENT VIRAL INFECTIONS AND IMMUNOLOGICAL AGING

Nancy Yang
(graduate student)

AFFILIATIONS

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ABSTRACT

Background/objectives: Over 38 million people are currently living with HIV. Despite antiretroviral therapies that have prevented transmission and increased lifespan, people living with HIV (PLWH) experience faster biological aging relative to their HIV-negative peers. Infection with chronic/latent viruses such as CMV, EBV, HHV-8, HSV-1, HSV-2, HCV, and HBV may accelerate immunological aging. Individually, these persistent viruses are associated with aging markers and/or age-associated diseases, but their cumulative effect is unknown. We characterized the number and type of seven non-HIV chronic/latent viruses in PLWH or not, of both sexes, enrolled in the Canadian CARMA (Children and women: AntiRetrovirals and the Mechanism of Aging) cohort, and investigated any association with leukocyte telomere length (LTL).

Methods: Among CARMA participants, PLWH (n=187, 105F/82M) and HIV-negative controls (n=189, 105F/84M) were selected, balanced for age, sex, and HIV group. CMV, EBV, HHV-8, HSV-1, and HSV-2 infection was determined serologically; HIV, HCV, and HBV were self-reported. Relative telomere length (LTL) was measured using qPCR. Associations between number of viruses, LTL, and sociodemographic factors were assessed using ordinal logistic and linear regression modelling.

Results: The median[IQR](range) number of non-HIV viruses was 3[2-4](0-6) for PLWH vs. 2[1-3](0-6) for HIV-negative participants ($p<0.0001$), and 3[2-4](0-6) for female participants vs. 2[1-3](0-5) for male participants. In multivariate analyses (adjustments for ethnicity, smoking status, and birth region), older age ($p<0.0001$), HIV-positive status ($p<0.0001$), and female sex ($p<0.0001$) were independently associated with having more viruses. In a sex-segregated analysis, older age and HIV+ status remained associated with more viruses in both sexes, however, Indigenous ethnicity ($p=0.0001$) and African continent of birth ($p=0.002$) were associated with having more viruses amongst only female participants. In all participants, a multivariable linear regression model for LTL showed that older age ($p<0.0001$), male sex ($p=0.0001$), and HIV+ status ($p=0.0006$) are associated with shorter LTL, and that compared to living with 0-2 non-HIV viruses, having 3-4 non-HIV viruses was independently associated with significantly shorter LTL ($B=-0.24$, $p=0.02$).

Conclusions: The increased prevalence of chronic/latent viruses with age and HIV-positive status is unsurprising, but the observed association with female sex is unclear. The sex-specific models reveal that some predictors of persistent viral infections are shared between sexes, for example older age and HIV, while others appear to diverge, among them ethnicity and region of birth. These findings warrant the need to investigate sex and related differences that would impact people's health differently, and possible sex-specific prevention, screening, and/or treatment approaches. Finally, our results suggest that persistent viruses contribute to immunological aging. Determining whether this is associated with comorbidities later in life could give insight into the value of treating and/or preventing specific viral infections.

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Joyce Zhang

SUPERVISOR(s): Dr. David Huntsman

INVESTIGATING THE TUMORIGENESIS PROCESS OF DICER1 SYNDROME WITH NOVEL TRANSGENIC MOUSE MODEL

Joyce Zhang
(graduate student)

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AFFILIATIONS

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ABSTRACT

Background/objectives: In 2012 our group discovered recurrent missense mutations in microRNA processing gene DICER1, in Sertoli-Leydig cell tumours of the ovary. These missense mutations are usually present in trans with a germline DICER1 null mutation, leading to a hemizygous state. "DICER1 syndrome" refers to tumours associated with germline DICER1 mutations, which can have pulmonary or extra-pulmonary manifestations. Given no cell line models exist, development of animal models is crucial. Many of DICER1 cancers are sarcomas; we therefore postulate the cell of origin is mesenchymal. To this end, we developed a tamoxifen inducible, Hypermethylated in Cancer 1 (HIC1)-creERT2 driven transgenic mouse strain. Hic1 marks mesenchymal progenitors. With this strain, we successfully created a model that histologically recapitulate the 3 renal tumours seen in DICER1 syndrome: Wilms tumours, anaplastic sarcomas, and cystic nephromas. We propose to construct the oncogenesis continuum to gain insights into early tumorigenesis events. Objective: to identify oncogenic events underlying Dicer1 mutation-driven murine kidney tumour development with single cell RNA-sequencing (scRNA-seq) technologies.

Methods: Prior to mapping temporal trajectory, we conducted pilot scRNA-seq with 2 murine renal tumours (result section). To build the tumorigenesis continuum, we will harvest control kidneys and tumours post tamoxifen injection at 0, 3, 6, 12 months (n=4 / time points) and perform scRNA-seq. Cell differentiation trajectory analysis will be performed. We will identify key genes/oncogenic pathways at pre-malignant stage and determine how they evolve along the course of tumour development.

