2023

HLI RESEARCH DAY

18 AUG 2023 | 8 am - 4:30 pm

DR. JENNIFER GARDY

Deputy Director, Surveillance, Data & Epidemiology, Bill& Melinda Gates Foundation

DR. KAREN CHEUNG

Professor, Electrical and Computer Engineering, UBC

POSTER PRESENTATIONS

ORAL PRESENTATIONS

KT RAPID FIRE

CULLEN THEATER, ST. PAUL'S HOSPITAL

Centre for Heart Lung Innovation
UBC and St. Paul's Hospital

a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

SCHEDULE

8:00 AM	**BREAKFAST**
8:30 AM	**OPENING REMARKS** Dr. David Granville
9:00 AM	**BRUCE MCMANUS LECTURE** Dr. Jennifer Gardy
9:45 AM	**POSTER SESSION (ODD NUMBERS)**
10:45 AM	**TRAINEE ORAL PRESENTATIONS** Rylan McCallum (Brunham Lab) Carly Lin (Luo Lab) Zeren Sun (Bernatchez Lab) Aileen Hsieh (Hackett Lab) Edward Li (Sin Lab) Samuel Leung (Wang Lab)
12:00 PM	**LUNCH**
12:45 PM	**PETER PARÉ LECTURE** Dr. Karen Cheung
1:30 PM	**POSTER SESSION (EVEN NUMBERS)**
2:30 PM	**TRAINEE ORAL PRESENTATIONS** Tiffany Chang (Luo Lab) Denitsa Vasileva (Daley Lab) Maria Elishaev (Wang Lab) Karolina Moo (Eddy Lab) Julia Brown (Bernatchez Lab) Anna Kovtunenko (Hackett Lab)
3:45 PM	**KT RAPID FIRE PRESENTATIONS**
4:00 PM	**COFFEE BREAK**
4:15 PM	**AWARDS & CLOSING REMARKS** Dr. Don Sin

HLI Research Day 2023 Opening Remarks

"Opportunities and Jobs in BC's Expanding Life Sciences Sector"

Dr. David Granville
PhD, FAHA
Professor, Pathology and Laboratory Medicine
Director, Wound Healing and Regenerative Medicine Laboratory, VCHRI
Principal Investigator, ICORD Centre and UBC Centre for Heart Lung Innovation
Founder and Chief Scientific Officer, viDA Therapeutics, Inc.

Friday, August 18th 8:30-9:00am
Hosted by *HLI Trainee Association*

David Granville is a Professor and Director of the Wound Healing and Regenerative Medicine Laboratory at the ICORD Centre and HLI. He is the former Executive Director of the VCH Research Institute (VCHRI) and Associate Dean of Research in the UBC Faculty of Medicine. In 2003, he was recruited to UBC as a Canada Research Chair and Michael Smith Foundation for Health Research Scholar. Dr. Granville is the co-Founder and Chief Scientific Officer of viDA Therapeutics.

In his early career at QLT (1994-2001), his research supported the approval of Visudyne®, the first treatment for age-related macular degeneration. In 2001 at the Scripps Research Institute in California, he discovered a novel pharmacologic approach for reducing myocardial infarction. Dr. Granville is an inventor on several patents pertaining to discoveries that led to the formation of Radical Therapeutix Inc. (San Diego, CA). In 2014, he was named a Fellow of the American Heart Association (FAHA) and in 2019 and was inducted as a Scholar of the Royal Society of Canada. He has received awards including the Canada Top 40 Under 40™ award, UBC Outstanding Young Alumnus Award and SFU Academic Alumnus Award. *The Granville Lab* is interested in aging and its impact on injury, inflammation, and impaired healing with the goal of discovering novel therapeutics for age-related chronic diseases. Dr. Granville's early work was focused on ischemic heart injury, atherosclerosis, allograft vasculopathy and aortic aneurysm. More recently, his research has extended to tissue injury and repair. His laboratory has identified a key role for granzymes, a family of 5 serine proteases, in tissue injury, inflammation, epithelial dysfunction, autoimmune blistering, pruritus, and aging-related impaired healing. Pre-clinical validation of novel inhibitors against granzymes and other targets is currently underway with the goal of entering the clinic in 2024.

HLI Research Day 2023 Bruce McManus Lecture

Dr. Jennifer Gardy

PhD

Deputy Director, Surveillance, Data, and Epidemiology

Friday, August 18th 9:00-9:45 am

Hosted by *HLI Trainee Association*

Jennifer Gardy joined the Bill & Melinda Gates Foundation's Malaria team as Deputy Director, Surveillance, Data, and Epidemiology in February 2019. In this role, she oversees the Foundation's strategy and investments, focusing on improving routine malaria surveillance and data quality, leveraging malaria molecular data as an alternative source of surveillance data, and supporting geospatial and mathematical modeling approaches to malaria planning and control efforts. Her overarching goal is to ensure that National Malaria Control Programs and other global malaria stakeholders, such as WHO and the Global Fund, are able to make high-quality programmatic and policy decisions using high-quality data and analytics. Since 2021, she sits on the leadership team for the Foundation's Institute for Disease Modeling.

Before joining the Foundation, Dr. Gardy spent ten years at the BC Centre for Disease Control and the UBC School of Population and Public Health, where she held the Canada Research Chair in Public Health Genomics. Her research focused on the use of genomics as a tool to understand pathogen transmission, and incorporated techniques drawn from genomics, bioinformatics, modelling, information visualization, and the social sciences. Her team was the first to use genomics to reconstruct an outbreak of infectious disease, and her subsequent work, she consulted with public health agencies around the world on how to stand up pathogen genomic epidemiology programs. In 2018, Jennifer was named one of BC's Most Influential Women in STEM by BC Business Magazine and was named one of the Government of Canada's 20 Women of Impact in STEM. In 2021, she was elected to the National Academy of Medicine in recognition of her contributions to establishing pathogen genomic epidemiology. In addition to her science work, Jennifer is also an award-winning science communicator, hosting many episodes of science documentary television, including *The Nature of Things* and *Daily Planet*, as well as authoring two science books for children.

HLI Research Day 2023 Peter D. Paré Lecture

"Organ-on-chip for physiology and development of new therapies"

Dr. Karen C. Cheung
Ph.D.
Professor, School of Biomedical Engineering,
Department of Electrical & Computer Engineering,
University of British Columbia

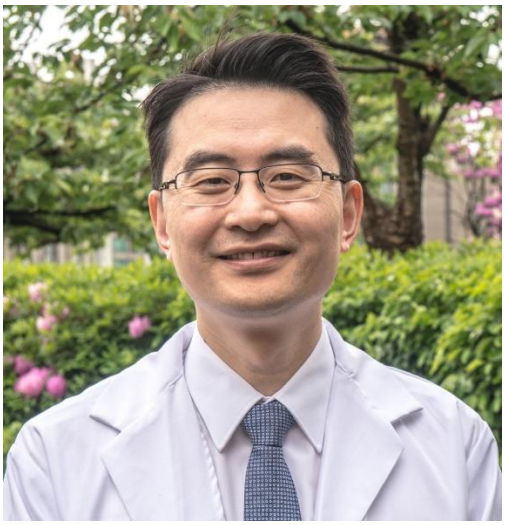
Friday, August 18th 12:45-1:30p.m.

Hosted by *HLI Trainee Association*

Karen Cheung received her B.S. and PhD. degrees in Bioengineering from the University of California, Berkeley. She did her postdoctoral work in microtechnologies at the École Polytechnique Fédérale de Lausanne, Switzerland. She is a Professor in the Departments of Electrical & Computer Engineering and School of Biomedical Engineering at UBC. She is also the Director of the School of Biomedical Engineering Graduate Program. She is a Fellow of the American Institute for Medical and Biological Engineering and serves on the editorial board of the IEEE Journal of Microelectromechanical Systems.

Her group has ongoing projects in tissue engineering, silicon photonic biosensors, inkjet dispensing of single cells for sequencing and proteomics, implantable microelectrode neural interfaces, and development of microfluidic organs-on-chip. Organ-on-chip platforms can mimic in vivo physiology, promising to enable more in-depth understanding of disease progression as well as permitting evaluation of novel therapeutics. Our team is developing a microfluidic model of the small airway that incorporates airway-specific cells supported by 3D extracellular matrix hydrogels to control the architecture in the microtissue. Our microfluidic model permits perfusion to expose cells to various environmental factors. Mend the Gap is an interdisciplinary project bringing together scientists, engineers, clinicians, and translation specialists to address the challenge of repairing the spinal cord after injury. The overall goal of Mend the Gap is to develop a treatment for spinal cord injury (SCI) using injectable biomaterials to support axonal growth across the spinal cord lesion. The hypothesis is that alignable structures within an injectable hydrogel will direct and extend axonal growth across the SCI lesion site and promote functional recovery. We test the properties of injectable biomaterial bridges with a variety of in vitro platforms. We have developed a spinal cord injury chip that allows us to examine 3D neurite growth in a biomaterial after injury.

HLI Research Day 2023 Closing Remarks



Dr. Don D. Sin

MD, FRCPC, MPH

Director & the De Lazzari Family Chair, Centre for Heart Lung Innovation (HLI)
Canada Research Chair in COPD
Professor of Medicine, The University of British Columbia

Friday, August 18th 4:15-4:30 p.m.

Hosted by *HLI Trainee Association*

Don Sin is the Director of HLI, Professor of Medicine at UBC, and Respirologist at St. Paul's Hospital (SPH). He holds a Tier 1 Canada Research Chair in COPD and the De Lazzari Family Chair at HLI. He has published over 550 peer-reviewed papers and has an H-index of 104. Expertscape.com ranks him as the top COPD expert in North America and 2nd in the world.

He obtained his medical degree at University of Alberta in 1991, completed a residency in general medicine and a fellowship in respiratory medicine at University of Alberta. This was followed by a Master's of Public Health from Harvard University (1997) and a research fellowship at University of Toronto. He returned to the University of Alberta as an Assistant Professor of Medicine in 1999, where he became a CIHR New Investigator (2001) and an Alberta Heritage Foundation Population Health Investigator (2002). He was recruited UBC/SPH in 2004 as a Tier 2 Canada Research Chair in COPD and the inaugural GlaxoSmithKline/St. Paul's Foundation Professor of COPD. He became a Professor of Medicine at UBC in 2009. During his career, he has received $30M in research funding as a Principal Investigator and $50M as a co-investigator, mostly from Tri-Council agencies, Genome Canada, Canada Foundation for Innovation (CFI), and the US National Institutes of Health. Over the past 10 years. He has directly supervised 13 master's and 3 doctoral students and 29 postdoctoral fellows. He currently serves on the Global initiative for chronic Obstructive Lung Disease (GOLD) scientific committee and is the section editor for the European Respiratory Journal and an editorial board member of the American Journal of Respiratory and Critical Care Medicine. His research focus is using "omics" data to discover novel biomarkers of disease activity and new therapeutic targets to reduce hospitalization and mortality in patients with COPD.

ORAL PRESENTATIONS

MORNING SESSION	
Rylan McCallum Brunham Lab	Assessing the Care of Indigenous Patients with Premature Atherosclerotic Cardiovascular Disease
Carly Lin Luo Lab	Understanding the Mechanism of Mitochondrial Dysfunction in Viral Myocarditis
Zeren Sun Bernatchez Lab	Of Mice and Men: Tweaking Rodent Lipoprotein and Cholesterol Metabolism to Better Mimic Human Muscular Dystrophy
Aileen Hsieh Hackett Lab	Mucus Plugged Airways in Asthma are Marked by Prominent Airway Remodeling
Edward Li Sin Lab	The Effect of Extracorporeal Radiofrequency Application on Emphysematous Lung Regions in a Unilateral Pig Model of Emphysema
Samuel Leung Wang Lab	Spatial Gene Expression Methods for Cross-sample Analysis and Quality Control
AFTERNOON SESSION	
Tiffany Chang Luo Lab	From Healer to Heartbreaker: The Pathogenic Role of cGAS-STING Activation in Viral Myocarditis
Denitsa Vasileva Daley Lab	Potential for Novel Epigenetic Clocks in Pediatric Targeted Methylation Sequencing Data
Maria Elishaev Wang Lab	Using a Novel Multiplex Imaging Platform to Visualize Inflammation and Cell Death in Human Atherosclerotic Lesions
Karolina Moo Eddy Lab	Clearing the Smoke on Healthy Lungs: Asssing the Structural Effects of Cannabis Inhalation and Vaping on the Lungs with Advanced Pulmonary Imaging
Julia Brown Bernatchez Lab	Characterization of Intramuscular Free Cholesterol Accumulation in Muscular Dystrophy Mice
Anna Kovtunenko Hackett Lab	Age-associated Gene Expression Differences in Healthy Lung Fibroblasts

Assessing the Care of Indigenous Patients with Premature Atherosclerotic Cardiovascular Disease

R.K. McCallum*, L. Akioyamen, D. Pinheiro Muller, I. Iatan M. Marchand, L. Arbour, L.R. Brunham

Background: Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of death worldwide and disproportionately impacts Indigenous populations. Indigenous populations in Canada continue to have poorer health outcomes than their non-Indigenous counterparts and are less likely to receive evidence-based cardiovascular care. Although ASCVD rates are rising in young adults, the experiences of Indigenous people with premature ASCVD remain unexplored.

Hypothesis: Evidence-based cardiovascular care in Indigenous patients with premature ASCVD will result in increased medication utilization and comparable cardiovascular risk reduction to non-Indigenous patients.

Methods: A mixed methods approach was used to assess the clinical care of Indigenous and non-Indigenous patients who received a diagnosis of premature ASCVD (men ≤ 50, and women ≤ 55 years of age) participating in the SAVE BC biobank. We collected data at baseline (time of diagnosis) and at the last clinical follow-up. Indigenous patients were invited to complete a survey to explore their experiences and perceptions regarding their diagnosis and treatment.

Results: Indigenous patients (n=19) with premature ASCVD had higher rates of diabetes, obesity, and history of smoking than non-Indigenous patients (n = 360). Rates of statin and high-intensity statin use were similar in both groups, as were reductions in LDL cholesterol from baseline to follow-up. Indigenous patients identified high cholesterol and genetic factors as major causes of their premature ASCVD and insufficient communication from healthcare providers and limited resources as major barriers in effective cardiovascular care.

Conclusions: Premature ASCVD significantly impacts Indigenous communities. Our study demonstrated among patients recieving specialized cardiovascular care, rates of heart medication, statin use, and LDL-C reduction did not differ between Indigenous and non-Indigenous patients. Tailored interventions, informed by increased healthcare provider awareness of Indigenous-specific issues and improved access to evidence-based care, could help reduce the burden of ASCVD in Indigenous populations.

This research is funded by the Canadian Institutes of Health Research and the British Columbia Network Environment for Indigenous Health Research.

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* Rylan McCallum is an Indigenous student currently completing his second year in the Master of Science in Experimental Medicine Program at UBC. During his spare time Rylan helps run the UBC Indigenous Undergraduate Research Mentorship Program and helps tutor in biology at the First Nations House of Learning

Understanding the Mechanism of Mitochondrial Dysfunction in Viral Myocarditis

C. Lin*, W. Hwang, Y. Mohamud, H. Luo

Background: Myocarditis, which is the inflammatory infiltration of the myocardium, is a leading cause of sudden death in young people, with approximately 1.5 million cases worldwide every year. Viral myocarditis is the most common etiology among all infectious causes, and coxsackievirus B3 (CVB3) is an extensively studied model system for viral myocarditis. Previous research on viral myocarditis demonstrated mitochondrial damage in the myocardium. Among all the viral proteins, 2BC, 3A, and 3D are known to be associated with membranes. Hence, there is a potential interaction between these viral proteins and the mitochondria, which are double-membraned cellular organelles and are highly abundant in the myocardium. The mitochondria are essential for many biological functions. Damaged mitochondria result in impaired energy production, increased reactive oxygen species (ROS) production, cell death, and inflammation. Nevertheless, the exact mechanism by which CVB3 damages mitochondria remains unclear. The present study aims to determine the role of viral proteins in mitochondrial dysfunction as well as to investigate the potential of antioxidant, L-sulforaphane to strengthen mitochondria by inducing antioxidant enzyme expression.

Hypothesis: CVB3 viral proteins 3A, 3D, and/or 2BC localizes to the mitochondrial membrane and causes mitochondrial dysfunction in viral myocarditis. Treatment of L-sulforaphane can effectively increase antioxidant enzyme expressions to counteract the overproduction of ROS resulting from damaged mitochondria and minimize CVB3-mediated mitochondrial injuries.

Methods: CVB3 viral protein constructs were transfected into HeLa cells to express viral proteins 3A, 3D, and 2BC. Localization of viral proteins was visualized using confocal microscopy. Mitochondrial function upon transfection of viral proteins will be assessed by detecting the production of ROS, quantifying ATP levels, and measuring mitochondrial membrane potential. Additionally, immunoprecipitation of viral protein 3D was conducted for mass spectrometry to identify protein-protein interactions, and the same protocol will be executed on the other two viral proteins. To determine whether L-sulforaphane can minimize CVB3-mediated mitochondrial injuries, HeLa cells were treated with 20 μM L-sulforaphane overnight, followed by an overnight infection of CVB3 (MOI = 10). Viral protein levels were examined using western blot, and qPCR was performed to measure the level of inflammatory cytokines and ROS to assess mitochondrial function.

Results: CVB3 viral proteins (3A, 3D, and/or 2BC) colocalize with mitochondrial outer membrane puncta. Viral protein 3D was observed to interact with coxsackievirus and adenovirus receptor based on mass spectrometry. Upon overexpression of the proteins, impaired energy production, overproduction of ROS, cell death, and inflammation are predicted. Overnight treatment with L-sulforaphane is expected to increase the expression of antioxidant genes, resulting in a significant decrease in the levels of ROS.

Conclusion: Localization of CVB3 viral proteins (3A, 3D, and/or 2BC) to the mitochondrial membrane and the subsequent impaired mitochondrial function contribute to viral myocarditis. Treatment with L-sulforaphane can successfully protect the mitochondria from overproduction of ROS.

This research is funded by CIHR and BioTalent and supported by the members of the Luo Lab.

* Carly Lin is a 3rd year UBC pharmacology student. She is currently completing her first co-op assignment at the Centre for Heart Lung Innovation.

Of mice and men: tweaking rodent lipoprotein and cholesterol metabolism to better mimic human muscular dystrophy.

Z. Sun*, Z. White, P. Bernatchez

Background: Mouse models of muscular dystrophy (MD) are notorious for their mild phenotype, which is in stark contrast to the loss of ambulation and death often observed in human MD. Since we have reported a high prevalence of dyslipidemia in MD patients - a new metabolic comorbidity caused by MD - and that apolipoprotein E (ApoE) gene knockout (KO) exacerbates mild rodent MD, we sought to examine whether other cholesterol or triglyceride metabolism regulators, such as the circulating high-density lipoprotein-associated cholesterol (HDL)/non-HDL ratio, circulating triglycerides, tissue 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) , low-density lipoprotein receptor (LDLR) and tissue free cholesterol accumulation, contribute to MD severity.

Hypothesis: Decreased circulating HDL/non-HDL ratio or increased circulating triglycerides will exacerbate muscle wasting in rodent MD models.

