Research Day Abstracts

2022

4th Year Honours Specialization Students

Presenter's Name: Huang, Sophia
Additional Authors: Schruder C, Kaiyum R, McCord C, Mermut O
Abstract Title: Evaluative Approaches for Supportive Housing Communities in St. Thomas, Ontario

Abstract: Substance use disorders and mental illnesses are mental disorders that affect a person's behaviour and can often hinder an individual's ability to complete daily tasks and exert control over substances; thus, posing a risk to their health. Homeless people are extremely vulnerable when considering their lack of access to stable housing and the social stigma surrounding their conditions. Homelessness has become an increasingly prevalent issue, especially in Southwestern Ontario. Despite the therapeutic benefits that companion animals may provide to recovery, homeless persons with companion animals have even less access to homeless services as many prohibit pets. As such, the challenge of solving homelessness is often related to the complex social and medical problems experienced by homeless people. Permanent supportive housing are housing communities that provide safe and stable housing to the homeless population that suffer from mental and substance use disorders. There is often support programming in these communities that equips users with resources to protect their well-being, including access to physicians and treatment. A challenge associated with these communities include a lack of standard approaches to evaluate these communities. As such, a scoping review of databases including MEDLINE, EMBASE, PsycINFO, SCOPUS and other grey literature sources will be performed to synthesize the body of knowledge in regards to factors that contribute to success, including the incorporation of pet-friendly housing, and evaluative approaches for supportive housing communities in Canada. This will further apply to the homeless population in St. Thomas, Ontario. Further, key criteria, stakeholders and programming for supportive housing communities will be discussed. Evaluative approaches for these communities can be separated into three categories: housing, support (mental disorders), and support (others). This study will provide supportive housing communities with approaches to evaluation in order to learn from other communities and make changes to better equip the homeless population to tackle substance use disorder and mental illness. The One Health approach recognizes the built environment of housing and social support services as well as companion animal health and other medical services as key parts in addressing homelessness and mental and substance abuse disorders. By protecting environmental health and animal health, human health outcomes are improved.

Presenter's Name: Nagano, Tyler
Additional Authors: Castellani CA
Abstract Title: Effect of CRISPR Induced Mitochondrial DNA Variation on the Nuclear DNA Epigenome and Transcriptome

Abstract:
Introduction: Mitochondrial DNA copy number (mtDNA-CN) is associated with several age-related chronic diseases and is a predictor of all-cause mortality. A previous study by Castellani et al. identified a role for mtDNA-CN variability in the regulation of nuclear gene expression via nuclear DNA (nDNA) methylation. What remains unknown are the underlying biological mechanisms controlling the effect of mtDNA-CN on nDNA gene expression. In this study, we hypothesized that site-specific differential nDNA methylation and differential gene expression resulting from in vitro reduction of mtDNA-CN would uncover shared genes and biological pathways important in the mechanisms mediating the effect of mtDNA-CN on disease.

Methods: To test this hypothesis, we generated epigenome and transcriptome profiles for three independent human embryonic kidney (HEK293T) cell lines harbouring a mitochondrial transcription A (TFAM) heterozygous knockout using CRISPR-Cas9, and matched control lines. Methylation data was generated using the Illumina Infinium Methylation EPIC BeadChip, RNA sequencing was performed using the Illumina HiSeq 2500 instrument and the data was analyzed to call differentially methylated sites (DMS), differentially methylated regions (DMR), and differentially expressed genes (DEG). Finally, we integrated the analyses and performed functional enrichment to determine genes and pathways which may facilitate mtDNA-CN effect on nDNA gene expression.

Results: Our results identified 4205 DMS associated with mtDNA-CN at epigenome-wide significance ($p<1\times10^{-7}$). 228 DMR associated with mtDNA-CN ($P<1.17\times10^{-5}$) and 164 DEG were also identified ($p<3.59\times10^{-6}$). Enrichment analyses demonstrated that the "neuroactive ligand receptor interaction", "GABAergic synapse" and "Nicotine Addiction" KEGG pathways were overrepresented in the DMS analysis ($p<6.37\times10^{-4}$, $p<1.54\times10^{-4}$, $p<1.42\times10^{-6}$), DMR analysis ($p<4.20\times10^{-3}$, $p<1.91\times10^{-4}$, $p<4.67\times10^{-3}$) and DEG analysis ($p<7.73\times10^{-3}$, $p<1.94\times10^{-2}$, $p<2.12\times10^{-3}$).