Results: Integration of 2 tumours revealed moderate overlap and diverse cell populations: immune cells and multiple tdTomato+ cell clusters reflecting different cell states. For instance, one epithelial-like cluster shows upregulation of cancer-stem cell markers and transcription factors only active in normal kidney development (ureteric bud). Another mesenchymal cluster shows high expression of markers for blastema - a histological component in Wilms tumours. We assigned this cluster to be muscle satellite cells based on marker genes. In addition, we identified a cluster of terminally differentially muscle cells. We will validate these findings with IHC on murine tumours.

Conclusions: With scRNA-seq, we begin to unravel heterogeneity of these murine tumours, paving the road for interrogating therapeutic susceptibility. Our proposed study will verify the utility of the first DICER1 syndrome-associated cancer model, enabling researchers to utilize it to improve patient management.

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Almira Zhantuyakova

SUPERVISOR(s): Dr. David Huntsman

DECIPHERING ABERRANT STING PATHWAY AND EXPLORING ONCOLYTIC VIRUSES THERAPY IN LOW GRADE SEROUS OVARIAN CARCINOMA

Almira Zhantuyakova
(graduate student)

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ABSTRACT

Background/objectives: There are two serous ovarian cancer histotypes, low and high grade (LGSOC and HGSOC), which are distinct clinical and biological entities. LGSOC is a rare histotype with a relatively stable genome, while HGSC is more common and genomically unstable. Somewhat surprisingly LGSOC expresses high levels of the stimulator of the interferon genes (STING). The STING pathway recognizes cytoplasmic double-stranded DNA and mounts innate cellular immunity through interferon-beta type I production. Our objective is to investigate the aberrant STING signaling in LGSOC and test the effectiveness of oncolytic viruses against LGSOC.

Methods: We used immunohistochemistry on tissue microarrays (TMAs) to assess STING protein expression in different ovarian cancer histotypes. Whole proteome analysis was applied to identify differentially expressed proteins in LGSOC and HGSOC patient samples (both n=9). Further, a semi-targeted proteomics approach was used to evaluate the expression levels of the STING pathway-related proteins in LGSOC, HGSOC, and LGSOC precursor tumors (each subtype, n=20). To evaluate the key transcription, phosphorylation, and translocation events in STING signaling, we treated LGSOC cell lines with an agonist (dsDNA90) and performed qPCR, immunoblotting, and immunofluorescence experiments, respectively. We tested the viability of the LGSOC cell lines in response to Vaccinia Virus (VV), and Vesicular Stomatitis Virus (VSV) based oncolytic vectors with or without immunostimulatory transgenes.

Results: Our results show that STING protein levels were consistently higher in LGSOC TMAs relative to other histotypes. Proteomics analysis showed that the half of the 16 most differentially expressed proteins were the effectors of STING signaling with unexpectedly lower expression in LGSOC, suggesting that despite the robust levels of STING in LGSOC tumors, the pathway is not fully active. Attenuated STING translocation and expression of IFNB1 and other cytokines in LGSOC cell lines confirm the aberrancy in the STING pathway. Semi-targeted proteomics revealed the considerable overexpression of STIM1 in LGSOC patient tumors, which has previously been shown to sequester STING in the endoplasmic reticulum. The treatment with VV and VSV oncolytic viruses significantly reduced the proliferation of LGSOC cell lines.

Conclusions: In summary, we find attenuated STING signaling in LGSOC, possibly due to overexpression of STIM1 preventing STING translocation. Although oncolytic viruses show promising results in LGSOC cell lines, more research is needed to determine the optimal treatment strategy, testing oncolytic viruses expressing various transgenes and combination therapies.

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Alicia Andrews

SUPERVISOR(s): Dr. Jessica Saunders

LOST IN TRANSLOCATION—A CASE REPORT OF A CIC-DUX4 SARCOMA WITH NOVEL LOSS OF H3K27ME3 EXPRESSION

Alicia Andrews
(Clinical Fellow)

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ABSTRACT

Background/objectives: The use of immunohistochemical (IHC) surrogates for molecular alterations can expedite the molecular classification of tumours in daily practice. Lack of specificity in these markers can therefore lead to diagnostic conundrums. Loss of IHC staining for trimethylation of lysine 27 of histone H3 (H3K27me3) is a sensitive marker for Malignant Peripheral Nerve Sheath Tumour (MPNST) when the morphologic differential diagnosis includes spindle cell lesions of soft tissue. However, there are increasing reports of other sarcomas with loss of H3K27me3 expression, which can make definitive classification difficult in cases where MPNST is a diagnostic consideration. The rarely reported CIC-DUX4 sarcoma, which occurs in the somatic soft tissue of young adults, is classically considered a small round cell sarcoma. Recently, a wider morphologic spectrum including round, epithelioid and spindle cells has been described. We herein describe a rare case of a young male, without clinical evidence of Neurofibromatosis-1, who presented with an aggressive spindled and round cell sarcoma of the thigh, ultimately diagnosed as a CIC-DUX4 sarcoma, whose concurrent loss of H3K27me3 represented a significant diagnostic pitfall during pathologic evaluation.