Methods: Mild dysferlin-deficient (Dysf) MD mice were used. The HDL/non-HDL ratio of mice was humanized by the transgenic expression of human cholesterol ester transfer protein (CETP), an enzyme not expressed in mice that converts HDL into non-HDL. Additionally, circulating triglycerides levels were raised in Dysf/ApoE double KO mice through cholesterol/triglycerides-rich diets. Circulating lipids were quantified. Muscle lesions were characterized by functional tests and histology. Intramuscular cholesterol metabolism was evaluated by Western blotting for HMGCR and LDLR, as well as filipin staining of histological sections for free cholesterol quantification.

Results: A ~30% reduction in HDL levels along with a ~60% reduction in the HDL/non-HDL ratio through expression of human CETP in Dysf mice failed to exacerbate muscle lesions. Dysf/ApoE mice on a cholesterol-rich diet caused hypercholesterolemia and a drastic, up to 10-fold exacerbation of muscle lesions and early ambulation dysfunction. In contrast, a triglyceride-rich diet in this model unexpectedly prevented muscle lesions despite increased circulating triglycerides. We also observed significant increases of intramuscular free cholesterol accumulation and HMGCR, LDLR protein expression in Dysf/ApoE mice with cholesterol-rich diet, which were normalized in triglyceride-rich diet.

Conclusion: MD causes cholesterol metabolism dysregulation. Transgenic or dietary approaches that cause hypercholesterolemia exacerbates mild rodent models of human MD, whereas hypertriglyceridemia may be protective. The HDL/non-HDL ratio does not appear to affect MD severity. Dietary interventions and cholesterol-lowering drugs should be considered for human MD.

This research is supported by Canadian Institutes of Health Research, Jain Foundation, and Centre for Heart Lung Innovation.

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*Zeren Sun is a PhD student at Faculty of Medicine of UBC and will be entering his third year of study. This is Zeren's third year at Centre for Heart Lung Innovation, St Paul's Hospital.

Mucus Plugged Airways in Asthma are Marked by Prominent Airway Remodeling

A. Hsieh*, M. Liegeois, M. Fouadi, C. Leung, J.V. Fahy, T.L. Hackett

Background: The hallmark features of asthma include mucus cell hyperplasia, mucus accumulation, reticular basement membrane thickening, and increased smooth muscle mass. Recently, airway mucus plugs have been identified as a persistent radiological feature on computed tomography lung scans of patients with moderate to severe asthma and are associated with worse clinical outcomes. To date, the relationship of airway remodeling within mucus-plugged airways in asthmatic subjects has not been investigated.

Hypothesis: Mucus-plugged airways in asthma have more prominent remodeling compared to non-mucus-plugged airways and controls.

Methods: The study included lungs from 9 fatal asthma cases, 5 non-fatal asthma cases (history of asthma but death from a non-asthma cause), 8 healthy control cases, and 7 Global Initiative for Obstructive Lung Disease (GOLD) 3 and 4 COPD patients who underwent lung transplantation, which served as disease controls for mucus plugs. Lungs were inflated, frozen, and randomly sampled for histology. Two or more airways with and without mucus plugs were assessed per case for a total of 175 airways. The thickness of total airway wall, airway epithelium, reticular basement membrane, and smooth muscle mass were quantified using Euclidean Distance Mapping to assess each wall measurement across the entire airway cross-section.

Results: As previously reported in the literature, total airway wall thickness of patients with asthma and COPD was significantly greater compared to age-matched controls ($P<0.05$) For the specific airway wall components, asthma patients had a significantly thicker airway epithelium, reticular basement membrane and smooth muscle layer compared to controls, whereas COPD patients had thicker smooth muscle. Interestingly, when the airways within the same patient were sorted into airways with or without mucus plugs, in all asthma patients, mucus-plugged airways had significantly greater remodeling of the reticular basement membrane and airway epithelium ($P<0.05$) compared to non-mucus-plugged airways. Further, the basement membrane in mucus-plugged airways from fatal asthma patients was significantly thickened compared to mucus-plugged airways from non-fatal asthma patients ($P<0.01$).

Conclusion: Heterogeneity of airway narrowing has been implicated as an important feature of fatal asthma episodes. This study demonstrates that airway wall remodeling is more prominent in mucus-plugged airways in fatal asthma compared to unplugged airways and highlights that mucus plugs may play an important role in airway narrowing and collapse in fatal asthma.

This research is funded by the Canadian Institutes of Health Research Canada Graduate Scholarship – Doctoral (CGS-D) and operating grant.

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*Aileen is a 2nd year PhD student in the Department of Anesthesiology, Pharmacology, and Therapeutics at UBC. During her spare time, she enjoys boxing, paddleboarding, and baking.

The Effect of Extracorporeal Radiofrequency Application on Emphysematous Lung Regions in a Unilateral Pig Model of Emphysema

E. Li*, T. Sasaki, D. Vasilescu, Y. Yasuda, C.Y. Seow, C.Y. Cheung, L. Mowbray, R. Hildebrandt, E. Goodacre, C. Myrdal, K. Wolff, E. Elizur, D.D. Sin

Background: Chronic Obstructive Pulmonary Disease (COPD) is a leading cause of death worldwide and commonly presents as emphysema, which is characterized by destruction of alveoli, loss of lung elasticity, and reduced blood flow to emphysematous lung tissue. Our previous studies have shown that porcine pancreatic elastase (PPE) instilled into rodent and pig lungs can produce reliable emphysema models. Extracorporeal radiofrequency (RF) treatment in rodents with emphysema caused mild fibrosis in emphysematous tissue and improved lung function. However, RF treatment in pigs with emphysema only produced minor improvements. Here we sought to improve RF treatment in pig models of emphysema by using pulmonary artery occlusion (PAO) to restrict blood flow to emphysematous lung tissue and make it more susceptible to thermal damage from RF.

Hypothesis: Extracorporeal RF application to pig lungs will improve lung function by selectively damaging emphysematous tissue with reduced blood flow from PAO.

Methods: 20 Yucatan pigs were split into four different conditions: no treatment control (n=3), PPE (n=6), PPE+RF (n=7), and PPE+RF+PAO (n=4). A unilateral emphysema model was created by intratracheally instilling PPE (725U/Kg body weight) into the left lung only. After 6 weeks, PAO was performed using a balloon catheter to restrict blood flow to the left lung, and RF (600W for weight-dependent time) was applied to the treatment groups. Pigs were sacrificed 6 weeks after RF application to harvest their lungs. Lung compliance was measured via water displacement and plethysmography. Each lung was then air inflated at 10cm H_2O, frozen in liquid NO_2 vapor, scanned by computed tomography (CT), sliced axially, and cored into 6 tissue samples through systematic uniform random sampling. Tissue cores were scanned by Micro-CT and images were measured for mean linear intercept (Lm) and alveolar surface density (Sv). Histological sections were assessed for fibrosis based on a modified Ashcroft scale.

Results: Water displacement and plethysmography tests showed all groups given PPE had significantly higher lung compliance than the control, but there was little difference between each experimental group. CT scans identified no significant difference in mean lung density between any groups. Micro-CT scans showed that compared to the control, all groups given PPE had a significant increase in Lm and decrease in Sv, indicating increased air space size and decreased alveolar surface density. However, treatment with RF or RF+PAO did not significantly improve either Lm or Sv. Finally, histological assessment revealed that RF produced mild fibrotic changes but no significant difference between groups.

Conclusion: Extracorporeal RF application after PAO did not significantly improve lung function. Our project protocols are currently being revised as we attempt to optimize treatment.

This research is supported by Ikomed Technologies and the Centre for Comparative Medicine.

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* Edward Li is a third year UBC student, majoring in Honours Cellular, Anatomical, and Physiological Sciences. He joined the Centre in May as a Co-op student.

Spatial Gene Expression Methods for Cross-sample Analysis and Quality Control

S. Leung*, B. Li, M. Elishaev, Y. Wang, A. Singh

Background: Formalin-Fixed Paraffin-Embedded (FFPE) tissue samples are the commonly used format for biobanking. Tools to obtain high-throughput spatial gene expression data for FFPE samples are commercially available, however there is a lack of benchmarking in how biobanking procedures influence analysis results. In the case of autopsy samples, a delay prior to preservation may lead to RNA degradation, creating artificial bias compared to explanted samples that are timely preserved. To quantitate the effect of RNA degradation, differences in spatial gene expression between explanted and autopsy samples need to be compared. Currently, pipelines for performing cross-sample comparisons of spatial gene expression data are not available. This project aims to create a pipeline to address this need, allowing for identification of key differentially expressed genes across different samples. These key differences can be used to design quality control probes that exclude low quality samples for spatial gene expression.

Hypothesis: Differences in spatial gene expression between explanted and autopsy samples can be identified by our pipeline.

Methods: Explanted and autopsy blood vessel samples were collected from the Bruce McManus Cardiovascular Biobank and sequenced by Visium, providing gene expression information spatially contextualized within tissue sections. As quality control for spatial comparisons of RNA degradation across samples, Seurat and GGplot2 packages in R were used to map spatial enrichment of mitochondrial genes, which were considered as areas of high RNA degradation. In order to ensure fair cross-sample comparisons of differentially expressed genes, regions compared need to be enriched with similar cell types. To estimate cell presence and abundance given local gene expression, cell deconvolution was performed on both samples using the SpaceXR module in R coupled with the Tabula Sapiens Vasculature reference dataset. After identifying similar cell regions with cell deconvolution, differential gene analysis was performed using the Seurat package to investigate highly expressed genes between these two samples.

Results: Inner blood vessel regions from the autopsy sample showed greater counts of mitochondrial genes, indicating RNA degradation of autopsy samples in general. Cell deconvolution showed these regions are enriched with smooth muscle cells in both autopsy and explanted samples. Comparing differences of gene expression between samples, we discovered that genes related to mitochondrial dysfunction and cell lysis were significantly increased in the autopsy sample.

Conclusion: Differences in gene expression were observed when analyzing similar cell-enriched regions of explanted and autopsy samples. Mitochondrial genes identified are potential markers for excluding low quality samples from spatial gene analysis. Applying this pipeline further can help generalize and correct biases found in spatial gene analysis using autopsy tissue samples.

Special thanks to New Frontiers in Research Fund - Exploration for providing data and funding

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*Samuel is a fifth-year undergraduate student majoring in Statistics at UBC. He is passionate about exploring biological problems using computational and statistical methods.

From Healer to Heartbreaker: The Pathogenic Role of cGAS-STING Activation in Viral Myocarditis

E.C. Chang*, Y. Mohamud, Y. Wang, G.K. Singhera, H. Luo

Background: Viral myocarditis (VM) is an inflammatory disease of the heart muscle caused by viral infections, leading to heart failure and dilated cardiomyopathy (DCM) in up to 30% of patients. The underlying mechanisms of VM are not fully understood. Excessive inflammation mediated by the innate immune system is known to induce cardiac damage in VM. However, attempts to suppress inflammation in myocarditis through clinical trials have yielded no significant improvements. Recent evidence implicates the involvement of the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway in various inflammatory diseases, including VM. The cGAS-STING pathway, a double-stranded DNA (dsDNA) sensor of the innate immune system, is triggered by pathogenic dsDNA or mislocalized host DNA (e.g., originating from cell damage). Although initially protective during acute viremia by facilitating viral clearance, excessive or prolonged activation of cGAS-STING may lead to hyperinflammatory states in the later stages of myocarditis. Previously, our lab demonstrated that cGAS or STING deletion reduces inflammation and reduces cardiac damage in mouse models of enteroviral myocarditis. This study aims to further validate the role of cGAS-STING signaling in the pathogenesis of VM by using myocardial tissue of heart transplant patients diagnosed with viral myocarditis.

Hypothesis: Activation of the cGAS-STING pathway by self-DNA leakage from viral-induced cardiac damage contributes to the pathogenesis of viral myocarditis by promoting prolonged and excessive inflammatory response in cardiomyocytes and inflammatory cells.

Methods: Explanted heart tissue samples from viral myocarditis patients (n=20) and healthy controls (n=10) were obtained from the Bruce McManus Cardiovascular Biobank transplant cohort. Molecular analysis, including RT-qPCR and immunohistochemistry, was performed on RNAlater™ solution-preserved myocardium to quantify cGAS-STING-mediated inflammation and compare pathway components ((dsDNA, cGAS, p-STING, p-TBK1, p-IRF3) and inflammatory markers (CD45, CD68, TNFα, INFβ1) between virus-infected and control tissues. De-identified clinical information and relevant findings were correlated to molecular analysis data to identify clinical correlates.

Results: The study found significant upregulation of the cGAS-STING pathway and inflammatory markers in the viral myocarditis group compared to the control group ($p<.05$). Moreover, cytosolic dsDNA levels were significantly higher in viral myocarditis tissue, and its level positively correlated with the severity of histopathological assessment of cardiac injury.

Conclusion: The identification of cGAS-STING as a contributor to viral-induced cardiac damage highlights its therapeutic potential as a treatment avenueDespite using tissue samples from explanted hearts of patients with advanced-stage VM, these initial findings provide valuable insight into the role of cGAS-STING in viral myocarditis, warranting further investigation. Future research should focus on exploring specific modulators of the pathway and assessing their efficacy in mitigating the progression of viral myocarditis.

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*Tiffany is a 5th year student completing her BSc. Behavioural Neuroscience program at UBC. She has gained valuable experience at HLI since joining the BMCB as a co-op student in 2021. Currently, she is conducting research in the Luo Lab funded by the UBC Faculty of Medicine Summer Student Research Program.

Potential for Novel Epigenetic Clocks in Pediatric Targeted Methylation Sequencing Data

D Vasileva*, M Wan, ES Chan, C Laprise, Y Asai, A Clarke, AJ Sandford, CMT Greenwood, D Daley

Background: DNA methylation (DNAm) is a dynamic epigenetic modification found at Cytosine-p-Guanine dinucleotides (CpGs). CpG sites that display differential methylation with age have been identified. The epigenetic clock leverages these age-informative CpGs to calculate epigenetic age (EA). The Horvath pan-tissue clock is the most prominent epigenetic clock; it was developed by applying penalized linear regression on DNAm array data with a focus on mid-life (mean age: 45 years). Epigenetic age acceleration (EAA, EA>chronological age) has been linked to increased risk of asthma. EAA is a potential biomarker that may differentiate persistent vs. transient childhood asthmatics. However, the clock does not account for logarithmic age-related changes in DNAm during childhood and underestimation of EA in older age groups (>65) has been reported. To be a biomarker, EAA should reflect biological processes, rather than prediction error.

Hypothesis: Incorporating non-linear methods and jointly increasing the number younger children and older adult 65-93 years) samples will yield more accurate epigenetic clocks.

Objectives: 1) identify age-informative CpG sites in DNAm sequencing data from across the lifespan and 2) assess the accuracy of linear and non-linear models in the development of epigenetic clocks.

Methods: The Illumina TruSeq MethylCapture EPIC targeted sequencing library (~3.5 million CpGs) was used to assess methylation from 932 samples from three studies-The Canadian Asthma Primary Prevention Study (CAPPS, n=632); the Saguenay- Lac- Saint- Jean study (SLSJ, n=180) and the Canadian Peanut Allergy Registry (CanPAR, n=120). In CAPPS, DNAm for 239 children was assessed on at least one of three occasions (birth, and/or 7 or 15 years). Data for 114 mothers (age:18-43 years) was also included. SLSJ consists of multi-generational triads from 60 French-Canadian families (age: 5-93 years). CanPAR is a registry of people with peanut allergy (age:5-38 years). After Quality Control 914 samples and 2067942 CpG sites were retained. **Identify age-informative CpGs**: in CAPPS and SLSJ using mixed effects linear regression with age as the dependent variable. We filtered CpGs with correlated DNAm using Pearson correlation (> 0.7 were grouped together and only the site with lowest p-value was retained). **Build epigenetic clocks**: using the 1000 remaining CpG sites with lowest p-values as input to elastic net (linear) and gradient boosted trees (non-linear) algorithms in CAPPS and SLSJ. Accuracy was assessed in CanPAR using absolute error (AE= |predicted-reported age|).

Results: We found >500,000 age informative CpGs (p-value range: 10^{-263}- 10^{-8}). Clocks developed using both linear (elastic net, 393 CpGs selected) and non-linear methods (trees) had lower mean AE (2.80 and 2.09 years) compared to the pan-tissue clock (8.55 years).

Conclusion and Significance: Clocks developed by incorporating childhood and older adult samples showed greater accuracy than the Horvath clock.

This project is funded by CIHR and the UBC 4-Year Fellowship.

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*Denitsa is a second year PhD student in the Bioinformatics program at UBC.

Using a Novel Multiplex Imaging Platform to Visualize Inflammation and Cell Death in Human Atherosclerotic Lesions.

M. Elishaev*, A. Zhou, B. Li, C. Lai, G.A. Francis, Y. Wang

Background: Cardiovascular events claim an estimated 17.9 million lives globally every year. Most cardiovascular events are attributed to advanced atherosclerotic lesions, which start with the formation of lipid-laden foam cells in the arterial inner layer known as the intima. Macrophages have long been considered the primary source of foam cells and inflammation in the intima, making them a target for therapeutic development. However, recent studies suggest that at least 50% of foam cells in atherosclerotic lesions may originate from smooth muscle cells (SMCs). Yet, it remains unclear whether SMC foam cells induce cell death and contribute to inflammation that drives disease progression and the onset of cardiovascular events. Conventional tissue staining methods used in previous studies can only visualize three protein markers per tissue section, which is insufficient for characterizing the features of foam cells and inflammation. In my current project, I am utilizing a novel multiplex imaging technology that employs barcoded antibodies and repeated sequential imaging cycles to visualize multiple protein markers simultaneously, thereby overcoming these limitations. This study aims to determine whether intimal SMCs are associated with increased cell death and inflammation, which necessitate therapeutic targeting to prevent cardiovascular events.

Hypothesis: Intimal SMCs are associated with the expression of inflammation and cell death markers.

Methods: We have selected ten early-stage lesion samples rich in foam cells from the Bruce McManus Cardiovascular Biobank for PhenoCycler multiplex imaging. Based on single-cell RNA-sequencing data, we have designed a unique 12-plex antibody panel to characterize cell phenotypes and inflammation markers. To generate this panel, selected antibody clones were conjugated to oligonucleotide barcodes and validated on adjacent tissue sections through immunostaining to confirm their antigen recognition after conjugation. Using PhenoCycler imaging, we have detected lipid accumulation in the intima and measured the expression of proteins associated with cell phenotype, cell death, and inflammation. We have subsequently conducted HALO cell segmentation analysis to characterize the foam cells.

Results: We identified that the vast majority (92±6%) of foam cells in early atherosclerotic lesions were of SMC origin. The results also indicate that both SMC and macrophage foam cells are associated with the expression of inflammatory and cell death markers. Furthermore, the number of SMC foam cells expressing the pro-inflammatory marker IL-1β is significantly higher than the number of macrophage foam cells expressing IL-1β. Additionally, both types of intimal cells contribute to cell death in early lesions, irrespective of their lipid accumulation status.

Conclusions: This is the first time the relationship between lipid accumulation 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 reveal that, in addition to macrophages, SMCs are another major source of inflammation and cell death in early lesions, which should be studied further for therapeutic development.