Discussion: These findings demonstrate that genes in the "neuroactive ligand receptor interaction", "GABAergic synapse" and "Nicotine Addiction" KEGG pathways may be related to the underlying biological mechanisms which facilitate mtDNA-CNs effect on nDNA methylation. Further, these results suggest that mitochondrial DNA variation signals to the nuclear DNA epigenome and transcriptome and may lead to changes relevant to development, aging, and complex disease.

MEDICAL BIOINFORMATICS

Presenter's Name: Qian, Brian
Additional Authors: Naghavi NH, Shooshtari P
Abstract Title: Collection of associations between cell types and complex disease including both bulk and single-cell open chromatin region data

Abstract:
Introduction: Several disease risk variants reside on non-coding regions of DNA, and particularly on open chromatin regions (OCR) of specific cell types. This suggests that disease risk may be driven by gene regulation rather than changing the coding sequences of protein coding genes, and therefore a combination of OCR data and genetic association data can help identify mechanisms of complex diseases. For most complex diseases, the known relevant cell types are highly heterogeneous; thus, further subsets of these cell types remain unexplored, and are potentially highly informative. In this study, I create a collection of associations between combinations of different cell types and an array of complex diseases, and in addition, use single-cell sequencing data to further expand these associations through potentially undiscovered cell subtypes.

Methods: I prepared open chromatin region (OCR) data from two curated databases: OCHROdb and scATAC.Explorer. OCHROdb is a quality-checked database of open chromatin regions gathered from multiple large-scale consortia-based projects. scATAC.Explorer is a curated collection of single-cell ATAC-seq datasets available in a standardized format. I integrated the OCR data and disease GWAS summary statistics and perform LD score regression analysis to estimate the amount of disease heritability attributing to OCR of each cell type. This resulted in prioritizing cell types that are likely to be relevant to complex diseases.

Results: I applied my method to GWAS of 27 diseases and the bulk OCR data, and found significant results (FDR < 0.05) for at least one cell type in eight complex diseases, including strong associations between immune cell types with rheumatoid arthritis and multiple sclerosis. With the integration of single-cell ATAC-seq data, there were significant correlations found in similar disease types, along with other diseases that showed little to no significance when initially integrated with bulk OCR data. This includes type 1 diabetes, primary biliary cirrhosis, and lupus, as these diseases were found to be significant with cell subtypes found in peripheral blood mononuclear cells.

Discussion: GWAS can be used to uncover associations between cell types and disease phenotypes, when coupled with OCR data. Furthermore, my results also suggest that single-cell data can further uncover new correlations through undiscovered cell-subtypes, providing more informative results versus bulk sequencing data.

Presenter's Name: Raval, Keval
Additional Authors: Poon AFY
Abstract Title: Automated mapping of an expertise network in a research group: application to Western Pathology

Abstract: Any academic department is an ensemble of expertise in research areas; for example, the department of Pathology at Western University itself contains sub-departments of research such as medical health Informatics, lung pathology, cardio pathology and more. The conventional approach to mapping an expertise network is to manually find the papers of faculty members in the academic department of interest, subjectively determine their overall topic of research, and then compare them to the others. This method is disadvantageous because it is time-consuming, there is an overwhelming amount of data to sift through, and it is a highly subjective process. Creating an expertise network greatly benefits researchers as it allows them to attract potential students interested in their research, external funding partners, and collaborators who work in a related research area. Streamlining the process of generating this network and producing it objectively by using text mining and clustering analysis is the novel method in this project, aiming to resolve the two main issues of the conventional approach. The inquiry can be summarized into four main steps. First, text mining of abstracts of the research papers from the researchers of the Pathology Department of Western University was performed to create unique sets of key terms per researcher. To perform this, the abstracts were pulled from the PubMed Application Programming Interface (API), and they were text mined using SpaCy, a Natural Language Processing (NLP) module in python. The key terms extracted were nouns, pronouns, verbs and adjectives as they are the parts of speech that most often characterize a body of text. Next, similarity scores between researchers' sets based on the cosine similarity metric were calculated to produce a matrix of results. Dimensionality reduction using Principal Component Analysis and clustering using various algorithms, such as Agglomerative and K-means, was performed on this resulting matrix. Finally, an optimal end goal is to build a website that has an interactive network that is intuitive to use, where users can easily search and find areas of interest to them. This inquiry proposed a novel method to streamline the tedious process of categorizing the research department of any faculty into areas of expertise. The applications of this method can allow researchers to effectively communicate their area of research, enabling an efficient and objectively accurate information exchange.