Methods: The patient underwent open biopsy, which was evaluated with H&E and an IHC staining panel consisting of H3K27me3 (Cell Signaling Technology; C36B11), BCOR, NTRK, AE1/AE3, high molecular weight cytokeratin, EMA, S100, CD34, desmin, myogenin, TLE-1, CD99, and NKX2.2. Nanostring pan-sarcoma panel was performed (BC Cancer Agency). The case was reviewed at sarcoma multidisciplinary conference and the IHC and morphologic differential diagnosis included an MPNST versus a high grade spindle and round cell sarcoma. Given the lack of supporting clinical and radiologic evidence for MPNST, further genetic work-up was performed via Trusight RNA-Sequencing (Mt. Sinai, Toronto).

Results: The biopsy showed a high-grade sarcoma composed of sheet-like growth of predominantly short spindled cells with areas of smaller, round to polygonal cells and abundant geographic necrosis. Mitoses were brisk but not atypical. IHC evaluation was uninformative with the exception of complete nuclear loss of H3K27me3 (Cell Signaling Technology; C36B11) in the lesional cells. Nanostring pansarcoma fusion assay did not reveal any identifiable translocation despite covering the most common break points for theCIC-DUX4 gene fusion. Radiologic findings showed no direct relationship between the mass and any large nerves. Follow-up Trusight RNA sequencing demonstrated a CIC-DUX4 gene fusion. The resection specimen showed similar morphology and loss of H3K27me3 staining.

Conclusions: We report a novel case of a CIC-DUX4 sarcoma with predominant spindled morphology and complete nuclear loss of H3K27me3 on IHC work-up, a finding not previously reported. The present case demonstrates a few of the challenges in diagnosis of CIC-DUX4 sarcoma, which can show a wide morphologic spectrum, and adds to the growing list of high grade sarcomas which can show loss of H3K27me3.

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Mehwish Anwer

SUPERVISOR(s): Dr. Cheryl Wellington

UNDERSTANDING THE RELATIONSHIP BETWEEN IMPACT DIRECTION AND TBI-RELATED NEUROPATHOLOGY USING CLINICALLY RELEVANT BLOOD BIOMARKERS AND NEUROIMAGING

Mehwish Anwer
(PDF)

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ABSTRACT

Background/objectives: Traumatic brain injury (TBI) is a leading cause of global death and disability. The heterogeneity in impact direction, impact intensity and consequent pathology poses a great challenge in pre-clinical modelling of TBI. The objective of this study is to understand the effect of direction of head rotation and acceleration on integrity of fibre tracts, neuronal networks and cerebrovascular function following a non-surgical CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration) TBI in mice.

Methods: Mice were randomly assigned to TBI (3.1J impact energy) or sham groups. TBI with complex multiplanar head motion was induced using an impactor positioned perpendicular to the mouse head. High speed videography was used to visualize head movement upon impact. Behavior was used to assess injury severity. Blood was collected 6 hours post-injury for biomarker assessment and the brain was harvested for neuropathological assessment and ex-vivo magnetic resonance imaging (MRI).

Results: Upon high-energy interface-assisted impact (3.1J), 27% acute mortality and increased loss of righting reflex was observed in TBI group. Plasma GFAP and NFL levels correlated with impact energy and were markedly higher in the TBI group compared to sham controls. MRI showed decreased neurite density (NDI) in the corpus callosum at 6 hours post-injury after lateral impact.

Conclusions: These data suggest that CHIMERA can be used to model lateral TBI with complex head motion in mice. Ongoing work comparing sagittal vs. lateral impacts will further characterize how impact directionality and head kinematics affects post-injury outcome.

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Maryam Asadi

SUPERVISOR(s): Dr. Ali Bashashati

DOMAIN GENERALIZATION IN DEEP LEARNING FOR MULTI-CENTER HISTOPATHOLOGY CANCER DIAGNOSIS

Maryam Asadi
(PDF)

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ABSTRACT

Background/objectives: The potential of Artificial Intelligence (AI) in clinical pathology is truly inspiring, as it offers immense opportunities to enhance diagnostic accuracy, improve efficiency, and contribute to better patient outcomes on a global level. Investigation of histopathology slides by pathologists is an indispensable component of the routine diagnosis of cancer. Variations in tissue preparation and staining protocols in different centers result in different color profiles for histopathology tissue slides (i.e., domain shift). Due to these variations, deep learning-based (DL) models tend to overfit when trained on the data from only one center, thereby underscoring the necessity to generalize deep learning networks for multi-center use. Several techniques have been suggested to generalize deep learning algorithms, such as the use of grayscale images, color normalization techniques, and Adversarial Domain Adaptation (ADA) networks. Despite the promising results of these approaches, there are limitations to their effectiveness and discriminability.

Methods: We develop a novel robust DL-based network for the classification of cancer subtypes from samples collected at different centers. In order to improve transferability and thereby discriminability, we propose the use of the Fourier-based Enhancer module, which allows the convolutional network to handle color variations more effectively by focusing on structured information that is highly similar between the two domains. We used two datasets of ovarian and pleural cancers. The Ovarian dataset comprises 1053 WSIs from 523 patients in the source domain and 60 WSIs from 60 patients in the target domain. Additionally, the pleural dataset consists of 194 WSIs from 128 patients and 53 WSIs from 53 patients in the source and target domains.