This research is funded by FoM New Faculty Research Award, Fondation Leducq, and CIHR.

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*I am a second-year Ph.D. student at the PALM Department. I enjoy playing chess, practicing yoga, and traveling.

Clearing the Smoke on Healthy Lungs: Assessing the Structural Effects of Cannabis Inhalation and Vaping on the Lungs with Advanced Pulmonary Imaging

K.C. Moo*, J.A. Leipsic, D.D. Sin, J.M. Leung, R.L. Eddy

Background: The recent and drastic popularization of vaping and cannabis smoking in Canada has brought about an urgent need to educate current and potential smokers on the possible risks of these habits. While the impact of cigarette smoking on the lungs is well established, little is known about the effects of cannabis inhalation and vaping on lung health. Our goal is to determine whether there are structural repercussions of these novel inhalation exposures using computed tomography (CT) imaging to capture quantitative indicators of possible lung damage.

Hypothesis: Cannabis smoking and vaping both contribute to structural changes in the lung that can be detected with advanced pulmonary imaging tools, before changes are detected with pulmonary function tests.

Methods: All participants were ≤ 35 years of age and were classified as only smoking cannabis, only vaping, or both. Age- and sex-matched control participants were identified as non-smoking, non-vaping participants with no lung disease or conflicting conditions. Quantitative analysis of thoracic CT images from the four study conditions (total n = 24) was performed using commercial VIDA Insights software (VIDA Diagnostics Inc., USA). Lung density, airway, and vasculature measurements from quantitative CT were then used to investigate lung structure. These measurements and other lung function metrics were compared between the study groups via one-way ANOVA with post-hoc Bonferroni corrections.

Results: Participant recruitment and selection, and data collection are complete. Data analysis and interpretation are ongoing. Initial analysis has revealed no statistically significant differences between participant groups when comparing lung densities (p=0.2-0.6), and air-to- tissue ratios (p=0.6). Further analysis is expected to reveal airway thickening in both cannabis smoking and vaping groups based on previous research, which would point to signs of possible structural lung changes.

Conclusion: We aim to show that cannabis smoking and vaping can lead to lung damage including large airway inflammation and small airways dysfunction detected by advanced imaging, hence putting the increasing population of people who smoke at risk for adverse health effects, and consequently a higher burden on the Canadian health care system. Further investigation into the lung functional repercussions of cannabis inhalation and vaping coupled with improved widespread education on lung health could alleviate the individual and societal burdens affiliated with vaping and cannabis smoking.

K.C. Moo is supported by the UBC Faculty of Medicine Summer Student Research Program via the Carman Crawford Browne Estate Fund for Medical Research and Providence Health Care Research Institute. This research is supported by the Canadian Institutes for Health Research.

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*Karolina Moo is entering her fourth year of Biomedical Engineering at UBC in the Applied Science Co-op Program. Karolina has been working at the Centre for Heart Lung Innovation with Dr. Eddy for the past two years, and this is her first summer at HLI as a co-op student.

Characterization of Intramuscular Free Cholesterol Accumulation in Muscular Dystrophy Mice

J. Brown*, Z. Sun, P. Bernatchez

Background: Muscular dystrophy (MD) causes muscle damage and wasting, leading to reduced mobility and premature death. Our team has shown the severity of MD is consistent with changes in cholesterol metabolism, but how this occurs is unknown. Understanding the mechanism between cholesterol and MD can lead to the development of novel therapeutics. Using a mouse model of limb-girdle MD type 2B (LGMD2B), a form of MD that causes muscle atrophy primarily in the proximal muscles around the hips and shoulders, we have observed heterogenous intramuscular free cholesterol (FC) accumulation in certain LGMD2B myofibers. The aim of this study is to determine the changes of FC at different stages of muscle damage and LGMD2B severity, and to correlate FC with common markers of dying myofibers.

Hypothesis: Higher FC in LGMD2B myofibers correlates with disease severity.

Methods: Quadriceps were collected from young (3 weeks), middle-aged (3 mo), and old (11 mo) wild-type (WT) and LGMD2B mice (mild) (n = 6). In addition, quadriceps from LGMD2B/apolipoprotein E double-knockout mice (severe) (n = 6) were collected. Muscle cross-sections were stained with fluorescent FC marker filipin and visualized by two-photon microscopy. Total cholesterol levels will later be quantified by the Amplex Red cholesterol assay kit. Muscle sections were histologically analyzed via Masson's trichrome, and stained with Draq7, laminin, and filipin to visualize the nucleus, sarcolemma and FC respectively. QuPath software (ver. 0.4.3) was used to quantify the FC level, and to detect co-localization of filipin FC and sarcolemma.

Results: Intramuscular FC levels in mild LGMD2B mice continuously increased with age, which was also aligned with disease progression. Compared to mild LGMD2B mice, muscles from severe LGMD2B mice had significantly decreased total cross-sectional area (~2 fold), increased fibro-fatty muscle damage area (~10 fold), and increased overall FC levels in the muscle tissue (~4 fold). Mild LGMD2B mice showed scattered accumulation of FC mostly in the sarcoplasm (71.8%) and aligned with the sarcolemma (28.1%), which indicated mild abnormalities in cholesterol transportation. By contrast, severe LGMD2B muscles showed different patterns of FC distribution (52.8% in sarcoplasm, 42.2% in sarcolemma, 5% in muscle interstitium). Compared to mild LGMD2B muscles, severe LGMD2B muscles presented significantly increased levels of FC accumulation in sarcolemma (~5 fold) sarcoplasm (~3 fold), and muscle interstitium (~200 fold).

Conclusion: The results indicate that LGMD2B progression is associated with incremental free cholesterol accumulation in muscle tissue, with a trend of excessive cholesterol efflux from myofiber to extracellular matrix. Treatments potentially reducing intramuscular FC accumulation will be tested in severe MD models for developing novel therapies.

This research is supported by Canadian Institutes of Health Research, Jain Foundation, and Centre for Heart Lung Innovation.

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*Julia Brown is a biochemistry student at UBC and will be entering her last year of study. This is Julia's first year at the Centre for Heart Lung Innovation and is working in Dr. Bernatchez lab as a summer student.

Age-associated gene expression differences in healthy lung fibroblasts

A. Kovtunenko*, K.O. Nwozor, K.Usman, Y. Yang, T.L. Hackett

Background: Fibroblasts within the lung have a critical role in maintaining the extracellular matrix (ECM) environment in health and in response to lung injury. With ageing, the risk for lung diseases such as chronic obstructive pulmonary disease (COPD) is significantly increased and is associated with ECM remodeling. During inflammatory episodes, IL-1α promotes a proinflammatory response in lung fibroblasts, whereas TGF-β isoforms act as critical regulator of tissue remodeling and repair. It is currently unknown if fibroblast gene expression is altered in response to inflammatory signals (IL-1α) and remodeling mediators (TGF-β), or how it changes with aging, and how it may affect fibroblast functions within the lung. We aim to study if gene expression is altered in fibroblasts by age and if this influences the response of fibroblasts to IL-1α or TGF-β1 stimulation.

Methods: Primary human lung fibroblasts (PHLFs) were obtained from parenchymal lung explants from 29 individuals aged 5-83 years with no history of respiratory disease. Fibroblasts were seeded and cultured to 90% confluency before being serum-deprived overnight. Each sample underwent a 72 hr treatment with either control media, 1ng/ml IL-1α or 50 ng/ml of TGF-β1. Cell lysate was used for RNA sequencing or protein extraction for western blot of ECM proteins. Cell-free supernatant was also used for ELISAs of inflammatory cytokines and ECM proteins.

Results: Lung fibroblasts derived from older healthy people were more pro-inflammatory expressing great levels of Interleukin (IL)-6 and 8 compared to younger people. Western blot analysis identified that fibroblasts from older people produce elevated levels of collagen 1 and decorin, which are elevated in lung fibrosis, compared to younger donors. In response to TGFb1 or IL-1a there was no difference in the expression of IL-6, IL-8, collagen or decorin expression between younger and older donors. For the RNA sequencing data, we obtained at least 30M paired-end reads from bulk RNA-Sequencing on all samples. Salmon was used to estimate the abundance of gene-level counts by aligning FASTQ reads to a human genome reference from the GENCODE project (GENCODE GrCh37, version 43). The gene count was normalized to log2 counts per million (CPM) using the limma package in R. Genes with log2 CPM < 1 in at least 10 samples were filtered out to leave only genes with high expression. Currently, linear mixed effect models are being applied to test associations between gene expression, age, and treatment. The Benjamini-Hochberg procedure will be used to correct multiple hypotheses testing. A false discovery rate (FDR) of <0.05 will be used as the threshold for significance.

Conclusion: Our results suggest that lung parenchymal fibroblasts in older healthy people have a pro-inflammatory and pro-fibrotic phenotype. Further, work focused on understanding the gene expression profile of fibroblasts with aging will help further understand the implications of the aging lung in the development of chronic lung disease.

POSTER PRESENTATIONS

1. Abhinav Kumar Checkervarty Tebbutt Lab	Sickle-Cell Trait Effects Early Life Immune Development, But Not Vaccine Response
2. Alexandra Schmidt Eddy Lab	Evaluating Quantitative Lung CT Measurements in Cardiac CT to Identify Novel Predictors for Acute Coronary Syndromes
3. Alyssa Yoo Yang Lab	Role of N-cadherin and NFAT5 in the Pathogenesis of Coxsackieviral Myocarditis
4. Arla Xiao Hackett Lab	Development of Better Bleomycin Murine Models for Testing Idiopathic Pulmonary Fibrosis Therapeutic Targets
5. Boaz Li Wang Lab	Quality Controls for Spatial Gene Analysis in Formalin-Fixed Paraffin Embedded Tissues
6. Cassie Gilchrist Leung Lab	Characterization of Respiratory Clinical Outcomes in Cannabis Smokers
7. Cathy Fu Luo Lab	Antiviral Potential of cGAS-STING Activation Against Enteroviral Infection
8. Christopher Yuen Bernatchez Lab	Early Endothelial Function Activation by Losartan Prevents Aortic Stiffness in a Model of Type 1 Diabetes
9. Elaine Ling Francis Lab	Effect of Lysosomal Acid Lipase Overexpression on Cholesterol Mobilization in Arterial Smooth Muscle Cells
10. Fatemeh Aminazadeh Hackett Lab	Investigating the Contribution of Sex Differences to Small Airways Disease in COPD Using Ultra-High Resolution Imaging
11. Cyril Helbling DeMarco Lab	Diagnostic Performance of α-Synuclein Seed Amplification Assays: A Meta-Analysis
12. Geoff Nonis Koelwyn Lab	The Role of Exercise in Reversing Sepsis-Induced Immune Suppression

POSTER PRESENTATIONS

13. Husan Aulakh Laksman Lab	Open-Source Analysis for Cardiovascular Research with Optical Mapping Data
14. Janette Chen Tan Lab	CanCOLD Re-Visit: Changes In Protocol To Address New Questions
15. Jeffery Tang Singh Lab	Benchmarking Methods for Gene-Regulatory Network Inference
16. Kauna Usman Hackett Lab	Interleukin-1 Alpha Counteracts Transforming Growth Factor-β1 and β2 Signaling in Lung Extracellular Matrix Remodeling
17. Kingsley Nwozor Hackett Lab	Single-cell RNA Sequencing Reveals Defective Bronchial Epithelial Cell Differentiation in Patients with Severe COPD in 3-Dimensional Organoid Cultures
18. Leiana Hoshyar Dorscheid Lab	
19. Vida Sussman/ Rachel Rosenblatt/ Ilana Guez Leipsic Lab	Artificial Intelligence-Enabled Quantitative Plaque and Hemodynamic Analysis in Coronary CT Angiography for Predicting Acute Coronary Syndrome Risk: the EMERALD II Study
20. Minzhi Chloe Huang Tan Lab	Population Attributable Risk of Chronic Obstructive Pulmonary Disease in Canadian Men and Women in a Cross-Sectional Multisite, Population-Based Study
21. Najmeh Assadinia Hackett Lab	Remodeling of Small Airways and Arterial Vasculature is a Hallmark of IPF
22. Oliver Haidari Sellers/Leipsic Lab	Predicted Versus Observed Valve to Coronary Distance in Valve-in-Valve TAVR: A Computed Tomography Study
23. Raveen Badyal Dunne Lab	The Effect of TGF-β Receptor Inhibition on Scleroderma and Idiopathic Pulmonary Fibrosis

POSTER PRESENTATIONS

24. Roy He Singh Lab	Using Cytokine-Specific Cell-Cell Interaction Networks to Predict Response to Allergen Exposure Using Graph Neural Networks
25. Samantha Chow Dorscheid Lab	CLOSURE - Computing Lesion Size of Monolayers Using a Convolutional Neural Network
26. Sanaz Ashraf Luo Lab	Understanding the Role of Coxsackievirus B3 in the Landscape of Immunogenic Proteins in Breast Cancer
27. Sarah Bradwell Koelwyn Lab	Peak ICU Performance Status and Long-Term Outcomes in Critical Care Survivors
28. Sheena Jiang Koelwyn Lab	Bioinformatic Exploration of Exercise and Age-Induced Changes to Innate Immune Cell Functions
29. Steven Chen Brunham Lab	Modulating High-Density Lipoprotein (HDL) Levels Improves Sepsis Outcomes
30. Tamara Nichvolodoff Walley Lab	High-Density Lipoprotein Levels in Patients with Septic Shock
31. Tucker Reed Sellers Lab	Impact of Bioprosthetic Surgical Valve Degeneration on True Internal Diameter (ID)
32. Victoria Baht Francis Lab	Effect of Overexpression of Lysosomal Acid Lipase on Cholesterol Mobilization in Cultured Human Aortic Smooth Muscle Cells
33. Wendy Hwang Luo Lab	Understanding the Role of Complement Regulator Proteins in CVB3 Induced Myocarditis
34. Wendy Liang Dorscheid Lab	The Effect of Senescence and Senolytics on Airway Epithelial Function
35. Cindy Wang Quon Lab	The Relationship Between In-Vitro and Clinical Responses to CFTR Modulators in Cystic Fibrosis Patients
36. Yolanda Yang **Dorscheid Lab**	Reference-free Deconvolution of Spatial Transcriptomic Data Using Semi-Supervised Topic Models

Sickle-cell trait effects early life immune development, but not vaccine response.

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Background: Sickle-cell trait (SCT) is a genetic condition that affects >300 million people worldwide, including vulnerable populations such as neonates. In SCT, individuals inherit one normal and one altered beta globin (*HBB*) gene and are usually free from any complications. However, under certain environmental stressors such as hypoxia, extreme exhaustion, and dehydration, they can experience sickling of their red blood cells, leading to blood flow obstruction, organ exhaustion, lowering of immune defenses against infections.

Hypothesis: Neonates with SCT can be identified by genotyping the RNA sequence reads mapped to *HBB*, which are often filtered out from blood-based transcriptomic studies. Additionally, SCT neonates exhibit distinct immune development during early life.

Methods: Study data were derived from neonatal blood samples collected over the first week of life from a NIH/NIAID-funded Human Immunology Project Consortium (HIPC) vaccination study of 720 neonates born in The Gambia, West Africa, a region with high prevalence for sickle-cell disorders. To determine the SCT status of neonates, we investigated RNA-seq reads mapped to the *HBB* gene. These were compared to the reference human genome GRCh38 to identify the T > A mutation at locus 5227002 on chromosome 11. Clinical data, including hepatitis B vaccine (HBV) immunization status, were integrated with systems biology data, including transcriptomics and proteomics, to investigate differences in the ontogeny and response to vaccination of neonates with SCT compared to the wild type (WT).

Results: Genotyping identified 118 out of 676 neonates (~17%) with SCT, while 4 neonates (~0.6%) were homozygous for sickle-cell disease (SCD). Anti-HepB antibody titres were not significantly different between neonates with SCT and WT, with or without the inclusion of the SCD neonates, either after a single dose of HBV (day 30) or the full immunization series (day 128). However, investigation of the proteomics data showed most pronounced differences in SCT group vs WT at day 3, with over ~25% of the proteins differentially expressed in SCT neonates, using a relaxed False Discovery Rate (FDR) of 0.2. This result is consistent with the supervised multivariate analysis conducted using single- and integrated multi-omics-based models, showing maximum accuracy (average cross-validated AUC > ~67%) at day 3. Gene set enrichment analysis of these proteins revealed pathways associated with erythrocytes and bone marrow disorders, platelet function, and the transcription factor RUNX1, involved in multiple hematological disorders.

Conclusion: Our findings suggest that compared to WT, SCT affects early life expression of proteins involved in blood-related functions and disorders altering hematologic and immune trajectories. Of note, SCT and WT neonates demonstrated similar HBV-induced Anti-HepB antibody titres. Overall, our approach provides a novel way of utilizing the globin reads that are usually removed from blood sample-based experiments to study disorders like sickle-cell disease, especially in areas with high prevalence of these conditions. This study provides new insights into the effect of sickle-cell trait on neonatal hematologic and immune development.
This research is funded by Mitacs Accelerate Fellowship, partnering with PROOF Centre of Excellence. The main neonatal immunization study is funded by NIH/NIAID U-19 grant.

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* Abhinav is a PhD Candidate in Tebbutt Laboratory at HLI centre, and a trainee bioinformatician at PROOF centre of Excellence, specializing in omics-based studies.

Evaluating Quantitative Lung CT Measurements in Cardiac CT to Identify Novel Predictors for Acute Coronary Syndromes

A. Schmidt*, L. Lidher, I. Guez, V. Sussman, R. Rosenblatt, E. Khasanova, C.J. Hague, D.D. Sin, L.J. Shaw, J.A. Leipsic, R.L. Eddy

Background: Cardiovascular disease is the leading cause of death worldwide and acute cardiac events like heart attacks are difficult to predict. Lung disease is strongly related to heart disease, but it is not usually evaluated as a predictor for cardiac events. Given the well-known relationships between cardiovascular disease and lung inflammation, we believe lung biomarkers may contribute to the prediction of acute coronary syndromes (ACS). In this study, we will evaluate lung structure within cardiac computed tomography (CT) images to determine whether measurements of structural lung disease can predict acute cardiac events.

Hypothesis: Participants with ACS will have a greater area of lung corresponding with emphysema or interstitial lung abnormalities (ILA) compared to control participants. Additionally, lung CT measurements will be significantly related to and predictive of future ACS.

Methods: Cardiac CT images from 468 participants included in the ICONIC (Incident COroNary Syndromes Identified by Computed Tomography) cohort were evaluated; 234 participants experiencing ACS over 3.4±2.1-year follow-up after a baseline cardiac CT were included and propensity-matched 1:1 to 234 within-site control subjects who did not experience ACS. CT images were quantitatively analyzed using commercial VIDAVision software (VIDA Diagnostics, Coralville, IA, USA) for parenchymal measurements including total lung volume and relative area of the lung in various ranges of Hounsfield Units (HU) to assess extent of parenchymal disease. Emphysema was quantified as the % of the lung area with voxels less than -950 HU (LAA950) and ILA were quantified as the % of the lung area with voxels between −601 and −250 HU, or high attenuating areas (HAA). Participants were further dichotomized as having emphysema or ILA as those with LAA950 or HAA greater than the mean for each group. Differences in lung CT measurements between participants with ACS and controls and between various types of ACS were evaluated by Kruskal-Wallis tests. Pearson's chi-squared test was used to determine if there were more ACS participants with emphysema or ILA compared with non-ACS participants.