MEDICAL BIOINFORMATICS

Presenter's Name: Safdar, Aisha
Additional Authors: Sidahmed A
Abstract Title: Immune Response to SARS-CoV-2 Infection in Immunocompromised Patients

Abstract:
Introduction: COVID-19 is an infectious respiratory disease caused by SARS-CoV-2, a new coronavirus strain, that was discovered in November 2019. COVID-19 can manifest itself in various ways and in severe cases, it can cause individuals to have respiratory failure, cardiac injury, or death. Research shows the immune response in individuals who get severe COVID-19 resembles a cytokine storm, characterized by an increase in pro-inflammatory cytokines such as IL-1B, IL-1RA, IL-7, IL-8, IL-9, IL-10, IL-12, IL-18, IFN-γ, TNF-α, GCSF and CXCL-10. In this study, we evaluated if immunocompromised patients with severe covid present with a cytokine storm, as compared to non-immunocompromised covid patients. We hypothesize that immunocompromised patients are protected from SARS-CoV-2 related cytokine storm due to their immune dysfunction.

Methods: To test this hypothesis, a cohort of 25 immunocompromised patients who were hospital-admitted for COVID had 2-3 blood samples collected during their time at the hospital. Chemokine and cytokine bead array kits were used to analyze cytokine levels in patients' serum samples. The patients were matched with controls, based on age and gender. The cytokine data was analyzed using GraphPad to conduct t-tests and assess if there was a significant ($p<0.01$) difference in cytokine levels of the study subject and their control. The data was assessed at a group level of patient vs control in R using a linear regression and the underlying data was visualized as boxplots. Subsequent analysis comparing cytokine levels will be performed in R.

Results: Our initial results show there is little difference in the cytokine response of immunocompromised patients and covid patients when compared at a group-level. At an individual matching level, there are some cytokines that are significantly lower ($p<0.01$) in immunocompromised patients, suggesting these patients may be protected from an increase in some cytokines that are associated with severe covid.

Discussion: These findings show that the varying nature of COVID-19 and the heterogeneity of each patient make it difficult to use single factors such as being immunocompromised to predict an individual's pathological response to COVID. Given the major implications associated with the potential immunomodulatory treatments needed for immunocompromised patients with severe COVID, further research should be conducted on smaller subsets of covid patients and their pathological responses.

Presenter's Name: Wang, Teng Qing
Additional Authors: Mohsenzadeh Y, Duerden E
Abstract Title: COVID-19:Managing Parent Attitudes and School Stress (COMPASS)

Abstract:
Introduction: The COVID-19 pandemic has left millions of children unable to access in-person learning, and brought about drastic changes to their home environment and learning delivery. These changes have increased stress for both parents and their children, which can negatively impact children's learning and cognitive development. In a previous study, we have found that screen time in children as well as parental stress have increased during the pandemic, and that parents experiencing more stress were significantly associated with increased screen time in children. This longitudinal study examines changes in children's cognition, home and school learning environments, and parental stress. We now hypothesize that changing school conditions (i.e. asynchronous lecture hours) are associated with cognitive and behavioural changes in children as well as parental stress, and that parental stress is associated with cognitive and learning outcomes.

Methods: Parents of children from 6-12 years old were recruited to complete 5 online surveys on a rolling basis. Major stressful life events in parents were measured using the Holmes-Rahe Life Stress Inventory and parenting stress was measured using the Parenting Stress Scale (PSS). Behavioural changes in the children were measured using the Strengths and Difficulties Questionnaire (SDQ). Parental involvement was assessed using the Alabama Parenting Questionnaire. Cognitive games created by Centivizer were completed by the children on a rolling basis and assessed common metrics of cognitive health, such as reaction time and working memory.

Results: As of the first round of data collection, we have found that increased processing speed in children was significantly associated with a child's age ($p < 0.001$), increased time spent reading every day ($p = 0.011$), and increased physical activity ($p = 0.011$). Furthermore, increased screen time in non-school contexts also significantly improved processing speed ($p < 0.011$).