Results: The experimental findings demonstrate that our method outperformed other approaches for both the source and target domains of the Ovarian and Pleural datasets across various measurements including the balanced accuracy, Cohen's Kappa, and F1-score. Specifically, our method yielded the highest balanced accuracy (75.82%), surpassing the performance of the baseline ($p < 6.0e-5$), color normalization technique ($p < 2.6e-3$), and ADA ($p < 1.08e-2$) for Ovarian dataset. Consistently, our approach demonstrated the best performance for the Pleural dataset, achieving a balanced accuracy of 82.56% compared to the baseline ($p < 9.1e-3$), color normalization technique ($p < 3.0e-2$), and the original ADA ($p < 4.1e-2$). The results of the study show that our proposed model not only outperformed the other approaches in the target domain but also in the source data for both datasets.

Conclusions: By utilizing a DL-based approach for the classification of histopathology images, we have achieved successful classification of various subtypes of ovarian and pleural cancers. This outcome highlights DL as a promising tool for the accurate diagnosis of cancers based on multi-center data. In the future, further research will be conducted to validate the effectiveness of our DL algorithm for the classification of histological subtypes of other types of cancers.

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Alexandre Aubert

SUPERVISOR(s): Dr. David Granville

TENASCINS AND LATENT TGF-BETA: MORE THAN A STICKY STORY

Alexandre Aubert
(PDF)

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ABSTRACT

Background/objectives: Transforming Growth Factor-Beta (TGF-Beta) is a pro-fibrotic cytokine produced and secreted in an inactive form within a structure called the Small Latent Complex (SLC), in which the cytokine is non-covalently attached to its latency associated peptide (LAP). Within the extracellular matrix, this conformation masks TGF-Beta active sites and prevents its interaction with cell surface receptors. One way to activate extracellular latent TGF-Beta is called conformational modification, a protease-independent mechanism that leads to the exposure of TGF-Beta bioactive sites, while maintaining interactions between TGF-Beta and its LAP prodomain. Tenascin-X, a glycoprotein of the extracellular matrix has been recently identified as an activator of latent TGF-Beta by conformational modification. Through its C-terminal fibrinogen-like (FBG) domain, Tenascin-X physically interacts with the SLC to promote latent TGF-Beta activation and subsequent downstream signalling in epithelial cells. Tenascin-X is a member of the Tenascin family composed of three other members: Tenascin-C, -R and -W. In contrast to Tenascin-X and -R that are constitutively expressed in adult tissues, Tenascin-C and -W are mostly absent in full grown organisms but are expressed de novo during physiological tissue remodeling. Increased expression of Tenascin-C has been also associated with fibrosis and keloid formation. Because FBG-like domains of the four Tenascins share more than 50% amino-acids similarity, we hypothesize that Tenascin-C, -R and -W also activate latent TGF-Beta through their conserved FBG-like domain.

Methods: Histidine-tagged recombinant full-length Tenascins and their respective FBG-like domains were produced in eukaryotic cells and purified by affinity chromatography. Similar to Tenascin-X, the three other members of the Tenascin family co-purified with components of the SLC (i.e. TGF-Beta and LAP) and promoted the activation of the latent cytokine, most likely by conformational modification. When used as a culture substratum, Tenascin-C, -R and -W as well as their FBG-like domains alone induced a TGF-Beta/Smad intracellular signalling pathway in epithelial cells leading to Smad2 phosphorylation and transactivation of a reporter gene under the control of a Smad-binding element. FBG-like domains of the four Tenascins were also able to induce a TGF-Beta-like response in epithelial cells, including cell cycle arrest (cytostasis) and epithelial-to-mesenchymal transition. New evidence recently obtained also indicate that Tenascin-C is cleaved in vitro by Granzyme B, a serine protease with emerging extracellular functions. GzmB-mediated cleavage of Tenascin-C led to the production of three protein fragments, one of which containing the FBG-like domain.

Conclusions: Tenascin-C, -W and -R share the ability of Tenascin-X to activate latent TGF-Beta through their conserved FBG-like domains and induce a TGF-Beta/Smad signalling in epithelial cells, leading to cytostasis and epithelial-to-mesenchymal transition. By cleaving Tenascin-C, Granzyme B may contribute to latent TGF-Beta activation and may explain how this glycoprotein contributes to fibrosis.

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Qudrat Aujla

SUPERVISOR(s): Dr. Suzanne Vercauteren and Dr. Jonathan Bush

SURVEY OF ADOLESCENTS REGARDING THEIR OPINION OF RESEARCH AND VACCINATION DURING THE COVID-19 PANDEMIC

Qudrat Aujla
(Staff)

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ABSTRACT

Background/objectives: The COVID-19 pandemic has emphasized the importance of research into disease biology, prevention, and treatment of emerging diseases and pathogens. Currently, there is limited understanding on adolescent perceptions towards biobanking, research participation, vaccinations, and how the pandemic has altered these perceptions. The BC Children's Hospital BioBank (BCCHB) surveyed Grade 8-12 students in British Columbia (BC) to determine perceptions before and after the pandemic about topics such as research, pediatric biobanking, vaccinations, and public health policies.