Results: There was no statistically significant difference between the participants with and without ACS when lung densities (p=0.6 – 1.0), lung volume (p=0.1), air volume (p=0.1) and tissue volume (p=0.1) were compared. Additionally, there was no difference in lung CT measurements between different types of ACS (p=0.3-1.0). Finally, there was no difference in the prevalence of emphysema or ILA for ACS participants versus non-ACS participants when defined by LAA950 (p=0.8) and HAA (p=0.7) group means, respectively.

Conclusion: Our results thus far did not detect an association between lung density measurements of emphysema and ILA on cardiac CT with ACS. However, ongoing analysis will account for additional clinical risk factors and the limited lung fields available on cardiac CT. Ongoing analysis will also include evaluating relationships between qualitative and quantitative lung and coronary vessel measurements. Further research has the potential to provide new ways to predict patients most at risk for ACS and reduce the burden and mortality of cardiovascular disease.

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*Alexandra Schmidt is a fourth year UBC student studying chemistry, physics and life science. She is currently completing her Directed Studies under Dr. Rachel Eddy and Dr. Jonathon Leipsic. Alexandra hopes to continue to pursue research in the future.

Role of N-cadherin and NFAT5 in the Pathogenesis of Coxsackieviral Myocarditis

A. Yoo*, G. Zhao, M. Zhang, D.C. Yang

Background: Coxsackievirus B3 (CVB3) is an etiological agent of viral myocarditis, which is an inflammatory disease of the heart muscle. Intercalated discs (ICDs) are specialized regions that connect cardiomyocytes, providing structural support and facilitating signal communication. N-cadherin (N-cad), an adhesion protein localized in ICDs, anchors myofibrils (heart contractile filaments) and connects actin filaments between cardiomyocytes. The *CDH2* gene encodes N-cad and is suggested to be targeted by the nuclear factor of activated T cells (NFAT5) transcription factor. Previous studies showed that CVB3 protease 2A cleaves NFAT5, inhibiting its functional role. Alterations in *CDH2* gene expression can disrupt the structural organization of ICDs, increasing the severity of viral myocarditis. Thus, the objective of the study is to identify the NFAT5 binding site and investigate its regulatory mechanism on *CDH2* gene expression.

Hypothesis: NFAT5 targets the *CDH2* promoter region and upregulates its gene expression. Mutations in the *CDH2* promoter region disrupt the binding site of NFAT5, causing its gene expression to be downregulated.

Methods: Position weight matrices of NFAT5 from JASPAR were used to predict the potential NFAT5 binding motif in the *CDH2* promoter region. The predicted target sequence was amplified and inserted into a PGL3-luciferase-firefly plasmid (wild-type *CDH2* luciferase reporter). HL-1 cell lines were transfected with constructed luciferase plasmids and then infected with CVB3 or sham-infected with PBS. Using site-directed mutagenesis, we also constructed a PGL3-luciferase-firefly plasmid with a mutant NFAT5-target sequence in the *CDH2* promoter region (mutant *CDH2* luciferase reporter). HL-1 cell lines were transfected with the mutant *CDH2* luciferase reporter and then infected with CVB3 or sham-infected with PBS. To determine *CDH2* reporter expression levels, luciferase assays were conducted. ChIP-assays were being conducted to confirm the NFAT5 binding site in the *CDH2* promoter region. The NFAT5 antibody pulled down the protein-chromosome complex and then qPCR detected the NFAT5 binding site.

Results: Upon transfection of the wild-type *CDH2* luciferase reporter, a detectable level of reporter activity is observed in sham-infected HL-1 cells. Transfection of the *CDH2* mutant luciferase reporter results in reduced reporter activity in sham-infected HL-1 cells. Compared to the sham-infected groups, CVB3 infection significantly inhibits reporter activity. ChIP-assays confirmed the NFAT5 binding site in the *CDH2* promoter region.

Conclusion: Our findings suggest that NFAT5 binds to the *CDH2* promoter region, which regulates *CDH2* gene expression. Further *in vivo* research is necessary to confirm the relationship between NFAT5 and *CDH2* gene expression. By examining this relationship, we can further understand the pathogenic mechanisms underlying CVB3 infections.

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*Alyssa Yoo is a fourth-year UBC Science student majoring in Integrated Sciences: Immunology and Genetics disciplines. This is her first summer at the Centre for Heart Lung Innovation.

Development of better bleomycin murine models for testing idiopathic pulmonary fibrosis therapeutic targets

A. Xiao*, N. Assadinia, L. Huang, C. Roskelley, P.A. Dean, F. Chu, P. Johnson, T.L. Hackett

Background: Idiopathic pulmonary fibrosis (IPF) is a fatal disease characterized by progressive, patchy scarring of the lung interstitium. Though it is thought to arise from a combination of genetic and environmental factors, the exact etiology is unknown. Because of this, it is difficult to create animal models that replicate all features of the human disease, hindering the development of therapeutics. The current standard *in vivo* IPF murine model is an acute single dose bleomycin exposure mouse model. However, this model is limited because the fibrosis induced by bleomycin is known to resolve. In this project, we assessed an acute versus chronic bleomycin murine model for tissue fibrosis and increased mucus production, which are two common features of the human IPF lung disease.

Hypothesis: Because IPF arises from persistent exposure to risk factors, a single dose of bleomycin may not be sufficient to induce IPF-like phenotypes. Therefore, the chronic mouse models, which will receive multiple doses over a period of time, may better resemble the human disease and induce lung fibrosis and mucus production that do not resolve.

Methodology: 6 to 12-week-old C57BL/6J mice were intratracheally instilled with either 2.0U/Kg bleomycin sulfate (bleo, Cayman Chemicals) dissolved in 50µL of phosphate buffered saline (PBS) or PBS control. Acute fibrosis mouse models received a single dose of bleo, whereas chronic fibrosis mouse models received a dose every 2 weeks until a total of 4 doses. Mouse lungs were harvested 7, 14, and 56 days following the last dose of bleomycin. Murine and human lungs were formalin-fixed, paraffin-embedded, sectioned, stained with either Masson's Trichrome (collagen) or Alcian Blue (mucus) stain, and digitally imaged. The number of positive pixels was divided by the total number of fixed area pixels detected via Aperio ImageScope to calculate the volume fraction ratio of collagen and mucus. The murine data were analyzed using an ANOVA with Tukey's post-test. The human data were analyzed with a Kruskal-Wallis test.

Results: In the acute bleomycin model, the % positive mucus staining per mm2 was increased at day 14, but returned to the baseline of naïve mice by day 56. In contrast, the chronic model's % positive mucus staining per mm2 remained elevated at day 7, 14 and 56 compared to naïve mice. In the acute bleomycin model, there was no significant elevation of % positive collagen staining per mm2 at any time point. In the chronic bleomycin model, there was a significant increase in % positive collagen staining per mm2 at day 56 compared to naïve mice. Over the subsequent weeks, we will be analyzing the human samples to compare the extent of mucus and collagen staining to the murine models.

Conclusion: IPF affects around 14 000 to 15 000 Canadians, and patients usually die 3 to 5 years after diagnosis, hence the need for better IPF models to develop new treatments. Our project demonstrates that repeated doses of bleomycin over a longer period of time may provide a more accurate IPF model to enable therapeutic testing of new IPF targets.

Quality Controls for Spatial Gene Analysis in Formalin-Fixed Paraffin Embedded Tissues

B. Li*, S. Leung, M. Elishaev, C. Ng, T. Chang, G. Singhera, B. Sahin, C. Lai, Y. Wang

Background: Gene expression assessment within the tissue context was made possible recently via Visium – a powerful application to find therapeutic targets and diagnostic markers based on formalin-fixed paraffin embedded (FFPE) tissue sections. Integrities of tissue morphology and RNA (i.e. RNA fragment length) are pre-requisites to obtain accurate results for Visium. Many cardiovascular biobanks archive post-mortem biospecimens, which may have degraded RNA that introduces bias in Visium results. The current quality control for RNA quality standardized by Visium is DV200, which is the ratio of RNA fragments above 200 base pairs to the total number of RNA fragments. This standard had never been previously benchmarked on post-mortem but mostly on explanted heart tissue. Explanted heart tissue was used as comparison to post-mortem as the former is freshly isolated, timely preserved, and less RNA-degradation due to being sourced from a living donor. The study will develop a protocol to quality control FFPE preserved coronary arteries to obtain high quality data from Visium.

Hypothesis: The DV200 of post-mortem biospecimens will remain similar relative to explanted heart while the former will suffer from greater total RNA degradation.

Methods: Explanted and post-mortem FFPE coronary arteries were withdrawn from the Bruce McManus Cardiovascular Biobank. Tissue biospecimens were sectioned (4 x 10 µm) with a Leica Biosystems microtome followed by genomic RNA isolation *via* the QIAGEN FFPE RNeasy RNA extraction kit. Isolated RNA was further concentrated *via* a laboratory freeze dryer to meet minimum concentration threshold for subsequent tests. A concentration test was performed using the extracted RNA using Qubit™ 4 Fluorometer and its compatible RNA High Sensitivity kit to determine the total RNA amount per tissue. Lastly, the RNA quality, indicated by DV200, was obtained via Agilent 2100 Bioanalyzer instrument using the Agilent RNA 6000 Pico kit. The total RNA amount and DV200 was compared between explanted and post-mortem tissue to monitor any changes in total RNA degradation and quality, respectively.

Results: The absolute RNA amount measured was 40 percent lower in post-mortem compared to explanted heart tissues, suggesting a total RNA degradation. However, this degradation was not indicated by DV200 as there was no significant difference found between post-mortem and explanted heart tissues. Visium sequencing showed a high percentage of mitochondrial contamination, which are associated with poor RNA quality, in post-mortem (30%) relative to the explanted heart (5%) in the intima of coronary tissue for tissues with acceptable DV200s.

Conclusion: DV200 is not an accurate quality control to determine if FFPE tissues meet the RNA quality standard for Visium spatial gene expression sequencing. The project informs spatial gene analysis researchers the issue with DV200 as a quality control along with a future direction of designing hybridization probes to reliably measure tissue RNA quality.

This research is supported by the New Frontiers in Research Fund - Exploration

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* Boaz Li is entering his last year at UBC, studying human physiology and cell biology. After completing the Co-op program, this summer project will be a continuation of his work at Centre for Heart Lung Innovation.

Breathing Beyond the Smoke: Characterizing Respiratory Clinical Outcomes in Cannabis Smokers

C. Gilchrist*, X. Li, R.L. Eddy, J. Yang, C Wong, C. Clark, E. Karlsen, J.A. Leipsic, D.D. Sin, J.M. Leung

Background: Cannabis is the most widely used drug worldwide. Recreational cannabis was legalized in Canada on October 17, 2018. Since legalization, cannabis use has been gaining popularity. In 2022, over 10 million Canadians reported using cannabis in the past year, over double the rate of tobacco users. The impact of smoking cannabis on lung health, however, is poorly understood. As the second country in the world to have legalized cannabis, it is important to identify potential risks of smoking cannabis on respiratory health.

Hypothesis: Cannabis smoking is associated with poor clinical lung health outcomes.

Methods: Clinical and biological samples were collected from the Canadian Users of Cannabis Smoke Study (CANUCK). Eligible participants had to be ≥19 years old. Respiratory symptom burdens were measured using the St. George's Respiratory Questionnaire (SGRQ) and the COPD Assessment Tool (CAT). Full pulmonary function tests were performed on each participant**.** A subset of participants underwent computed tomographic (CT) imaging which was scored for lung abnormalities, such as emphysema, by a clinical radiologist. Blood samples were collected, and complete blood counts were measured. Clinical data was compared across smoking groups with one-way ANOVA, adjusted for age, sex, BMI, and smoking status.

Results: The CANUCK cohort currently has 53 current cannabis smokers (CS) and 27 non-smokers (NS). Our cohort is mostly female (64%), Caucasian (61.8%) and young (mean age 33.92 years). SGRQ scores were higher in CS (indicating greater respiratory symptom burdens) compared to NS (med. total score 11.55 vs. 7.03, p=0.019). CAT scores were higher in CS compared to NS (med. score 8 vs. 6, p=0.046). CS have preserved lung function when compared to NS but display higher diffusing capacity % predicted than NS (med. 111% vs. 103%, p = 0.014). Heavy CS (> 20 joint-years) was associated with a 13.4% reduction in forced expiratory volume/ forced vital capacity ratio (FEV_1/FVC) compared to mild CS (< 5 joint-years, p=0.029). Complete blood count analysis revealed that CS have 0.08 cell/ µl more monocytes compared to NS (p=0.007), and 0.06 cell/ µl more eosinophils than NS (p=0.079). Qualitative CT emphysema scores are comparable between CS and NS groups.

Conclusion: Analysis of clinical data showed cannabis smokers have significantly higher respiratory symptoms than non-smokers but display preserved pulmonary function and lung structure on CT imaging. Cannabis users have increased diffusion capacity, potentially due to increased cardiac output observed from cannabis smoking. Monocyte levels are significantly elevated in blood and there is potential association with cannabis smoking and raised eosinophil levels, suggesting that cannabis smoking may induce a pro-inflammatory state. Cannabis smokers should be aware of these potential harms associated with their smoking habits.

This research is supported by the Canadian Institutes of Health Research.

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*Cassie Gilchrist began her Masters of Experimental Medicine at the University of British Columbia in Dr. Janice Leung's lab. She enjoys spending her free time outdoors, preferably in the mountains hiking or skiing.

Antiviral Potential of cGAS-STING Activation against Enteroviral Infection

C. Fu*, Y.M. Fan, Y.L. Zhang, Y. Mohamud, H. Luo

Background: Enteroviruses are a genus of single-stranded, positive-sense RNA viruses lacking envelopes. They are known to target the heart and pancreas, instigating inflammatory diseases such as myocarditis, pericarditis, and pancreatitis. Currently, there are no FDA-approved drugs that treat enteroviral infection; therefore, identifying molecular pathways that can be targeted for the development of anti-enteroviral drugs is important. One such possible pathway, the cyclic GMP–AMP synthase–stimulator of interferon genes (cGAS-STING) pathway, is a cytosolic DNA-sensing pathway that induces type-I interferon (IFN) response as well as autophagy in independent processes. While type-I IFN signalling is responsible for regulating the transcription of 1000s of genes involved in various immune functions, autophagy plays a role in host defense through the targeted degradation of pathogens. The Luo lab has previously demonstrated that induction of the cGAS-STING pathway significantly inhibits enteroviral replication. In the current project, we aim to explore the possible antiviral mechanism involved.

Hypothesis: Mechanistically, cGAS-STING activation fights enteroviral infection by the induction of type-I IFN response, rather than by stimulating autophagy.

Methods: Using Coxsackievirus B3 (CVB3) as a model enterovirus, the antiviral mechanisms of activating STING with exogenous agonists, 2'3'-cGAMP and diamidobenzimidazole (diABZI), were studied *in vitro*. siRNA knockdown of interferon-α/β receptor (IFNAR), a membrane receptor integral to type-I IFN signalling, was used to study the involvement of type-I IFN response in the antiviral effect of STING activation in HeLa cells. To examine the effects of STING activation on autophagy, human embryonic kidney cells (HEK293) possessing low baseline autophagy were transfected with STING and treated with cGAMP in the presence or absence of an autophagy inducer or inhibitor. Western blotting and qPCR were used, respectively, to observe protein and RNA expression of components of the pathways under study, as well as to quantify viral replication.

Results: Activation of the cGAS-STING pathway by exogenous STING agonists cGAMP and diABZI was verified by Western blot analysis to inhibit CVB3 replication *in vitro*. siRNA knockdown of IFNAR resulted in increased expression of viral protein, even in the presence of cGAMP. Additionally, cGAMP treatment of STING-transfected HEK293 showed an increase in lipidated LC3, which is required for the targeting and formation of autophagic membranes in autophagy.

Conclusion: The results suggest type-I IFN signaling as the likely mechanism by which activated cGAS-STING defends host cells against CVB3 infection, as the knockdown of IFNAR attenuated the effects of activating STING. STING activation by cGAMP alone also appears to stimulate autophagy. Further studies are required to eliminate the possible role of autophagy and the inhibition of viral translation to confirm or reject the hypothesis. These results show a potential for initiating type-I IFN response via agonist activation of STING in the development of enteroviral drugs.

This research is supported by BioTalent Canada and CIHR (PJT-186101)

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*Cathy Fu is an undergraduate student at UBC, majoring in combined Biochemistry and Chemistry. This is her second of two Co-op terms at HLI, after which she begins her 4th year of undergrad.

Early endothelial function activation by losartan prevents aortic stiffness in a model of type 1 diabetes

C. Yuen*, Z. White, A.M. Devlin, P. Bernatchez

Background: Type 1 diabetes (T1D) is an autoimmune disease that causes insulin-producing pancreatic β-cell destruction. Despite insulin replacement therapy (IRT), T1D increases the incidence of cardiovascular diseases (CVD) and at an earlier age. It has been speculated that at the time of diagnosis and IRT initiation, T1D has already damaged the vasculature whereas early prophylactic pharmacotherapy may prevent the long-term complications of T1D, although how this can be achieved is unknown. In addition to their antihypertensive properties, we have shown that angiotensin II receptor blockers (ARBs) such as losartan, are effective in activating nitric-oxide (NO)-dependent endothelial function, a poorly understood biological property of the vascular endothelium to promote vasodilation and promote vascular quiescence. Whether losartan can increase endothelial NO release in T1D vasculature and prevent early changes in vascular homeostasis remains unknown.

Hypothesis: Early endothelial function activation by losartan will prevent T1D induced aortic stiffness and markers of vascular damage.

Methods: T1D Akita male mice with a heterozygous insulin 2 gene variant were assessed **1)** at T1D onset (blood glucose ≥ 16.6mmol/L) **2)** 4 weeks post-T1D onset and **3)** 12-weeks post-T1D onset. Losartan treatment (0.6g/L drinking water) was initiated at 4 weeks post-T1D onset and its effect on mean arterial blood pressure (MABP) and aortic pulse wave velocity (PWV), indicator of aortic stiffness, were measured non-invasively. At termination, endothelial function was measured by wire myography of isolated aortic rings. Nitric oxide synthase inhibitor L-NAME was used to assess basal NO production.

Results: Early (4 weeks) after T1D onset, Akita mice displayed a 51.7% increase in aortic PWV, a 70.2% increase ($p<0.04$) in response to phenylephrine (PE) induced constriction and a 24.5% reduction ($p<0.01$) in acetylcholine (Ach) induced endothelial NO-dependent vasorelaxation compared to control mice. This confirms that the development of aortic stiffness and alterations in endothelial function in the vasculature occur early. At a later (12 weeks) stage, T1D Akita mice had a 241.1% increase ($p<0.01$) in aortic PWV, a 16.2% higher PE constriction and a 20.2% Ach reduction ($p<0.01$) compared to control, signifying further deterioration in vascular function. Treatment with Losartan lowered PWV in Akita mice to control levels, preventing aortic stiffness fully. Post-termination myography experiments showed that Losartan reduced PE contractility in Akita (46.1%) and control (71.1%) mice ($p<0.01$) and attenuated the impaired Ach-induced vasodilation, suggesting an overall increase in basal NO production and availability while restoring homeostatic vascular protective properties.

Conclusion: Early intervention with losartan prevents aortic stiffness while rescuing endothelial protective properties by increasing NO production, which may be an effective treatment option to reduce long term risk for T1D associated cardiovascular complications.

This research is supported by the Canadian Institutes of Health Research

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*Christopher is in his second year of his PhD in Pharmacology at UBC.

Effect of Lysosomal Acid Lipase Overexpression on Cholesterol Mobilization in Arterial Smooth Muscle Cells

E. Ling*, V. Baht, T. Chan, G.A. Francis

Background: Unregulated uptake of lipoproteins in the artery wall by macrophages and smooth muscle cells (SMCs) leads to formation of cholesterol-overloaded "foam cells", the biochemical hallmark of atherosclerosis. Lipoproteins ingested by these cells transit to lysosomes for hydrolysis of the cholesteryl esters (CE) they carry to release free cholesterol from the lysosome, allowing its trafficking within the cell or removal by cholesterol efflux mechanisms. Our laboratory discovered that expression and activity of the sole lysosomal lipid hydrolase, lysosomal acid lipase (LAL), encoded by the gene *LIPA*, is markedly low in arterial SMCs compared to macrophages in both humans and mice. This results in trapping of lipoprotein CE in the lysosomes of SMC foam cells and inability of these cells to release excess cholesterol. Supplementing SMCs with exogenous LAL *in vitro* increases the ability of these cells to release or "efflux" excess cholesterol. This occurs despite the observation that SMCs also express lower levels of the membrane cholesterol transporter ABCA1 when compared to macrophages. It is known that the removal of cholesterol to apolipoprotein AI (apoAI), the main protein of high-density lipoproteins (HDL) requires ABCA1, and that cholesterol can also be removed from cells to HDL by non-ABCA1-dependent mechanisms. This project will test whether overexpression of the *LIPA* gene in cultured SMCs in order to increase LAL is capable of fully correcting CE hydrolysis in SMC lysosomes, and how well this increases the amount of cholesterol available for removal from the cells by both ABCA1-dependent and -independent mechanisms.

Hypothesis: Overexpression of LAL will mobilize lysosomal cholesterol and increase availability of cholesterol for removal from arterial smooth muscle cells by different mechanisms

Methods: Human aortic SMCs were transfected with an adenoviral vector encoding either *LIPA* or mCherry (control) for 6 hours at an MOI of 500. Western blot, and LAL activity assay were used to confirm overexpression of LAL protein, and LAL activity respectively. Following infection, the removal of cholesterol to apolipoprotein AI (ApoAI), the main protein of high-density lipoproteins (HDL), which requires ABCA1, will be tested. Cholesterol removal by HDL particles partially digested via trypsin, which utilizes the non-ABCA1-dependent cholesterol efflux pathway, will also be tested. In this way, both major pathways of cholesterol removal from cells present in the human body will be tested following overexpression of LAL by cultured SMCs.

Results: Infection of human aortic SMCs was successful, with an efficiency of up to 95% as visualized using mCherry controls via fluorescence microscopy. Increased LAL expression was detected via Western blots, RT-PCR, and LAL activity assay post infection. Cholesterol efflux experiments are now underway as described.

Conclusion: These data will determine the ability of LAL supplementation toy correct cholesterol removal from SMCs via ABCA1-dependent or -independent pathways. These results are highly relevant to understanding the benefit of potential supplementation of LAL in vivo as a means to reduce SMC foam cells in atherosclerosis.

This research is funded by the Canadian Institutes of Health Research.

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* Elaine Ling is entering her last year at UBC and will be completing the B.Sc. Pharmacology program. This is Elaine's second summer working with the Francis Lab.

Investigating the Contribution of Sex Differences to Small Airways Disease in COPD Using Ultra-High Resolution Imaging

F. Aminazadeh*, K. Wu, D. Cooper J, JC. Hogg, M. Vasilescu, T. L. Hackett

Background: Over 380 million people with chronic obstructive pulmonary disease (COPD) are challenged every day with the simple task of breathing. In Canada and other developed countries worldwide, the number of hospitalizations and deaths due to COPD is now greater for females than males. In the developed world, tobacco smoke is the biggest risk factor for disease development. In general, females smoke fewer cigarettes than males, but are more susceptible to a faster annual decline in lung function and experience greater symptoms such as dyspnea. We recently demonstrated ultra-high resolution (7μm) computed tomography (micro CT) imaging, that over 41% of the smallest conducting airways (terminal bronchioles) are destroyed by the time a patient is diagnosed with mild COPD, but it is unknown if there are differences in small airways disease between females and males.

Hypothesis: Female smokers have greater remodeling of their terminal bronchioles and associated terminal bronchiole vessels than males, making them more predisposed to developing severe early-onset COPD

Methods: A cross-sectional cohort of lungs from female and male ex-smokers with a matched smoking history and normal lung function (n=20) were compared to matched donors with mild/moderate COPD (n=40) or very severe COPD (n=20). Lungs were inflated, frozen and eight systematic uniform random samples were taken per lung. Samples were scanned using microCT to assess the wall area, wall thickness, luminal area, and alveolar attachments of terminal bronchioles (TB) and terminal bronchiole associated vessels (TBV).

Results: Our preliminary results presented are from 44 donors within the study. There was a trend that terminal bronchial alveolar attachments were lower in females with mild/moderate COPD compared to males. Further, the terminal bronchial wall area was thicker in males with mild/moderate COPD compared to females with a matched smoking history ($p<0.05$). Furthermore, when we assessed the terminal bronchial associated vessels (TBVs), female patients with mild/moderate COPD have fewer alveolar attachments tethering the TBV wall open than male patients with mild/moderate COPD ($p<0.05$). Further, the wall area of TBVs was thicker in the male control group compared to females ($p<0.01$). With the disease progression, TBV wall thickens in both females and males with mild/moderate COPD, but it is still thicker in males than females ($p<0.001$).

Conclusion: Our data suggest, female patients with COPD have fewer alveolar attachments on the terminal bronchiole and TBV walls, this could explain why females have worse airflow obstruction compared to male COPD patients for the same smoke exposure due to loss of alveolar attachments and radial tethering, of airway and vessels.

This study is funded by the Canada Institute of Health Research and the Canadian lung association.

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* Fatemeh Aminazadeh is a first-year PhD student in the Department of Anesthesiology, Pharmacology and Therapeutics.

Diagnostic Performance of α-Synuclein Seed Amplification Assays: A Meta-Analysis

C. Helbling*, S. Yeung, M.L. DeMarco

Background: Parkinson's disease, dementia with Lewy bodies and multiple system atrophy—collectively termed synucleinopathies—are defined pathologically by the aggregation of α-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 α-synuclein pathology is highly desired. Seed amplification assays 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 seed amplification assays, 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.

Hypothesis: Seed amplification assays detecting α-synuclein pathology will perform similarly across synucleinopathies and samples matrices in clinically relevant cohorts.

Methods: A database search was conducted for α-synuclein seed amplification assay studies on Parkinson's disease, dementia with Lewy bodies, or multiple system atrophy 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 and exclusion criteria. Seed amplification assays demonstrated the following average diagnostic accuracies by specimen types and control groups. Using CSF, seeding assay 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, seed amplification assays 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, seed amplification assays differentiated synucleinopathies from DM and NC with diagnostic accuracies of 0.83 [0.76-0.89] and 0.77 [0.67-0.86], respectively. **Conclusion:** α-Synuclein seed amplification assays 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 seed amplification assays 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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*I'm a first year PhD in the DeMarco Lab in the department of Pathology and Laboratory Medicine.

The Role of Exercise in Reversing Sepsis-Induced Immune Suppression

G.M. Nonis *, C.A. Lawrence, E.L. Lu, S.M. Bradwell, N.D. Eves, J.H. Boyd, G.J. Koelwyn

Background: Sepsis is defined as a dysregulated host immune response to infecting leading to organ failure; and in 30-50% of patients, death. Of those who survive sepsis, risk for re-infection and rehospitalization remains high due to chronic immune suppression. While host adaptations leading to immune suppression can occur across a variety of cell types and organ systems, a central regulator is cells of the innate immune system, such as monocytes. Following sepsis, monocytes become 'tolerant', resulting in blunted cellular functions, including reduced phagocytosis, cytokine production and migratory capacity, which persist for months following hospital discharge. This dysfunction is due to epigenetic changes, termed innate immune memory. To protect against reinfection and reduce rehospitalization, therapies that can reverse tolerant phenotypes and accelerate the trajectory of monocyte recovery following sepsis are urgently required. Intriguingly, emerging evidence suggests that appropriately dosed, repeated exposure to exercise can enhance immune function; however, it remains unknown whether exercise can also reverse monocyte tolerance.

Hypothesis: Chronic exercise will improve monocyte function thereby protecting against secondary infection following sepsis. This exercise-induced protection will result in increased survival in post-sepsis mice when challenged with a secondary infection or recurrent sepsis and will be associated with reversed monocyte tolerance as measured by phagocytosis, cytokine production and migratory capacity.

Methods: This study will utilize the *'two-hit'* model of sepsis induced immune suppression that we are currently validating in the lab. Briefly, 50 C57BL/6 mice will first be interperitoneally injected with cecal slurry (CS) (n=30) or sham control (n=20) – *the first hit*. Surviving animals will then be randomized to 6 weeks of aerobic exercise training (1h/day, 5days/wk), or sedentary control. Following the exercise program all animals will be challenged with a respiratory infection (*S. Pneumoniae* or *L. Pneumophila)*, or recurrent sepsis – *the second hit* – and 10-day survival will be monitored. A second cohort of animals (n=40), having undergone the same CS injection and exercise program, will have monocytes isolated 24h post pneumonia to assess for functional changes in phagocytic ability, cytokine production and migratory capacity. These functional assays aim to associate the expected changes in survival with exercise induced adaptations in monocytes.

Results: Currently, we are validating the mouse model in our lab. We previously showed that our model of sepsis reduces exercise capacity and that our exercise program stimulates physiological changes associated with improved immune function in mice. Ongoing trials are aimed at confirming immune suppression following sepsis in our model by challenging mice with *S. Pneumoniae* (to be completed July 2023), *L. Pneumophila,* and recurrent sepsis (both to be completed August 2023). Future trials (to be completed Fall 2023/Summer 2024) aim to profile the effects of exercise on monocyte function following sepsis and determine if exercise can improve survival to secondary infection and/or recurrent sepsis.

Conclusion: The results from the proposed study are intended to identify the role of exercise in enhancing immune responses following sepsis. These results will also be used to inform translational studies determining the biological efficacy of exercise training in improving immune function in sepsis survivors to improve patient outcomes.

This study was made possible by the generous funding from NSERC, Providence Research and the Canada Research Chair Program.

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* Geoffrey Nonis is an undergraduate student studying biological physics at SFU.

Open-Source Analysis for Cardiovascular Research with Optical Mapping Data

H. Aulakh*

Background: Proper characterization of cardiac tissues is essential for advancing research in cardiovascular regenerative medicine. However, the complexity of cardiomyocytes, which possess both metabolic and transcriptional machinery, as well as unique electrical and mechanical properties, makes this a challenging task. Optical mapping, a fluorescence microscopy technique utilizing fluorescent voltage and calcium sensitive dyes and high-framerate imaging, offers valuable temporal and spatial data at high resolution, making it ideal for studying electrical behaviors in cardiac tissues. Unfortunately, limited accessibility to data analysis software for optical mapping hinders many research groups from adequately characterizing electrical activity, thereby limiting its utility in regenerative medicine applications.

Hypothesis: Our hypothesis is that our open-source analysis package will enable researchers to effectively and accurately analyze optical mapping data, facilitating the study of cardiac dynamics and the evaluation of regenerative approaches.

Methods: To address the limitation of accessible data analysis software, our research group has developed an open-source analysis package for optical mapping data. Built using Python and leveraging popular open-source packages such as numpy, scipy, opencv, and scikit-image, our tool empowers researchers to explore data for parameter optimization and perform automated batch analysis. The flexibility of our package allows it to be adapted to various model systems, and it can be easily scaled to leverage modern cloud computing infrastructures, enabling the analysis of high-throughput experiments.

Results: To validate the performance and effectiveness of our analysis system, we conducted experiments using three distinct model systems commonly used in cardiovascular research: cardiac monolayers, embryoid bodies, and engineered heart tissues. Our analysis package successfully extracted feature space representations for repolarization and depolarization dynamics from time-series recordings obtained through optical mapping. Importantly, it demonstrated accurate differentiation of different biological preparations, including distinguishing between atrial and ventricular cells. Moreover, we introduced novel feature engineering techniques that allowed the discrimination between pacemaker and working cardiomyocytes based on their diastolic depolarization characteristics.

Conclusion: By providing an open-source analysis tool for optical mapping data, we aim to empower cardiovascular researchers with a comprehensive solution for characterizing cardiac electrical behaviors. Our user-friendly software bridges the gap between data acquisition and analysis, enabling a broader range of researchers to effectively utilize optical mapping in their studies. The accessibility and versatility of our package will contribute to accelerating discoveries in the field of cardiovascular regenerative medicine.

I would like to thank Jeremy Parker and Mishal Ashraf for their valuable contributions and support throughout this project.

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*I am a fourth-year Engineering Physics student at the University of British Columbia (UBC). Currently, I am working as a computational biologist with the Laksman lab.

CanCOLD Re-Visit: Changes In Protocol To Address New Questions

J. Chen*, W. C. Tan

Background: The Canadian Cohort of Obstructive Lung Disease (CanCOLD) is a prospective, longitudinal population study examining Chronic Obstructive Pulmonary Disease (COPD) progression within Canadian men and women. COPD is the third leading cause of death worldwide, often associated with smoking. The first phase of CanCOLD, the "COLD" study followed 1500 individuals across 9 Canadian data collection sites including Vancouver. The findings revealed that COPD prevalence in Canada is 4-fold higher than previous estimates suggested, with 70% of the Canadian population underdiagnosed. This underdiagnosis suggests a need for further examination of the disease development to improve COPD diagnosis and management. The second phase of CanCOLD, CanCOLD 2.0 introduced a new framework and changes in protocol with an extended follow-up period until December 2024 that will help enrich the dataset, facilitate research collaborations, sub-studies, and address new questions within the community.

Objective: To re-visit CanCOLD and examine the changes in the study protocol with the extended follow-up period. Continued data collection in the longitudinal study will allow for further analyses which will address emerging questions about COPD development.

Methods: A comparison of the protocols and questionnaires from the first and second phases of CanCOLD was used to identify the changes. In phase one, normal participants (non-smokers) with post-bronchodilator spirometry of FEV_1/FVC <70%, normal participants who are current or former smokers classified as "at risk" (AR), as well as COPD participants were administered all questionnaires. Normal participants with post-bronchodilator spirometry of FEV_1/FVC ≥70% completed all questionnaires excluding the McGill COPD-specific and Food Frequency module. All participants completed spirometry, blood draws, pulmonary function test (PFT), and cardiopulmonary exercise test (CPX). Only AR and COPD participants completed CT Scans. CanCOLD 2.0 consists of shortened questionnaires, that all participants (COPD, AR, and normal) complete along with spirometry blood draws, PFT, CPX and CT Scan procedures. Only the St. George Respiratory Questionnaire is completed by COPD, AR or normal participants with FEV_1/FVC <70%. In both phases, all participants answer a quarterly exacerbation questionnaire by phone interview.

Results: Over 100 publications, 40 conference presentations, and 80 completed or ongoing sub-studies have resulted from data collection, with 5 new publications as of 2023. There are 115 participants that have completed their visit and 124 remaining participants yet to be scheduled to begin their fourth follow-up visit and tests at the Vancouver site.

Conclusion: The protocol changes are necessary for the extended follow-up period to gather more longitudinal data. With 124 participants yet to be scheduled, continued data collection, collaborative opportunities and research questions can be anticipated. The advancements in CanCOLD can serve as a model for other population-based studies and contribute to a better understanding of COPD.

Acknowledgement: *All participants, the COLD and CanCOLD Collaborative research group and sponsors.*

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*Janette is a fourth-year Health Sciences student at SFU. This is her second Co-op work term practicum, working as the CanCOLD Study Coordinator in the Tan-Hogg Lab.

Benchmarking methods for gene-regulatory network inference

J. Tang* and A. Singh

Background: Gene regulation is a key component of cellular development and maintains a variety of activities including cell signalling, ligand-receptor binding, chromatin accessibility. The interplay between genes, chromatin, and transcription factors (TF) have been represented as gene regulatory networks (GRN). With recent advances in single-cell technology, GRNs are estimated with better accuracy and with cellular specificity. There are many methods for estimating GRNs, each benchmarked on different "ground truth" datasets and applied to different real-world datasets. Therefore, it is unclear which methods are superior with respect to identifying true cell-specific molecular interactions.

Hypothesis: By benchmarking GRN methods using a set of "ground-truth" single-cell multiomics data we will identify the methods which can consistently identify the true cell-specific molecular interactions.

Methods: We will aggregate "ground truth" datasets from published studies focused on cell differentiation and TF/drug perturbations. We will apply GRN inference methods such as SCENIC+, scMTNI, and CellOracle and estimate their ability to uncover known TF-target interactions using the F1 score. Will also assess the stability of these method using 5-fold cross-validation repeated 50 times. All analyses will be performed in Python using UBC's Advanced Research Computing environment (ARC Sockeye).

Results: We have created a database of uniformly processed "ground truth" datasets from ChiP-seq and perturbation experiments as well as disease (e.g. breadth of application can range from mucociliary epithelial differentiation to lung adenocarcinomas) datasets with associated TF knock-down data. Based on our benchmarking of many GRN methods we expect to identify the top-performing methods for GRN inference.

Conclusion: Single cell multiomics technology has paved the way for the construction of more accurate gene regulatory networks. This study will identify the top-performing methods and may provide guidance for the development of additional methods for GRN inference.

***** Jeffrey is currently working at the CompBio Lab focusing on benchmarking existing tools for differential network analyses of single cell sequencing data. Previously he had experience with exome sequencing data and machine learning classification. Jeffrey is currently interested in furthering knowledge in deep learning, localized cell-to-cell interactions and predicting clinically actionable outcomes.

Interleukin-1 Alpha Counteracts Transforming Growth Factor-β1 and β2 Signaling In Lung Extracellular Matrix Remodeling

K. Usman, M. Fouadi, K. Okechukwu, P. Nair, E.T. Osei, T.L. Hackett.

Background: The Lung extracellular matrix (ECM) forms an essential scaffold for cells within the lung and also serve as a reservoir for cytokines and growth factors. Repetitive lung injury caused by inhaled particulate matter and pathogens is associated with the persistence of inflammatory immune cell infiltration, the production of growth factors and remodeling of the ECM, leading to lung fibrosis. Epithelial-derived Interleukin-1 alpha (IL-1α) is important in mediating fibroblast inflammation within the interstitium, while transforming growth factor-β (TGF-β) is a potent inducer of fibroblast ECM production and cell-ECM interactions. The dynamic interplay between IL-1α and TGF-β is crucial for ensuring normal tissue repair versus tissue fibrosis as both are known to be released during lung injury. The goal of this project was to assess the cross-talk of IL-1α and TGF- β signaling as these mediators have only been studied in isolation.