Methods: A voluntary, anonymous, 15 question online survey was distributed through student emails, online school portals, and parent newsletters. Demographics such as age, grade, gender, biological sex, location, ethnicity and whether English is their first language were collected. Phase 1 of the survey was conducted from May to June 2021 and Phase 2 was conducted from Sept 2021 to June 2022. Questions were edited between Phase 1 and 2 to reflect vaccine availability for adolescents, and the emergence of new variants of COVID-19.

Results: We received a total of 1,022 completed survey responses, from participants aged 12-19 years from 13 BC school districts and compared the findings to a previous completed school survey in 2016 conducted by the BCCHB, asking some similar questions [1]. The majority of participants (94%) agreed that COVID-19 is a serious disease and 95% stated that the pandemic has shown them how important medical research is. The desire to contribute to research was reflected by the 80% of participants willing to donate an extra blood sample during a medical procedure for biobanking purposes during the COVID-19 pandemic, compared to 64% from a 2016 school survey conducted among the same age group [1]. 79% of participants agreed to donate leftover samples to research, such as blood and saline gargles, and 72% stated that they would be comfortable donating extra samples, even if it requires an extra procedure such as a blood draw. Participants (97%) agreed that research participation would allow them to help others. Of the adolescents surveyed, the majority (85%) were comfortable with making the decision on their own to donate a sample specifically to research. Considering the current public health climate, 92% of participants stated that they will be vaccinated, for reasons such as knowing vaccines are safe, allowing them to engage in social activities, and protecting them from COVID-19.

Conclusions: Adolescents across BC were more willing to participate in research and donate both leftover and extra samples towards biobanking after living through the COVID-19 pandemic and knowing the importance of research in disease diagnosis and treatment. The results of this survey will help implement future public policies surrounding vaccination effects and research for this age group in the future.

Reference: [1] Kong CC, Tarling TE, Strahlendorf C, Dittrick M, Vercauteren SM. Opinions of Adolescents and Parents About Pediatric Biobanking. J Adolesc Health, 2016; 58(4):474-480. doi: 10.1016/j.jadohealth.2015.12.015. PMID: 27013273.

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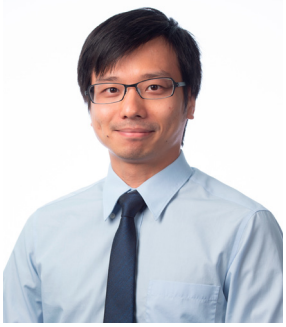

Wai Hang (Tom) Cheng

SUPERVISOR(s): DR. CHERYL WELLINGTON

CHRONIC NEUROPATHOLOGIES IN A TRANSGENIC MOUSE MODEL OF TAUOPATHY, USING CHIMERA INTERFACED OR DIRECT IMPACTS

Wai Hang (Tom) Cheng
(PDF)

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ABSTRACT

Background/objectives: We recently reported the effects of TBI in 4-mo rTg4510 tauopathy mice, using the Closed Head Injury Model of Engineered Rotational Acceleration (CHIMERA). A single interfaced impact (4.0J) induced significant mortality, white matter injury and increased GSK-3 beta activity at 2-mo post-injury. However, the effect on tau pathology was inconsistent. In the current study, younger rTg4510 mice were experimented with 2 TBI paradigms, and aged for longer. In this study, our objective is to induce CHIMERA TBI (a single interfaced head impact, or repetitive direct impacts) to rTg4510 mice, and investigate neuropathologies at 1-d, 1-mo, and 6-mo post-injury.

Methods: We included 2 CHIMERA TBI paradigms: in the high-energy single TBI arm (sh), 2-mo male rTg4510 received either interfaced head impact (3.4J, n=69), or sham (with anesthesia and body restraint, but no head impact; n=49). In the low-energy repetitive TBI arm (rl), mice received either 6 direct head impacts (6x0.7J, n=51) within 12 days, or 6 sham (n=50). Brain tissues and cardiac plasma samples were collected at 1-d, 1-mo, or 6-mo post-final injury. Longitudinal saphenous plasma samples were collected every 2 weeks until sample harvest.

Results: In sh-TBI, 27.5% of TBI mice (19/69) died immediately after TBI or reached humane endpoint. Surviving mice had significantly prolonged LRR duration compared to sham (4165 sec vs 139 sec, $p<0.001$). In rl-TBI, there was 0% mortality and no significant increase in LRR (245 sec vs 233 sec, $p=0.5902$). Iba-1 immunostaining was increased in optic tract at 1-mo post-injury, in both TBI paradigms.

Conclusions: In 2-mo rTg4510 mice, a single high-energy CHIMERA interfaced head impact (1x3.4J) induced mortality and increased LRR. This was not observed in repetitive, low-energy direct head impacts (6x0.7J).On-going analyses include immunohistochemistry for brain tau and Mesoscale Discovery assays for plasma tau.