Hypothesis: Dysregulation of interleukin-1α and transforming growth factor-β signaling leads to lung fibrosis.

Methods: Primary human lung fibroblasts (PHLF) were seeded (100,000/well), grown to 80% confluence and treated with media control, or 1ng/ml IL-1α with or without 50ng/ml TGF-β1 or TGF-β2. PHLF were also treated with media control or 12.5nM miR-146a mimic with or without 50ng/ml TGF-β1 or 50ng/ml TGF-β2. Using a 3D collagen gel model, PHLF were seeded (40,000/gel) and weighed at 72 hours following treatment to assess ECM remodeling. Protein samples were extracted after 1, 6 and 72 hours for Western blot and miRNA samples were extracted at 6, 24 and 48 hours for qPCR. The spent media was collected for ELISA.

Results: Stimulation of lung fibroblasts with TGF-β1 and TGF-β2 induced collagen I and fibronectin protein expression ($P<0.05$) but attenuated decorin expression, these were inhibited when IL-1α was present ($p<0.05$). Treatment of TGF-β1 and TGF-β2 also induced myofibroblast differentiation and the expression of α-smooth muscle actin, which was inhibited by IL-1α ($P<0.05$). Further, the presence of IL-1α led to the inhibition of collagen I gel contraction ($P<0.05$). Stimulation with IL-1α induced the expression of IL-6, IL-8 and thymic stromal lymphopoietin ($P<0.05$), which were not inhibited by TGF-β1 or TGF-β2. IL-1α and not TGF-β1 and TGF-β2 led to the up-regulation of miR-146a and the downregulation of IL-1 receptor-associated kinase-1 (IRAK1), tumour necrosis factor receptor-associated factor-6 (TRAF6) and TGF-β receptor 2 (an essential component of TGF-β signaling) protein expression.

Conclusion: Our data shows that the inflammatory mediator IL-1α is essential for modulating the response of TGF-β1 and TGF-β2 and that this crosstalk may be important for balancing tissue repair versus tissue fibrosis. Future work will focus on the dysregulation of IL-1α and TGF-β cross- talk in lung diseases involving fibrosis.

Funding: Canada Institute of Health Research and MITACs. Kauna is a third year PhD student who enjoys nature and trying out new recipes.

Single-cell RNA sequencing reveals defective bronchial epithelial cell differentiation in patients with severe COPD in 3-dimensional organoid cultures

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Background: Impaired airway epithelial barrier function and repair are thought to be key drivers in chronic obstructive pulmonary disease (COPD) pathology, however, the mechanisms involved remain not fully understood.

Objective: Assess bronchial epithelial cell differentiation in an organoid model by COPD severity using single-cell RNA sequencing (ScRNAseq).

Methods: Bronchial epithelial cells were obtained from bronchial brushings of ex-smokers with normal lung function, mild-moderate (MM)-COPD, and severe COPD (n=6 per group) and cultured in an organoid model using matrigel to allow self-organization and differentiation. Organoids were harvested at days 3, 10 and 17 and processed for scRNAseq. The number and size of organoids were quantified on day 12.

Results: There was no difference in the number of organoids formed from each group. However, organoids from severe COPD patients were significantly larger than those from MM-COPD and ex-smoker donors (p=0.032). The scRNAseq identified 5 different cell types: basal, dividing basal, suprabasal, secretory and ionocytes. We observed a significantly larger suprabasal cell population in the severe COPD group compared to the MM-COPD and ex-smokers on day 17 (p=0.041).

Conclusions: In summary, bronchial epithelial cells from patients with severe COPD form larger organoids which contain more suprabasal cells indicating defective differentiation which could impair epithelial repair and homeostasis in severe COPD.

Background: The ongoing COVID-19 pandemic, caused by the SARS-CoV-2 virus, presents significant research challenges. Due to its classification as a Risk Group 3 human pathogen, studying its interaction with the airway epithelium, the body's first line of defense against inhaled pathogens, and susceptible host cells typically requires Biosafety Level 3 Laboratories. An alternative approach involves using SARS-CoV-2 Spike-pseudoviruses in Biosafety Level 2 Laboratories. These pseudoviral particles effectively mimic viral entry, one of the many processes involved in infection, and the virus's interaction with the airway epithelium.

Spike pseudotyped lentiviruses are used in various research areas, such as vaccine development, assessing the efficacy of neutralizing antibodies against SARS-CoV-2 variants, studying virus-receptor interactions, understanding cellular tropism, and facilitating drug discovery. However, previous experiments have shown that airway epithelium cell lines have low transduction efficiency and may experience cell death when exposed to pseudovirus.

Objective: To develop an optimized protocol for SARS-CoV-2 lentiviral pseudoviruses that minimizes cell death and maximizes transduction efficiency.

Methods: We produced SARS-CoV-2 Spike-pseudotyped lentiviral particles by co-transfecting HEK-293FT cells (highly transfectable immortalized human embryonic kidney cells) with the HIV-1 three-plasmid packaging system consisting of a packaging plasmid, a transfer plasmid, and a membrane plasmid. To compare the infectivity of the pseudoviruses, three different spike membrane plasmids were used simultaneously. These membrane plasmids include an overexpressing spike plasmid (PCMV3XFLAG Spike), one with the deletion of the last 21 amino acids responsible for endoplasmic reticulum (ER) signaling (HDMdelta21), and finally, the delta variant carrying a D614G mutation and the deletion of ER retention signaling. The supernatant containing pseudoviral particles was then centrifuged to eliminate cell debris and concentrated using ultracentrifugation techniques. We aim to find the optimal ultracentrifugation speed that results in highest viral titer. The concentrated pseudoviral particles were used to transduce susceptible host cells. Initial data generation was performed using ACE2-293T cells (HEK293T cells over-expressing ACE2 on their surface), while further transduction experiments were conducted on airway epithelial cell lines, such as 16HBEs, based on the obtained results. The viral titer was evaluated indirectly by measuring luciferase activity of transduced cell lines, and the direct titer was determined by conducting a p24 ELISA on the supernatant containing viral particles.

Results: In this study, we present a thorough and simple protocol for generation and transduction of spike pseudotyped lentiviruses in airway epithelium cell lines. We expect to conduct optimization experiments to identify the spike plasmid that yields the highest transduction efficiency while minimizing cell death. We will also investigate whether generation of high titer lentivirus is dependent upon ultracentrifugation speed during viral concentration.

Conclusion: The optimized protocol described facilitates the generation of spike-pseudotyped lentiviruses, which can be used to mimic the interaction between airway epithelium and SARS-CoV-2. By achieving this, researchers can enhance their ability to investigate the virus's behavior and interactions with the airway epithelium, ultimately contributing to the development of effective interventions and therapies against COVID-19.
This project is funded by the Faculty of Medicine Summer Studentship Research Program (FoM SSRP).

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*Leiana Hoshyari is studying Biology at the University of British Columbia. She recently finished her third year of studies and will be starting her co-op at the Dorscheid Lab in the fall.

Artificial Intelligence-Enabled Quantitative Plaque and Hemodynamic Analysis in Coronary CT Angiography for Predicting Acute Coronary Syndrome Risk: the EMERALD II Study

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Background: While acute coronary syndrome (ACS) is humanity's leading cause of mortality, comprehensive risk prediction models for ACS events have not been well-established. We aimed to investigate 1) plaque and hemodynamic features that provide incremental ACS prediction beyond the current diagnostic scheme of coronary CT angiography (CCTA), 2) the performance of a model incorporating purely quantitative features, and 3) individual and aggregated impact of these features on ACS risk for each lesion using artificial intelligence (AI)-enabled quantitative coronary plaque and hemodynamic analyses in CCTA.

Hypothesis: Incorporating plaque and hemodynamic features will help determine the culprit lesion to further improve the prediction of ACS events, compared to the current diagnostic scheme of CCTA.

Methods: Clinical, CCTA, and invasive coronary angiography (ICA) data were collected and analyzed in ACS patients who had undergone CCTA within 1 month to 3 years prior to ACS presentation. CCTA images were analyzed using the CAD-RADS 2.0 reporting system, and a reference-risk prediction model was defined based on CAD-RADS grading and high-risk plaque (HRP) features. XGBoost - a machine learned algorithm helping with data interpretation - was used as a framework to train and validate the prediction model. Patients were assigned to either a derivation or an independent and blinded test cohort, based on matching criteria including age, sex, diabetes, diagnosis of ACS, and the interval from CCTA to ACS event. In the pre-specified training cohort with 1495 lesions, the best plaque and hemodynamic features were selected from four categories: lumen, plaque, hemodynamics, and myocardial mass at risk. In the independent test cohort with 956 lesions, the diagnostic performance of the trained model was tested. Finally, the SHapley Additive exPlanations (SHAP) - a machine learned statistical analysis tool - calculated the predictive value of each individual plaque or hemodynamic feature for defining the culprit lesion.

Results: According to the AI plaque and hemodynamic quantification model, the four best predictors of ACS culprit lesions were %diameter stenosis, non-calcified plaque volume (NCPV), changes in fractional flow reserve across the lesion ΔFFR_{CT}, and % total myocardial mass risk (%MMR). In the independent test cohort, the model incorporating these four factors along with CAD-RADS grading and high-risk plaque (HRP) showed significantly higher predictability compared to the model with CAD-RADS grading and HRP only. A SHAP analysis revealed that ΔFFR_{CT} was the most significant variable in risk prediction. **Conclusion:** The addition of these new features from AI-enabled plaque and hemodynamic quantification significantly improved the accuracy of predicting ACS culprit lesions, beyond the current CCTA reporting method. ΔFFR_{CT} can be a primary target to alleviate ACS risk. Further, the estimated aggregate and individual contributions of plaque and hemodynamic features may help guide decision-making for individualized treatment strategies. Integration of the AI model in clinical practice can offer improved prevention for ACS as well as optimize treatment strategies for patients with CAD.

We would like to thank Dr. Jonathon Leipsic for his guidance and support.

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*Ilanais a third year student in Biomedical Engineering at UBC. She loves to cook for her friends and family, sing and be outdoors exploring the city. *Vida is in her second year of Kinesiology at McMaster University. During her free time, she enjoys taking her dog on hikes, playing sports, and traveling. *Rachel is in her second year in the school of Arts and Sciences at Rutgers University. She enjoys doing activities outdoors and spending time with her dog.

Population Attributable Risk of Chronic Obstructive Pulmonary Disease in Canadian Men and Women in a Cross-sectional Multisite, Population-based Study

M. Huang *, W. Tan

Background: Chronic Obstructive Pulmonary Disease (COPD) is a major global health concern, being the third leading cause of death worldwide and causing a substantial burden of disability. With millions of deaths reported annually, understanding the prevalence and the impact of risk factors is crucial for the prevention of COPD.

Objective: To measure the prevalence of COPD and its risk factors in the Canadian population.

Materials and Methods: 4,893 complete and acceptable spirometry data sets were analyzed, comprising 2,096 males and 2,797 females. All analyses were performed using Standard SAS 9.4 software, well-suited for medical statistics. Hypothesis testing was carried out using a two-tailed approach with a set significance level at 0.05. The population attributable risk (PAR), a quantifiable characteristic of COPD, was defined as the proportion of disease occurrence within a population that can be attributed to a specific risk factor and calculated with estimated relative risk. We considered ten risk factors, namely heavy smoking (20+ pack years), poor education, passive smoking, history of tuberculosis, dusty job (10+ years), underweight, asthma, childhood hospitalization, use of biomass cooking fuel, and the presence of heart disease, systemic hypertension, or diabetes (HD/HT/DM). Descriptive statistics were calculated using a weighted proportion to account for demographics. Chi-square test, for dichotomous variables, and Kruskal-Wallis test, for continuous variables, were used to compare demographics across sites.

Findings: Across all sites, the weighted average prevalence of COPD was 10.9% for males (n=241) and 11.4% (n=316) for females. COPD status was determined by comparing the ratio of two lung volume measurements: forced expiration in one second and exhalation with maximal forced effort. A value less than the lower limit of normal, which is 0.7, indicated the presence of COPD. Heavy smoking was estimated to contribute to 5.33% (95% CI: 4.18, 6.30) of COPD cases in males and 3.77% (95% CI: 2.82, 4.61) in females, ranked as the most influential risk factor for COPD development in both genders. When considering specific sites, Montreal had the highest prevalence of COPD among males, with a rate of 16.0%, while Kingston had the highest prevalence among females, with a rate of 13.4%. At Saskatoon, heavy smoking was estimated to contribute to 7.89% (95% CI: 3.48, 10.4) of COPD cases for males and at Toronto, it was estimated to contribute to 6.10% (95% CI: 3.06, 7.83) of COPD cases for females. The findings reinforce heavy smoking as the most influential risk factor for COPD development in both genders across sites or by site.

Interpretation: While heavy smoking remains the primary contributor to COPD in both males and females, it does not fully explain all variations in disease prevalence. Other factors, such as HD/HT/DM, which contributes to 2.09% of COPD cases in males, and poor education, which contributes to 1.49% of COPD cases in females, play a notable role as the next most influential risk factors for COPD development in the respective sexes.

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*Minzhi Huang is a final-year statistics student at SFU. This is her second work term at HLI where she is working as the CanCOLD Data Management and Analyses co-op student.

Remodeling of small airways and arterial vasculature is a hallmark of IPF

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Background: Idiopathic pulmonary fibrosis (IPF) is one of the most aggressive forms of interstitial lung disease (ILD), characterized by chronic, progressive fibrosis and respiratory failure. The prevalence of IPF in Canada is 42 persons /100,000, with a reported median survival of 3-5 years from diagnosis. IPF is currently diagnosed using clinical computed tomography (CT), whereby radiological patterns of honeycomb cysts and basal reticulation are assessed by a radiologist within the lung. Such lesions can also be confirmed by lung biopsies and pathological diagnosis. However, it is not clear what are the pathological mechanisms driving these structural changes in IPF. A precise understanding of the infiltration of inflammatory cells and structural components involved in IPF-associated lesions is needed to understand the disease pathobiology.

Hypothesis: This research aims to distinguish the cellular microenvironment of IPF lesions to understand the disease pathobiology and identify new therapeutic targets for this deadly disease.

Methods: In total, 8 normal donor lungs and 8 IPF lungs obtained following the transplant were inflated to 10 CM water and frozen over liquid nitrogen. Eight unbiased systematic uniform random (SUR) tissue samples were taken across the height of each lung and imaged using micro-CT to segment the airway tree and calculate the volume fraction of parenchymal tissue as a measure of fibrosis. Histological tissue sections were then taken and stained for CD163, CD68, MUC5AC, MUC5B and collagen. The volumetric airway tree was then used to segment conducting airways, respiratory airways, vessels and honeycomb cysts within histological tissue sections to then assess the percentage of positive staining for each antibody marker in different tissue components.

Results: Having all IPF samples imaged using microCT, we identified that honeycomb cysts are formed from the remodeling and cystic dilation of respiratory bronchioles. There was a significant increase in the percentage of positive staining of CD68 (M1-like) and CD168 (M2-like) macrophages in the parenchyma ($p<0.05$) and honeycomb cysts ($p<0.001$) in IPF lungs compared to donor controls. Interestingly, macrophages in the IFP lung formed aggregates of 5 or more cells, and when this was quantified, we found significantly more macrophage aggregates in the parenchymal tissue ($p<0.05$) and honeycomb cysts ($p<0.05$). Compared to MUC5AC, there was a rise in the Vv% of MUC5B positive staining in IPF lung. The study revealed that the percentage of positive staining for MUC5B is higher in the terminal bronchioles ($p<0.05$), respiratory bronchioles ($p<0.05$), and honeycomb cysts ($p<0.001$) than it is for MUC5AC.

Conclusion: M2-like macrophage biology is associated with the formation of IPF honeycomb cysts and parenchymal fibrosis reticulation lesions. Elevated MUC5B and macrophages in the remodeled respiratory bronchioles with microcystic changes (honeycomb cysts) indicate the significant importance of these structures in the pathobiology of IPF. This new knowledge will be used to identify new strategies to target macrophage biology and honeycomb cyst formation in the IPF lung to improve patient outcomes.

This project does not have any direct funding currently.

*Najmeh Assadinia is a first-year MSc student in the Department of Anesthesiology, Pharmacology and Therapeutics, and this is her first summer at the center.

Predicted Versus Observed Valve to Coronary Distance in Valve-in-Valve TAVR: A Computed Tomography Study

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Background: Valve-in-valve (ViV) transcatheter aortic valve replacement (TAVR) has emerged as a treatment strategy for patients with degenerated bioprosthetic surgical heart valves (SHV). Pre-procedural CT work-up with assessment of virtual transcatheter heart valve (THV) to coronary ostia (VTC) and sinus (VTS) distances is recommended to assess the risk of coronary obstruction following ViV TAVR.

Objective: We sought to investigate 1) the agreement of predicted VTC and VTS distances and observed post-TAVR anatomy on CT and 2) their relationship with THV expansion and deployment conditions.

Methods: Fifty-one patients who underwent balloon-expandable ViV procedure were included in this study. The expansion of the THV stent-frame was evaluated at four levels: THV-inflow, SHV-sewing ring, SHV-outflow, and THV-outflow. Assessment of the VTC/VTS distances were performed on the pre-TAVR CT and THV distance to the coronary ostia (TC) and to the sinus (TS) were assessed on the post-TAVR CT. The relationship between pre- and post-TAVR distances were assessed using linear regression and Bland-Altman analyses.

Results: Following ViV, THV stent-frame flared towards the outflow but was generally under-expanded at all levels, particularly at the SHV-sewing ring level. Post-dilatation (additional balloon expanding after valve deployment) impacted the extent of THV expansion resulting in greater expansion than nominal balloon filling at all four THV-levels ($p<0.001$). Observed TC distances were systematically larger than predicted by the VTC (mean difference 1.25 ±1.28mm) in patients (n=47) with nominal balloon filling but systematically smaller in cases (n=17) of post-dilatation (mean difference -0.45 ±0.52mm). Observed TS distances were larger than predicted by the VTS (mean difference 0.95mm ±0.93) in nominal balloon volumes, and smaller than predicted by the VTS (mean difference -0.77mm ±0.83) in patients with post-dilatation.

Conclusion: With nominal balloon filling, VTC and VTS distance underestimate postprocedural distances due to THV frame under-expansion. However, post-dilatation may lead to distances smaller than predicted due to THV overexpansion at the outflow level. Careful pre-procedural planning and/or patient-specific simulation with bail-out procedures should be considered in cases with borderline VTC/VTS distance measurements where post-dilatation strategy is intended.

Funding: none

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*Contributed equally to this manuscript

**Oliver Haidari is entering his third year in the UBC Neuroscience undergraduate program. This is his second year working at HLI.