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Amal EL Naggar

SUPERVISOR(s): Dr. David Huntsman

FROM ORIGIN TO METASTASIS: THE CRITICAL ROLE OF CYSTATHIONINE GAMMA-LYASE IN CLEAR CELL CANCER OF THE OVARY

Amal EL Naggar
(RA)

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ABSTRACT

Background/objectives: Clear cell ovarian cancer (CCC) is the 2nd most common ovarian cancer and is histologically and clinically distinct from other subtypes. Late-stage CCC have a worse prognosis than other ovarian cancer histotypes as they are inherently resistant to the standard platinum/taxane chemotherapy. CCC usually arises directly from endometriosis. Recently, we found that cystathionine gamma-lyase (CTH), a key enzyme in the transsulfuration pathway, is highly expressed in CCC but not other ovarian cancer subtypes. Whether and how the transsulfuration pathway, notably CTH, enables CCC to adapt to an endometriotic cyst's hostile microenvironment and ultimately promote metastasis remains unanswered.

Methods: We generated CTH knockout (KO) cells using CRISPR/Cas9. We assessed the effects of CTH loss in vitro -under ambient and stress conditions- and in vivo on cell viability, cell proliferation, ROS levels, migration, invasion, and metastasis. Further, we investigated the impact of CTH modulation on the hypoxia response, a salient feature of CCC.

Results: We found that CTH expression is enhanced under stress conditions, notably hypoxia, and this is critical for optimal HIF1alpha response. Further, CTH loss has significantly enhanced cell death and ROS accumulation. CTH loss significantly inhibits CCC cells' progression and metastasis in vitro and in vivo. Further, CTH is critical for optimal hypoxia response through enhancing HIF1 alpha expression.

Conclusions: In conclusion, CTH is a primary stress adaptation factor contributing to hypoxia response, a key feature of CCC. Further, CTH is essential for CCC progression and metastasis in vivo. Therefore, targeting CTH might represent a novel therapeutic opportunity for patients with CCC.

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Bengul Gokbayrak

SUPERVISOR(s): DR. YEMIN WANG

GENOMIC PROFILING OF DEDIFFERENTIATED ENDOMETRIAL CANCER

Bengul Gokbayrak
(PDF)

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ABSTRACT

Background/objectives: Dedifferentiated endometrial cancer (DDEC) is a rare and aggressive subtype of endometrial cancer with a poor prognosis. It consists of a mixture of low-grade endometrioid and undifferentiated carcinoma components. Previous findings suggest a clonal origin between the differentiated endometrioid and the undifferentiated component of dedifferentiated endometrial carcinoma. Recent studies suggest that mutations in specific genes, including SWI/SNF components, may drive dedifferentiation in endometrial cancer. This can cause a loss of differentiation and promote a more aggressive tumor phenotype. Further research is needed to fully understand the molecular mechanisms behind dedifferentiation in DDEC. For this purpose, we aimed to investigate the mutational signature of undifferentiated components in DDEC.

Methods: In this study, we performed panel sequencing and immunohistochemical analysis in a series of dedifferentiated endometrial carcinomas (n=46). In 30% of cases (14/46), we have sequenced both well-differentiated and undifferentiated components from the same patient. Each case was evaluated by immunohistochemistry (IHC) for p53 status, mismatch repair (MMR) deficiency and several components of SWI/SNF complex including SMARCA4/2, ARID1A/B and SMARCB1. POLE status was determined by Sanger sequencing for a few cases. POLE mutations from panel sequencing were evaluated for the remained cases. Sequencing data was cleaned for mutations that might occur from sample preparation and for single nucleotide polymorphisms.

Results: We observed a good correlation between mutational status and IHC results for tested genes as proof of principle. In accordance with previous reports, we observed a higher mutation burden in cases with POLE hotspot mutations. The majority of DDEC samples displayed MMR deficiency. Frequent truncating mutations were detected for PTEN, PIK3CA, PIK3R1, KMT2C and FBXW7- genes commonly mutated in gynecologic cancers. Mutations in SWI/SNF components are enriched in undifferentiated cases. We are currently working on identifying enriched mutations specific to the undifferentiated component in comparison to well-differentiated samples.

Conclusions: Due to its rarity, the mutational profiling of DDEC cases was limited to a number of cases. This cohort is the largest reported cohort of DDEC with 46 individual cases. Overall, DDEC cases in this study represent the mutational signatures of gynecologic cancers and typical DDEC characteristics reported in previous studies. Currently, we are working on identifying genetic alterations specific to undifferentiated components, which might help better understand the disease.

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Farhia Kabeer

SUPERVISOR(s): Dr. David Huntsman & Dr. Yvette Drew

THE EVOLUTION OF HIGH GRADE SEROUS OVARIAN CANCER UNDER THE PRESSURE OF NON-CYTOTOXIC AND CYTOTOXIC TREATMENT

Farhia Kabeer
(PDF)

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ABSTRACT

Background/objectives: High-grade serous ovarian cancer (HGSOC) is driven by loss of TP53 and genome instability . Despite the recent success of PARP inhibitors, advanced BRCA mutant and homologous recombination repair deficient (HRD) HGSOC, chemotherapy remains the first line treatment. Little is known about how chemotherapy exposure alters tumor heterogeneity and subsequent response to targeted therapies. Certain mutations may survive the exposure of chemotherapy better than others. The question of whether targeted therapies should be given before or after response to chemotherapy also remains unanswered. We use the principles of natural selection to investigate how HGSOC evolves over time and selection operates on clones in the context of cytotoxic/non-cytotoxic combination therapies, and what changes in genomes/transcriptomes at the single cell level drive tumor progression.