The Effect of TGF-β Receptor Inhibition on Scleroderma and Idiopathic Pulmonary Fibrosis

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Background: Systemic sclerosis (SSc), also known as scleroderma, is a rare autoimmune disorder characterized by multiorgan fibrosis impacting 44 per 100,000 people in Canada. The mechanisms underlying SSc are not well understood. However, widespread tissue abnormalities are associated with excess collagen production caused by fibrocyte, fibroblast, and myofibroblast dysfunction due to upregulation of TGF-β, among other factors. Fibrocytes are mesenchymal progenitor cells, and they are precursors to fibroblasts and myofibroblasts. All three of these cell types are found in tissues and blood and play roles in wound healing and tissue repair. Uncontrolled upregulation of TGF-β can lead to dysfunction of these cells and dysregulate collagen and matrix deposition involved in fibrosis. This study includes patients with limited cutaneous and diffuse cutaneous forms of SSc, with and without interstitial lung disease (ILD), healthy controls, and fibrosis controls with idiopathic pulmonary fibrosis (IPF), a form of ILD with unknown cause. IPF is the most prevalent form of ILD and is estimated to have a prevalence of 12 per 100,000.

Hypothesis: TGF-β receptor inhibitors reduce or reverse fibrosis of tissues in patients with SSc and IPF.

Methods: Human Lung Fibroblast (HLF) and Human Dermal Fibroblast (HDF) cell lines were utilized to optimize treatments with the TGF-β receptor inhibitor G*alunisertib* (LY2157299) before working with primary samples from patients with SSc or IPF, and healthy controls. Primary samples include dermal fibroblasts from 4-5 mm punch biopsies and circulating fibrocytes from the peripheral blood mononuclear cell fraction of whole blood. To accurately phenotype the cultured cells before and after treatment, light microscopy will be used to detect changes in cell morphology, and western blot, flow cytometry, and qPCR will be used to determine the expression of markers such as *collagen* I, alpha-smooth muscle actin, CD45, CD90, and CXCR4.

Results: It is expected that the pharmaceutical agent G*alunisertib* will reduce the expression of fibrotic markers, such as collagen I and alpha-smooth muscle actin, in dermal and lung fibroblast cell lines. Treated primary fibrocyte and dermal fibroblast cultures will be further characterized with respect to changes in the fibrotic nature of these cell types.

Conclusion: Cell culture and treatment protocols will be assessed as to the effect of TGF-β receptor inhibition on fibrocytes, fibroblasts, and myofibroblasts from SSc and IPF patients.

This research is funded by The Scleroderma Association of BC, Saint Paul's Foundation, The University of British Columbia, and The University of Northern British Columbia.

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*Raveen Badyal recently graduated from UBC with a BSc in Biology. She is a research assistant in the MPCL and is a student researcher with the Scleroderma Association of B.C.

Using Cytokine-Specific Cell-Cell Interaction Networks to Predict Response to Allergen Exposure Using Graph Neural Networks.

R. He*, A. Singh

Background: This project aims to use graph neural networks (GNN) to predict outcomes using cell-cell interaction networks mediated by specific cytokines. Cytokines are chemical messengers produced by human tissues for cell-cell communication. Cell-Cell interaction networks can be represented as directed graphs, a mathematical structure consisting of nodes (vertices) connected by edges (links) representing relationships between the nodes. GNNs are a type of neural network designed to work on data represented as graphs. Here, each node represents a cell-type, and each edge represents cell-cell communication.

Hypothesis: Leveraging the cell-cell communication network will allow prediction of response to allergen exposure and contribute to our understanding of which cytokine-specific cell-cell networks are activated by allergen exposure.

Methods: The goal is to estimate cytokine activity using gene-expression and to predict disease. Blood gene expression data from 14 patients was obtained both before and after allergen inhalation challenge. Data was then fed through a GNN trained to predict whether a given gene expression is from before or after exposure. We will benchmark this algorithm using gene expression datasets based on cytokine treatment responses (*e.g.* TGF-b treatment). We will then apply this algorithm to a previously published gene-expression dataset from our laboratory to identify key cytokines that are perturbed after allergen inhalation challenge in asthmatic individuals. We will validate these results by measuring levels of cytokines like HGF and CCL2 in the same individuals and comparing this data to which cytokine networks were most predictive.

Expected Results: In the benchmarking experiments we expect our method to identify the correct cytokines for the gene expression generated under different cytokine treatment responses. We also expect to identify cytokines known to recruit immune cells into the airways such as IL-1, GM-CSF and TFG-b.

Conclusion: After the cytokines are ranked by accuracy, it will take field research to determine whether predictiveness has any correlation with prominence in actual reactions. At the end of the project, everything will be packaged in an open-source Python library, so that anyone with gene expression data and patient groups labels can quickly create a prediction network and data visualizations. Ultimately, the project could provide immediate gains in our understanding of biology and could one day be useful beyond its original purpose, such as predicting BMI, diet, or lifestyles.

This research is supported by BioTalent Canada.

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* Roy He is entering his fourth year at UBC. This is Roy's first summer at the Centre for Heart and Lung Innovation, following work done during the school year as a part time researcher.

CLOSURE – Computing Lesion Size of Monolayers Using a Convolutional Neural Network

S. Chow*, T.J.F. Guo, G.K. Singhera, D.R. Dorscheid

Background: The airway epithelium protects against inhaled substances. Repairing it after injury is vital for restoring normal function. *In vitro* studies of repair can involve intentionally injuring cultures of the airway epithelium and observe the repair process through time-lapse imaging. Analyzing these images to quantify wound closure is time-consuming and prone to observer bias. To overcome these challenges, we will develop the CLOSURE program, which is trained on a Mask Region-Based Convolutional Neural Network (Mask R-CNN) model to accurately assess wound size and shape. Mask R-CNN is a type of instance segmentation, which is a technique that allows computers to localize and identify objects. A convolutional neural network will be used as it specializes in analyzing images. Like a virtual brain, it can analyze information, learn from it, and make predictions and decisions. The program will be developed using Python and the Detectron2 framework. For evaluating the model's performance, we will utilize common metrics such as average precision (AP) and average recall (AR). AP evaluates the precision-recall trade-off, measuring how well the model localizes and identifies objects at different confidence thresholds. On the other hand, AR measures recall performance at various IoU (Intersection over Union) thresholds, where IoU is a measure of how well the predicted bounding box overlaps with the ground truth bounding box.

Hypothesis: By training the Mask R-CNN model on annotated images of wounded airway epithelial monolayer cultures, the program will be able to detect and segment wounds more efficiently compared to manual analysis.

Methods: 16HBE immortalized airway epithelial cells were cultured as a monolayer until 90% confluent on 12-well plates. Using a dental pick, crosshatch wounds were made on the monolayer and imaged using light microscopy. Subsequent images were taken 6-, 12-, and 24-hour post-wounding. This dataset of images of wounds will be annotated and used to train and evaluate the model. Performance evaluation will be conducted using common object detection metrics such as AP and AR.

Results: The model achieved an overall accuracy of 76%, with 94% in loose evaluation (AP) and 91% in strict evaluation (AR) based on the evaluation metrics. The model performed favorably on large wounds, achieving 76% accuracy. However, it was unable to evaluate the performance on small and medium wounds.

Conclusion: By streamlining the process of wound detection and segmentation, the CLOSURE program removes the necessity for manual analysis and offers an automated approach for wound analysis. In the future, the CLOSURE program will be optimized to quantify wound volume of three-dimensional air-liquid interface cultures, which more closely resembles the structure and function of the *in vivo* airway epithelium.

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*I am a fifth-year undergraduate student studying Computer Science at Simon Fraser University and currently a directed studies student with the Dorscheid Lab. During my spare time, I like to explore different parks in Metro Vancouver.

Understanding the Role of Coxsackievirus B3 in the Landscape of Immunogenic Proteins in Breast Cancer

S.Ashraf Nouhegar*

Background: The oncolytic property of coxsackievirus B3 (CVB3) virus has been previously studied in the context of cancer immunotherapy and has shown promise as a potential treatment option for breast cancer. It has shown strong oncolytic activity against different types of cancer, including breast cancer. Cancer cells are known to take advantage of immune checkpoint proteins and immunosuppressive exosome cargo to fight the immune system. Exosomes are extracellular vesicles that are released by all cells into the extracellular space and can be taken up by neighboring cells. Increasing evidence indicates that exosomes derived from cancer cells carry immunomodulatory cargo that can alter the function of immune cells in the tumor microenvironment. This project aims to investigate the effect of miR-CVB3 treatment on immunogenic proteins in breast cancer cells, their exosomes and within the tumor microenvironment. We will examine the effects of micro-RNA modified Coxsackievirus B3 (miR-CVB3) on the expression of some key immunomodulatory molecules.

Hypothesis: Based on the oncolytic properties of CVB3, we hypothesized that miR-CVB3 treatment would affect breast cancer cells and exosomes harvested from them. We aim to investigate the immunomodulatory cargo carried by these exosomes. Additionally, we anticipate changes in the tumor microenvironment such as the expression of key immunomodulatory molecules in mice.

Methods: This study was conducted using different in vitro and in vivo assays. In vitro, exosomes were isolated from the medium of cultured 4T1 breast cancer cells infected with miR-CVB3(MOI=1) overnight. The cell lysate and exosomes were collected for further analysis. For the in vivo, immunocompetent BALB/C mice bearing 4T1 breast tumors received intertumoral miR-CVB3 injections (MOI=10E7). Tumor, tissues, and blood samples were collected 10 days after infection. We utilized Immunohistochemistry (IHC) , Western blotting and statistical analysis tools to conduct our results and conclusion

Results: The miR-CVB3 infection led to a significant downregulation of immune checkpoint proteins in both infected 4T1 breast cancer cells and the exosomes they released. Additionally, miR-CVB3 treatment triggered a mechanism that led to the release of immunostimulatory molecules via exosomes.

Conclusion: The findings of this study demonstrate that miR-CVB3 treatment modulates the expression of immune checkpoint proteins and triggers the release of immunostimulatory molecules via exosomes in breast cancer cells. These results highlight the potential of miR-CVB3 as a candidate for breast cancer therapy by influencing immune responses within the tumor microenvironment. The implications of these findings may contribute to the development of innovative strategies in cancer immunotherapy, harnessing exosomes as therapeutic vehicles for enhanced treatment outcomes.

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*My name is Sanaz Ashraf Nouhegar, I am a third-year Combined Major Science student at UBC. Currently, I am immersed in my Directed Studies at the HLI Centre, working diligently in Dr. Luo's lab. My project is centered around cancer immunotherapy, specifically utilizing oncolytic viruses to combat cancer. I am driven by my passion for cancer research and aspire to contribute significantly to our understanding of cancer while forging a career in this field.

Peak ICU performance status and long-term outcomes in critical care survivors

S. Bradwell*, J. Gelinas, A. Barber, H. Fisher, S. Jiang, J. Boyd, G.J. Koelwyn

Background: Critical illness is a broad term to encompass the sickest individuals who require high levels of care in the intensive care unit (ICU). Unfortunately, those who survive critical care face high rehospitalization rates (e.g., subsequent infections, cardiovascular events) and mortality risk, and therefore identifying biomarkers to predict these events are urgently required. Performance status scoring tools such as mobility scores are prognostic for adverse outcomes in numerous diseases, however, their prognostic utility following critical care is less clear. Using a retrospective dataset of patients admitted to the ICU at St. Paul's Hospital, we aim to assess how peak mobility score across ICU admission associates with clinical outcomes in critical care survivors.

Hypothesis: Individuals with a high peak mobility score during their ICU stay will have lower 28-day and 90-day rehospitalization rates and mortality compared to patients with a low peak mobility score.

Methods: We are currently performing a retrospective analysis of a critical care patients entering the ICU at St. Paul's Hospital between 2020 and 2022. Using a case control design, we aim to compare rehospitalization and mortality rates of survivors with high peak mobility scores (4/5: equivalent to standing/walking) achieved during ICU admittance to survivors with low mobility scores (1: supine), matched on age, sex, and disease severity (APACHE II) at ICU admission.

Results: Preliminary results are expected for August, 2023.

Conclusion: Critical illness can result in long-term challenges for survivors. A higher risk of rehospitalization due to infection or cardiovascular events, as well as death, may be predicted by highest mobility score achieved in the ICU. This can help to identify patients who may be at a higher risk of complications following critical illness and inform follow up decisions by medical staff.

This research is supported by CIHR-Canada Graduate Scholarship, Simon Fraser University, and UBC.

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*Sarah Bradwell is completing her first year at Simon Fraser University in the Faculty of Health Sciences. She is a member of Dr. Koelwyn's Precision Exercise Research Lab and her research interests lie within the fields of exercise physiology and immunology.

Bioinformatic Exploration of Exercise and Age-Induced Changes to Innate Immune Cell Functions

S. Jiang*, S. Bradwell, G. Nonis, C. Lawrence, G. Koelwyn

Background: Aging is associated with a deterioration of immune function, resulting in imbalanced inflammation and decreased protection against disease. Conversely, recent mouse studies have shown that exercise directly improves cellular function, and even reverses some of these age-related immune changes. To date, few studies have examined cell-specific changes in immune function following exercise exposure, or how these differ with age. Using publicly available single-cell transcriptomic data, we hope to examine exercise-induced changes in specific immune cells (i.e. circulating monocytes and macrophages), and the potential to ameliorate age-related immune dysfunction.

Hypothesis: We hypothesize that exercise will protect from age-related changes seen at the transcriptomic level in monocyte and macrophage populations. Further, these changes will be unique based on tissue compartment (e.g. circulation, lung, bone marrow), cell subset (e.g. classical vs. non-classical monocyte, alveolar vs. interstitial lung macrophage), and following microbial challenge (lipopolysaccharide).

Methods: Monocyte and macrophage responses to exercise and/or age will be evaluated using publicly available single-cell transcriptomic data (GSA: CRA007207, GSE196364). Ingenuity Pathway Analysis (IPA), a gene enrichment software, will be utilized to assess canonical pathways, upstream regulators, and predicted biological functions. IPA analysis will be based on differentially expressed genes in monocytes and macrophages from young/old mice either exposed or not exposed to exercise.

Results: Completed results expected in August 2023.

Conclusion: Results from this analysis provide foundational knowledge on the role of exercise in mitigating age-related immune dysfunction. Such results will inform future trials to further elucidate the immune-modulatory effects of exercise in mouse models and future translation to humans.

This research is funded by the St. Paul's Foundation at St. Paul's Hospital.

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*Sheena Jiang is entering her second-year at UBC, in the Honours Cellular, Anatomical and Physiological Sciences B.Sc. Specialization. This is her first summer at the centre, where she is currently undertaking CAPS 448 (Directed Studies in Physiology).

Modulating high-density lipoprotein (HDL) levels improves sepsis outcomes

L.Q. Chen*, H.Y. Deng, M. Trinder, P.C.N. Rensen, J.H. Boyd, K.R. Walley, L.R. Brunham

Background: Sepsis is defined as the life-threatening organ dysfunction caused by a dysregulated immune response to infection and is a leading cause of death in hospitals globally. Previous studies demonstrated that low levels of high-density lipoprotein (HDL) cholesterol are associated with higher risks of sepsis mortality and that inhibiting cholesteryl ester transfer protein (CETP) increases HDL levels and decreases sepsis mortality in mice. Other studies have suggested that CETP inhibition may affect the host immune response via its regulation of HDL levels, however, the underlying mechanisms of how this happens remain unclear. Our goal is to investigate the mechanisms by which CETP inhibition improves sepsis outcomes

Hypothesis: Inhibiting CETP using anacetrapib will preserve HDL levels leading to improved sepsis outcomes by attenuating the severity of the host's pro-inflammatory response.

Methods: In our pre-sepsis treatment model, 2 groups of APOA1.CETP mice, a model with human-like lipoprotein metabolism, were fed a high-fat diet for 3 weeks, and then treated with anacetrapib (5 μg/kg) or a placebo for 1 week. Mice were then administered 10^7 PFU *Streptococcus pneumoniae* to induce sepsis and monitored every 24 hours post-treatment for percent survival. Mouse tissues were also collected to examine protein and transcriptional expression of pro-inflammatory cytokines via ELISA and RT-qPCR, organ damage through H&E staining and macrophage activation through IHC staining. In our post-sepsis treatment model, 2 groups of APOA1.CETP mice were fed a high-fat diet for 3 weeks and then inoculated with 10^7 PFU *S. pneumoniae* to induce sepsis. Mice were then treated with anacetrapib (5 μg/kg) or placebo at 6-, 30- and 54-hours post-inoculation and monitored every 24 hours post-inoculation for percent survival. *In vitro* studies were conducted using THP-1 monocytic cell lines to examine protein levels of established monocyte markers, such as COX-2 and Caspase-1.

Results: CETP inhibition significantly increased plasma HDL levels and survival in pre-sepsis treatment mice. CETP inhibition also significantly decreased protein levels and transcription of pro-inflammatory cytokines like IL-1β, IL-6 and TNF-α in the lung, liver and plasma of pre-sepsis treatment mice. Additionally, CETP inhibition decreased the pathological score of lungs in pre-sepsis-treated mice by 2-fold, indicating less extensive pneumococcal infection. IHC staining shows CETP inhibition significantly increased macrophage infiltration during early-phase sepsis in the lung. Furthermore, CETP inhibition significantly increased COX-2 and Caspase-1 protein levels *in vitro* suggesting that CETP inhibition promotes monocyte activation. Finally, CETP inhibition significantly increased plasma HDL levels and survival in post-sepsis treatment mice, suggesting that CETP inhibition can improve sepsis outcomes in a more clinical setting.

Conclusion: Our findings suggest that CETP inhibition preserves HDL levels leading to significantly improved sepsis survival in mice. Improved survival is likely due to CETP inhibition increasing HDL levels and promoting monocyte and macrophage infiltration during early phase sepsis. This leads to decreased bacterial burden which attenuates excessive host pro-inflammatory cytokine production, preventing dysregulation of host immune responses. Further studies are needed to investigate how CETP inhibition activates host innate immunity.

This research is supported by the UBC Faculty of Medicine Summer Student Research Program, Michael Smith Health Research BC and Canadian Institutes of Health Research.

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* I am a 3rd year UBC student and it is my first summer at the centre as a summer student.

High-Density Lipoprotein Levels in Patients with Septic Shock

T. Nichvolodoff*, H. Kong, J. Russell, K. Walley

Background: Septic shock is the most severe form of sepsis characterized by low blood pressure despite fluid replacement. This results in impaired perfusion and subsequent organ failure. In sepsis, pathogenic lipids function as ligands that contribute to severe systemic host inflammatory responses and organ damage. Clearance of these lipids from the blood via the liver is facilitated by incorporation into lipoproteins such as high-density lipoproteins (HDLs). In addition, HDLs have protective qualities including anti-inflammatory and antithrombotic effects. Plasma levels of HDL cholesterol (HDL-C) decrease during sepsis and may play a role in modulating survival. HDL-C levels in patients with septic shock specifically have not been investigated. Therefore, we measured plasma HDL-C from a previous cohort of patients with septic shock to investigate the association of HDL with septic shock mortality.

Hypothesis: Low plasma HDL-C levels are associated with higher mortality in patients with septic shock.