Methods: Cell lines ID8 *Trp53 −/−; Brca1* −/− and WT were used to represent defective /proficient homologous recombination were given intraperitoneally to C57B6 mice to develop HGSOC models. Transfected Luciferase expression in the cells used to monitor tumor response to olaparib (Ola)+/- Bevacizumab (BEV-various doses) +/- Atezolizumab (ATz) combinations by bioluminescence imaging (BLI). Ascitic fluid and mouse organs were harvested for the evidence of seeding and identification of biomarkers. In addition, 10 treatment naïve HGSOC PDX were developed in immunodeficient mice and treated with either cisplatin or olaparib. Single-cell whole-genome sequencing (scWGS) was performed using direct library preparation (DLP+). Hierarchical clustering and *Sitka* are used to identify the clonal structure of each condition following treatment. Phylogenetic tree was computed using copy number data.

Results: In the BRCA WT HRp study group, the greatest response was seen in the triplet Ola+BEV+ATz combinations and interestingly no significant differences were observed using a lower dose of BEV. Furthermore, from scWGS data of PDX passages we captured initial clonal heterogeneity leading to emergent clones. We found evolving copy number changes on chromosome 19, 8, 3 and loss of heterozygosity of TP53.

Conclusions: The triplet combination of low-dose-intensity bevacizumab with other non-cytotoxic drugs was an effective regimen for BRCA WT syngeneic mouse tumors. In HGSOC PDX, we are able to capture diversification between HGSOC PDX passages– specifically after drug exposures.

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Katie Mayne

SUPERVISOR(s): DR. JACQUELINE QUANDT

THE EXPRESSION AND LOCALIZATION OF NPAS4 AND ARNT2 IN THE CNS FOLLOWING IMMUNE-MEDIATED DEMYELINATION

Katie Mayne
(PDF)

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ABSTRACT

Background/objectives: Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS) characterized by areas of focal demyelination, neuroinflammation and neurodegeneration. Treatment options for MS patients have greatly progressed since the discovery of various disease modifying therapies, however options are still limited for those with progressive MS. The neuronal PAS domain-containing protein 4 (NPAS4) is expressed in the CNS, almost exclusively by neurons, and is involved in mediating neuronal functionality and survival. The aryl hydrocarbon (Ah) receptor nuclear translocator protein 2 (ARNT2) is the major heterodimeric partner of NPAS4 and has been shown to be involved in key processes in the CNS, including neuronal health and development. NPAS4/ARNT2 complexes regulate the transcription of brain-derived neurotrophic factor (BDNF), important for neuronal differentiation, plasticity and survival. Previous literature and preliminary data highlight potential roles for NPAS4 and ARNT2 both independently and as a complex in mediating responses to inflammatory demyelination.

Methods: The expression of NPAS4 and ARNT2 in the brain and spinal cord at different timepoints over the disease course was evaluated in the murine model of autoimmune-mediated demyelination, experimental autoimmune encephalomyelitis (EAE). Myelin oligodendrocyte glycoprotein (MOG)35-55 chronic EAE was induced in female C57BL/6 mice aged 9-13 weeks old. mRNA and protein expression levels of NPAS4 and ARNT2 were evaluated by qPCR and immunohistochemistry in healthy, sham-immunized (no MOG peptide), and EAE mice prior to the onset of symptoms, peak disability, and during recovery.

Results: Preliminary experiments show that NPAS4 protein is expressed in neurons of the brain of healthy mice and prior to onset, peak and recovery timepoints of EAE, however is not detected in the spinal cord with immunohistochemistry. NPAS4 was localized to neurons in the motor and sensory cortex, piriform cortex, and the hippocampus. ARNT2 is also expressed in the brain and spinal cord of healthy and EAE mice, although expression was not restricted to neurons, as we previously published. Co-expression of NPAS4 and ARNT2 is more common in the cortex but is rarely observed in hippocampal regions. The mRNA expression of npas4 and arnt2 mice undergoing EAE was significantly increased in the brain prior to disease onset. In contrast, expression levels of npas4, arnt2, and bdnf significantly decrease in the spinal cord through disease onset, peak and recovery timepoints compared to healthy and sham-immunized controls.

Conclusions: Levels of npas4 and arnt2 are elevated in the CNS prior to the onset of EAE, highlighting potential roles in response to inflammation and immune-mediated demyelination. As levels of npas4, arnt2, and bdnf decrease following disease onset, these factors may be important in mediating neuroprotection. Ongoing studies in neuronal culture models and during EAE will determine the functional relevance of NPAS4 and ARNT2 to neuroprotective and neurodegenerative processes in neuronal and glial populations, respectively.

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Layla Nabai

SUPERVISOR(s): David J. Granville

HEALING TIME AND SCARRING IS REDUCED IN CUTANEOUS LESIONS OF GRANZYME B KNOCKOUT MICE INOCULATED WITH LEISHMANIA MAJOR PARASITE

Layla Nabai
(PDF)

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ABSTRACT

Background/objectives: Scar formation following tissue injury is a challenge that might result in severe disfigurement. Further elucidation of contributors to tissue damage and scarring paves the way for development of targeted treatments. Our group has previously shown that extracellular granzyme B (GzmB), a serine protease, is involved in inflammatory skin conditions and delayed wound healing. Cutaneous leishmaniasis (CL) is a parasitic disease with chronic skin lesions that leave severe scarring. Here, we hypothesize that GzmB is elevated in CL caused by Leishmania major (L. major) and contributes to impaired healing through the cleavage of adhesion and/or extracellular matrix proteins.

Methods: Various histological methods were utilized to determine the presence of GzmB, its cellular source(s), and severity of the degradation of its known substrates in skin biopsies from patients with confirmed L. major infection. Cell- free in vitro cleavage assay was used to identify new GzmB substrates. Further, 12 wild type and 11 GzmB knockout C57BL/6 mice were inoculated with L. major and the clinical course of the disease was documented over 8 weeks. Samples were collected for histological analysis at study endpoint.

Results: Immunohistochemical and immunofluorescent staining of human samples showed that GzmB is highly expressed in CL compared to normal skin, and more than 90% of GzmB expressing cells were mast cells. Levels of GzmB substrates such as E cadherin, collagen VII, and collagen XVII were reduced in areas with increased number of GzmB+ cells. Cleavage of desmoglein 4 and annexin A2 by GzmB was confirmed in vitro. In vivo study revealed that in 54% of GzmB KO mice the skin lesions healed without visible scarring while all WT mice showed clinical lesion/scarring at the study endpoint.

Conclusions: Conclusions: GzmB-mediated proteolysis may contribute to CL pathogenesis. Topical inhibition of GzmB may reduce tissue injury and subsequent scarring in CL.

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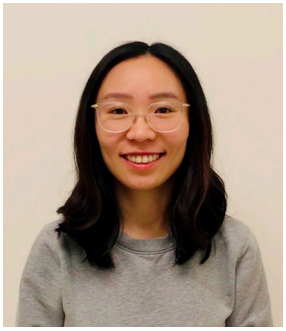

Meng Wang

SUPERVISOR(s): DR. MARI DEMARCO

NEXT-GENERATION SEROLOGY TEST: QUANTITATIVE IMMUNOGLOBULIN PROFILING OF ACUTELY ILL COVID-19 PATIENTS

Meng Wang
(PDF)

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ABSTRACT

Background/objectives: Severity and disease outcomes for patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections are associated with the antibody response of the infected individual. Previous serology studies lacked a detailed understanding of each humoral response, including isotype and subclass generation, mainly due to the unavailability of an advanced analytical tool. To investigate the humoral response in COVID-19 patients, we needed to resolve the total generated antibody to a detailed antibody response profile. We did this by generating a new blood test capable of quantifying all anti-SARS-CoV-2 antibody isotypes and subclasses (individually) from < 5 µL of blood. Using this antibody response profile, we assed the associations between antibody profiles and outcomes in acute COVID-19 hospitalized patients.

Methods: Plasma samples collected from COVID-19 patients (n=137) at day 4 and day 7 after hospitalization were analyzed using the newly developed blood test (ImmunIQ). Through immunoprecipitation and high-performance liquid chromatography tandem mass spectrometry detection, ImmunIQ provided quantification of anti-receptor binding domain (RBD) IgG, IgA, IgM, IgD, IgE, IgG1-4, IgA1-2 in a single analysis using 4.2 µL of plasma. Statistical analyses of the antibody profiles and patient outcomes (28-day mortality, in-hospital mortality, and organ dysfunction which was characterized by the use of mechanical ventilation and vasopressor) were performed using logistic regression, and odd ratios (OR) were calculated for each association.

Results: There was a 2.5-fold increase in anti-RBD IgG (95% confidence interval (CI) 2.1–2.9) in plasma from acutely ill COVID-19 patients from day 4 to day 7 after hospitalization; a doubling in anti-RBD IgG from day 4 to day 7 was associated with decreased likelihood of death by day 28 (OR 0.56, 95% CI 0.32–0.93). There was a 2-fold increase in anti-RBD IgA (95% CI 1.7–2.3) from day 4 to day 7 after hospitalization, but no association was found between this increase and mortality and disease severity. There was a 1.7-fold increase in anti-RBD IgM (95% CI 1.5–1.9) from day 4 to day 7 after hospitalization; a doubling in anti-RBD IgM from day 4 to day 7 was associated with reduced likelihood of invasive mechanical ventilation after day 7 (OR 0.15, 95% CI 0.02–0.67) and with reduced likelihood of vasopressor use after day 7 (OR 0.17, 95% CI 0.03–0.69). Dexamethasone use (n=19) was associated with increased anti-RBD antibody response.

Conclusions: Development of the ImmunIQ test enabled accurate quantification and detailed resolution of an individual's specific humoral response to COVID-19 using minimal sample volume. Utilizing this tool in the investigation of acutely ill COVID-19 patients, we found that early increases in specific anti-SARS-CoV-2 antibody isotypes were associated with lower mortality and decreased uses of mechanical ventilation and vasopressor.

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