Methods: Validation of the following methodology was performed on samples from a previous cohort of emergency department (ED) admission patients with suspected sepsis (n=12) whose baseline HDL-C had previously been measured with an analyzer. HDL-C was measured from EDTA plasma stored at -80°C using an assay kit based on PEG precipitation to separate the lipoproteins, followed by an enzymatic reaction step and colorimetric detection. Here, samples from the Vasopressin Versus Norepinephrine Infusion in Patients with Septic Shock (VASST) cohort (n=419) were used to investigate HDL-C levels in patients with septic shock. The VASST cohort consisted of a randomized controlled trial with septic shock patients whose mortality data is known. Plasma used was from blood collected at baseline, 6, or 24 hours.

Results: For validation of the methods, the correlation analysis comparing previous and present HDL-C measurements in the ED patients gave an r^2 value of 0.312 and a p-value of 0.059. One sample in particular gave unexplained biological variation that was consistent upon repeating the experiment. While not significant, a trend was observed and we are satisfied that the values are representative of their biological values. VASST cohort HDL-C results are pending.

Conclusion: If the results show association of low HDL-C with mortality, HDL may contribute to survival in patients with septic shock. Future directions include investigating how HDL-C levels mechanistically cause altered septic shock mortality using Mendelian randomization strategies, elucidating how host genotype may influence septic shock outcomes.

This research is funded by the Canadian Institutes of Health Research. We thank all the patients involved in the VASST study.

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* Tamara Nichvolodoff has a BSc in Microbiology & Immunology from UBC and is entering her second year at BCIT in the Medical Laboratory Science program. This is Tamara's first summer at the Centre for Heart Lung Innovation.

Impact of Bioprosthetic Surgical Valve Degeneration on True Internal Diameter (ID)

T. Reed*, D. Meier, G. Tzimas, A. Lai, H. Gill, M. Akodad, J.A. Leipsic, P. Blanke, G.W. Payne, J.G. Webb, J. Sathananthan, and S.L. Sellers

Background: Heart valve replacement serves as the primary therapeutic option for valvular heart disease, most commonly using a bioprosthetic valve via open heart surgery or transcatheter implantation. Unfortunately, all bioprosthetic valves will eventually degenerate. In the case of failed surgically implanted bioprosthetic heart valves (SHVs), a transcatheter heart valve (THV) can be placed inside the failed valve. This is known as a valve-in-valve (VIV) procedure. A crucial component of VIV is optimizing size selection of the THV suitable for the failed SHV. Currently, this sizing is primarily reliant on bench modeling utilizing manufacturer provided SHV dimensions and non-degenerated SHV specimens. Inaccurate sizing of the THV suitable for VIV procedures can lead to unfavourable clinical outcomes, and therefore, is crucial to understand how valve degeneration impacts the true size of SHVs.

Hypothesis: Sizing of SHVs for VIV procedures is impacted by SHV degeneration; different modes of SHV degeneration will impact the effective true internal diameter (ID) of SHVs.

Methods: Patients who received a SAPIEN 3 (S3) THV into a failed SHV between 2016 and 2022, and had pre- and post-procedure CT scans, were evaluated. THV expansion was measured at three points: the point of blood flow entry (THV-inflow), the point where blood flow exits the valve (THV-outflow), and at the point where the THV is attached to the SHV (SHV-sewing ring), and compared to the expected THV expansion based on the manufacturer's reported true ID. Bench modelling with explanted SHVs was used to show the impact of degeneration on effective true ID; four SHVs with different modes of degeneration (inflow pannus, extrinsic or intrinsic calcification, and mixed degeneration) were modeled. A 25mm Z-MED-II-X balloon at 5 atm was placed in each failed SHV and imaged by micro-CT. CT analysis was used to assess and visualize the effective true ID of the SHV and areas of restriction.

Results: Fifty-one patients were included (mean age 77 ±11 years, 68.6% male). Median time interval from SHV implant to VIV procedure was 10.7 years. Balloon post-dilatation was performed in 17 (33.3%) patients. For these cases, the most constricted portion of the THV was found at the level of the SHV sewing ring; mean THV expansion based on nominal diameter was 84±6%. THV expansion for VIV was based on true ID was expected to be 89±7% and THV was on average 1.3±1.6mm smaller than predicted. When looking only at THVs that did not undergo post-dilatation (34 patients), mean THV expansion was 81±4% despite an expected THV expansion of 90±4% and THV being an average of 2.1±0.8mm smaller than predicted. The benchtop model's micro-CT showed that SHVs with valvular degeneration impeded the full balloon expansion including at the level of the SHV sewing ring in all modes of degeneration.

Conclusion: Various modes of SHV degeneration creates a smaller true ID causing THV under-expansion during VIV procedures, which can lead to leaflet pinwheeling. This offers valuable insights into the selection of THVs specifically tailored for degenerated SHVs, with the ultimate aim of improving clinical outcomes.

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*This is my second summer at HLI in the Dr. Sellers/Sathananthan lab and I am entering my 4th year at the University of Calgary studying Biological Sciences, and Health and Society.

Effect of Overexpression of Lysosomal Acid Lipase on Cholesterol Mobilization in Cultured Human Aortic Smooth Muscle Cells

V. Baht*, K. Besler, T. Chan, G.A. Francis

Background: Smooth muscle cells (SMCs) comprise the majority of foam cells in human atherosclerosis. SMCs express low levels of lysosomal acid lipase (LAL) and ATP-binding cassette transporter A1 (ABCA1), which both contribute to foam cell formation. LAL, encoded by the *LIPA* gene, is the sole enzyme responsible for the hydrolysis of cholesteryl esters to free cholesterol in the lysosome. The addition of exogenous LAL *in-vitro* has been shown to decrease lysosomal cholesteryl ester accumulation and increase efflux of free cholesterol out of SMCs.

Hypothesis: Increasing expression of LAL via an adenoviral vector containing *LIPA* will reduce lysosomal cholesterol accumulation and increase cholesterol efflux through ABCA1-dependent and non-ABCA1-dependent mechanisms.

Methods: Human Aortic Smooth Muscle Cells (HASMCs) were infected with AdmCherry, an adenoviral vector containing the gene for fluorescent protein mCherry, to assess infection efficiency. Cells were subsequently infected with AdmCherry or AdLIPA using Lipofectamine™ and assessed for LAL activity and protein to confirm increased expression of LAL over cells infected with AdmCherry and non-infected cells. To assess lipid localization, infected cells were loaded with aggregated LDL (agLDL), known for its efficient uptake by lysosomes. Subsequently, cells were stained with lysosomal marker LAMP1 and lipid dye BODIPY to evaluate colocalization by confocal microscopy.

Results: HASMCs were successfully infected with AdmCherry with an infection efficiency of 98%. We obtained a 2-fold increase in LAL activity and a 1.5-fold increase of LAL protein at 48-hours after infection in cells infected with AdLIPA compared to cells infected with AdmCherry and non-infected SMCs. Visual inspection of confocal images confirmed *LIPA* infection resulted in marked clearance of cholesterol from lysosomes, but no change in cholesterol accumulation in cells infected with AdmCherry relative to non-infected SMCs.

Conclusion: Our findings suggest that adenoviral infection of *LIPA* in vascular SMCs is successful in increasing LAL activity and clearing stored cholesteryl esters out of lysosomes. Further experiments are required to assess the ability of SMCs to release cholesterol after increasing LAL expression and activity. These include testing efflux of cholesterol to 1) apolipoprotein A1 (apoA1), which facilitates cholesterol transport to HDL particles through ABCA1-dependent mechanisms and 2) HDL and trypsinized HDL via non-ABCA1-dependent mechanisms allowing cells to release cholesterol independently of ABCA1.

This project is supported by a CIHR grant from 2018-2023.

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* Vittoria Baht is entering her last year in the Biochemistry program at UBC, completing her Co-op Term at the Centre for Heart Lung Innovation.

Understanding the Role of Complement Regulator Proteins in CVB3 Induced Myocarditis

W. Hwang*, C. Lin, Y. Mohamud, H. Luo

Background: Myocarditis, or inflammation of the heart muscle, accounts for up to 20% of deaths among young adults. Virus infections are the leading cause of myocarditis, among which is coxsackievirus B3 (CVB3). CVB3-induced viral myocarditis can mediate various mechanisms including modulating the host cell inflammatory response and autophagy. The innate immune system plays an integral role in inflammation and defense against pathogens. Specifically, the complement system is part of the innate immune system that detects and removes pathogens and infected cells upon activation by antigen recognition. A cascade of events leads to inflammation, opsonization for phagocytosis, and formation of the membrane attack complex (MAC) for cell lysis. Complement regulator proteins CD55, CD59 and CD46 protect the host from excessive complement activation by regulating MAC formation and inflammation. Many viral infections alter the expression of complement regulator proteins to supress host immune response and regulate inflammation. Given that myocarditis has autoimmune causes, complement regulator proteins may be an important mechanism for viral myocarditis. However, there is little known about the impact of CVB3 on the complement system. This project aims to confirm whether CD55, CD59, and CD46 are regulated at the RNA and/or protein level, and the functional consequences of the downregulation in CVB3-induced viral myocarditis.

Hypothesis: CVB3 downregulates the complement regulator proteins CD55, CD59, and CD46, which increases inflammatory responses and MAC formation on host cells, contributing to the progression of viral myocarditis.

Methods: To determine complement activation in human myocarditis, immunohistochemical staining of MAC was performed on human cardiac tissue with viral myocarditis. To determine whether the regulation is at the RNA and/or protein level, western blot was conducted on mice cardiomyocytes and HeLa cells infected with CVB3 and stained for CD55, CD59 and CD46, to visualize the protein levels at different time points after infection. Quantitative polymerase chain reaction (qPCR) will be performed on uninfected and CVB3 infected cells, with primers for the complement regulators. To investigate functional consequences of the downregulation, MAC levels will be examined with confocal microscopy, following overexpression of the regulator proteins in HeLa cells and cardiomyocytes. Inflammatory cytokine gene expression will be investigated using qPCR.

Results: Significant increase of MAC was seen in CVB3 infected human cardiac tissue compared to healthy control, indicating an activated complement system. MAC levels also increased after CVB3 infection in HeLa cells, and colocalized with a viral protein. Decrease in CD55, CD59 and CD46 protein levels was observed 12 hours post infection in mice cardiomyocytes. No decrease in protein levels was seen 5 to 7 hours after infection. Noticeable decrease in MAC was seen in HeLa cells after overexpression of CD59 compared to control.

Conclusion: The complement system is activated during CVB3 induced myocarditis, and the complement regulator proteins are downregulated later in CVB3 infection. Mechanisms of downregulation should be studied further to investigate possible therapeutic targets.

This research is supported by members of the Luo Lab and funded by CIHR and BioTalent.

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*Wendy Hwang finished her third year at UBC with a major in biology and minor in microbiology. This is her first co-op term at the Centre for Heart Lung Innovation.

The Effect of Senescence and Senolytics on Airway Epithelial Function

W.Y. Liang*, T.J.F. Guo, G.K. Singhera, D.R. Dorscheid

Background: The airway epithelium is the first line of defense against pathogens from the inhaled environment. In response to persistent damage from inhaled particles, the epithelium undergoes proliferation as part a component of its repair mechanisms, facilitating recovery and restoration processes. However, in asthmatic patients, the airway epithelium exhibits impaired repair abilities and dysfunction, resulting in the development of bronchitis and breathing difficulties. Thereby, improving decreased epithelial regeneration may reduce disease outcomes associated with asthma. Cellular senescence is a response characterized by irreversible cell arrest, which limits cellular regeneration and airway repair, and release of pro-inflammatory senescence-associated secretory phenotype (SASP) factors, such as pro-inflammatory cytokines. These SASP factors contribute to airway inflammation and remodeling, but also epithelial dysfunction when overly abundant. Consequently, targeting senescent cells is a potential therapeutic strategy to restore regenerative functions of the airway and reduce respiratory disease outcomes. Senolytic agents, such as dasatinib and quercetin, can selectively eliminate senescent cells by inducing apoptosis. Hence, these agents hold promise for alleviating disease outcomes. Previous clinical studies have shown that dasatinib and quercetin administration improves physical functions in individuals with idiopathic pulmonary fibrosis. However, the specific effects and mechanisms of senolytic agents in the airway remain poorly understood. This study aims to investigate the underlying cellular mechanisms of senescence and the impact of senolytics on epithelial cells, which play a critical role in airway defense, repair, and in asthma development.

Hypothesis: Dasatinib and quercetin reduce the abundance of senescence cells and SASP molecules, consequently restoring epithelial repair in the airway.

Methods: We have induced cellular senescence in the airway epithelium with the herbicide, paraquat, in an immortalized airway epithelial cell line, 16HBE14o-. Markers of cell cycle arrest (p21, p16) and proliferation (Ki67) were detected via western blot and pro-inflammatory SASP cytokines using ELISA. The paraquat-induced senescent model will be subsequently treated with dasatinib and quercetin. To explore their effects on restoring epithelial regeneration in airway repair, cells will be mechanically wounded after treatment to quantify repair over 48 hours by examining epithelial repair rate in wound closure under light microscopy.

Results: Preliminary data demonstrate significantly increased p21, decreased Ki67 expression, and elevated pro-inflammatory cytokines, interleukin-6 and interleukin-8, after 24 hours of paraquat treatment. Proliferation was reduced over time upon paraquat administration, which was found to be influenced by culture confluency. These data show that paraquat induces senescence in epithelial cells, establishing our senescence model. In the following experiments, we anticipate reversed paraquat-induced senescence, reduction of SASP molecules, and restored epithelial regeneration post-wounding.

Conclusion: We have demonstrated that paraquat induces senescence in the airway epithelial cell line, 16HBE14o-. We anticipate the senolytics, dasatinib and quercetin, to mitigate senescence and restore epithelial regeneration in the airway.

*****Wan Yi (Wendy) Liang is a second-year microbiology and immunology undergraduate at UBC with experience in studying inflammation. She is a research assistant in the Dorscheid lab.

The Relationship Between *In-vitro* and Clinical Responses to CFTR Modulators in Cystic Fibrosis Patients

X. Wang*, N. Potter, B. Quon

Background: Cystic fibrosis (CF) is a recessive genetic disorder characterized by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is an anion channel expressed in several epithelial tissues such as the sweat glands, airways and gastrointestinal tract. Impairment of CFTR causes dysregulation of epithelial fluid secretion and mucus thickening leading to organ dysfunction. The most devastating effects are seen in the lungs, characterized by severe lung disease. Lung disease is the usual cause of death in most CF patients (85% of mortality), leading to a median age of death of 57.3 years in the CF population in Canada in 2021.

Over the past decade, CFTR modulators, which improve ion transport and augment CFTR activity, have been highly effective for patients with the common mutation F508del, but 5-10% of patients remain ineligible for CFTR modulators since their rare genotypes have not been assessed in clinical trials. With the introduction of newer era modulators, more individuals with rare mutations have been granted access to modulators based on in vitro testing, which measures ion fluxes driven by patients' CFTR proteins. Although this is a promising avenue to help more individuals with CF access treatment, data looking at the relationship between clinical outcome and in vitro outcome for these individuals tends to be reported only on a case-by-case basis. To better understand if the in vitro model is representative of the clinical model for patients with rare mutations, we will examine the relationship between the in vitro and clinical response to modulators for CF patients that have been granted access to modulators.

Hypothesis: We hypothesize that the response observed in in-vitro models will be positively correlated with the clinical response after administration of CFTR modulators.

Methods: A systematic review will be conducted independently by two researchers. Outcomes relating to clinical and in-vitro models of CF will be collected from 5 databases, namely MEDLINE, EMBASE, Web of Science, CINAHL and Cochrane Central Register of Controlled Trials. The clinical outcomes include changes in sweat chloride and FEV1 while the in vitro outcomes include changes in short-circuit current, organoid swelling assay measurement, transepithelial current and patch clamp measurement. The searching and screening process follows the PRISMA flow diagram, which summarises the number of articles retained at each stage of the systematic review. For each class of CF mutation, the reported clinical and in vitro response changes will be collected and compared to that of the non-CF controls (if applicable).

Results: The protocol of the systematic review has been published on PROSPERO. The collected papers have been uploaded to Covidence and the screening is in progress. The data will be categorized based on the types of mutations. F508del mutation has been heavily researched, and we expect there will be enough data to conduct a meta-analysis using STATA SE17. The data on rare mutations is expected to be limited and will be reported in a tabular format.

Conclusion: The systematic review will provide insight into the value of in vitro models to predict clinical outcomes. The study will highlight gaps in the field such as the need for standardization of in vitro models and techniques as well as clinical parameters.

This research is funded partially by UBC Faculty of Medicine Summer Student Research Program (SSRP) grant.

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*Xindi Wang is entering her last year as a UBC Pharmacology student. This is Xindi's second summer in HLI and her first summer joining the Quon lab.

Reference-free deconvolution of spatial transcriptomics data using semi-supervised topic models

C. Yang*; D.D. Sin; R.T. Ng

Background: Recent technological advancements have enabled spatial transcriptomic (ST) profiling of tissues. However, each measured spot usually contains a mixture of multiple cell types, which hinders our understanding of the spatial organization and cell type-specific transcriptomics signature of these cells. *In silico* deconvolution is a promising approach to resolve the cellular composition at each spot. A number of reference-based deconvolution methods have been developed, but they require a reference cell type-specific transcriptomic profile, usually generated from single-cell transcriptomic studies. Despite the emerging number of scRNA-seq studies, the desired reference profile may not be available for certain cell types or conditions. To address this challenge, a few reference-free methods were developed, which simultaneously infer the cell type-specific transcriptomic signature and cell type compositions. Subsequently, the inferred cell types were labeled with a cell type name by comparing the cell type-specific signature against known cell type markers. However, the performance is usually lower compared to reference-based methods and is highly variable between runs.

Hypothesis: In our study, we propose to develop Spatial transcriptomics deconvolution using MARker-gene-assisted Topic models (SMART), a reference-free deconvolution method based on semi-supervised topic models to incorporate prior knowledge into the deconvolution process. We hypothesize that SMART achieves superior performance in comparison with existing reference-based and reference-free methods.

Methods: To evaluate SMART, we simulated ST datasets from single-cell ST data. Then, we applied SMART and some of the best-performing ST deconvolution methods to the simulated data. The Pearson's correlation coefficient (PCC) and the root mean square error (RMSE) between the predicted and ground truth cell type proportions were used as the evaluation metrics. To evaluate SMART's compatibility on different ST platforms, we also validated SMART on real ST datasets.

Results: SMART demonstrated improved performance and lower variability on multiple simulated datasets, even when compared to some of the best-performing reference-based methods ($P<0.05$ using per-spot RMSE). A PCC > 0.9 was obtained across all cell types and a PCC > 0.7 was obtained in individual cell types. With the real ST datasets, SMART demonstrated compatibility with both the high-resolution and the low-resolution ST platforms.

Conclusion: Upon successful development of the method, SMART will be a powerful tool to unravel the heterogeneity of ST data and to identify potential therapeutic targets at a single-cell type resolution with spatial information.

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* I am a staff statistician at the HLI and a second-year PhD student in the bioinformatics program of UBC. During my spare time, I like singing, music recording, and 3D design and modeling.

THANK YOU!

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Providence Research
We're all in.

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