Discussion: These results may show that increased screen time in children from ages 6-12 as a result of the COVID-19 pandemic may provide some benefits to their cognitive development. However, the negative changes induced by the pandemic on both parents and their children, such as reduced access to physical activities, could negatively impact the cognitive development of children.

MEDICAL BIOINFORMATICS

Presenter's Name: Brar, Sukham
Additional Authors: Vanin S, Hardy DB, Arany E
Abstract Title: Evaluating the effects of cannabidiol use during pregnancy on offspring pancreatic development and function in rats: potential impacts for human and environmental health

Abstract:
Introduction: fj9-tetrahydrocannabinol (fj9-THC) and cannabidiol (CBD) are the two main components of Cannabis sativa. In the United States, 1 in 20 women report consuming cannabis while pregnant, which is likely an underestimate due to self-reporting. A recent study has shown that in utero fj9-THC exposure impairs female offspring glucose homeostasis and endocrine pancreatic development in the rat. Yet, contributions of in utero CBD exposure in pancreatic development and function remains largely unknown. This is concerning since CBD has become increasingly accessible and consumed since the legalization of cannabis in 2018 in Canada.

Project Goal and Objectives: A one health approach was used to investigate the potential effects of CBD use during pregnancy on offspring pancreatic development and function in a rat model, the effects of CBD consumption on other animal species and the impacts of CBD production on the environment. Key stakeholders interested in CBD consumption and production were identified and mapped.

Methods: Pregnant rats per treatment received daily intraperitoneal (i.p) injections of either a vehicle (1:18 cremophor: saline i.p), a low dose of CBD (3 mg/kg i.p), or high dose of CBD (30 mg/kg i.p) from gestational day 6 to parturition. Pancreatic development and function was assessed in male offspring at 3 weeks and 3 months of age through a glucose tolerance test and assessment of pancreas morphometry by immunofluorescence. A scoping review was conducted to provide an overview of current key findings related to the effects of cannabis cultivation on the environment and the effects of CBD as a therapeutic agent in domestic dogs. Key stakeholders were identified by reviewing grey literature and mapped using Kumu software.

Results: The preliminary findings at 3 months showed a significant increase in the area under the curve for blood glucose in offspring exposed to a low dose of CBD. No significant changes in fasting insulin concentration and indices of insulin resistance (HOMA-IR, HOMA-B, QUICK-I) were observed. It is expected that cannabis cultivation will negatively impact the environment and CBD consumption will have mixed effectiveness as a therapeutic agent in dogs.

Discussion: These findings indicate that fetal CBD exposure may predispose offspring to develop glucose intolerance. The potential adverse effects of CBD makes its consumption and production concerning for the health of humans, animals and the environment.

Presenter's Name: Hung, Anton
Additional Authors: Sun G, Northover A, Frisbee SJ
Abstract Title: Optical Coherence Tomography for Evaluating Oral Cancer

Abstract:
Introduction: Optical imaging within tissues is promising for the medical screening and diagnosis of various diseases, such as oral cancer and pre-cancer. Clinically identifying oral cancers during routine screening is challenging and there is disagreement regarding its effectiveness at reducing mortality. Optical Coherence Tomography (OCT) is a non-invasive interferometry technique that allows micron-scale resolution images of complex structures in oral tissue. From the images generated by OCT, we hope to quantitatively map the attenuation of the light signal throughout the tissue. The computed maps might then be used for differentiating between cancerous and non-cancerous oral tissues, enhancing clinicians' capacity to screen for oral cancer.

Methods: We acquired 248 tissue samples from prospective oral patient biopsies of a variety of pathological diagnoses and used OCT to capture 3 images per tissue. Using MATLAB, we developed computational models to create optical attenuation maps from our OCT data. Our calculations are built upon the principles of the Beer-Lambert Law. One variation of the Beer-Lambert model incorporates an additional parameter, the confocal point spread function, to account for the confocal properties of the OCT apparatus. We compared the accuracy of our computational models by testing with intralipid phantom samples.

Results: We were able to successfully write a program to generate attenuation maps from the OCT scans of our oral tissues. Both the regular Beer-Lambert model and our modified Beer-Lambert model can be used to visualize light attenuation in different areas of oral tissue. Our maps can be used to accurately calculate attenuation coefficients for our tissues.

Discussion: Using OCT to measure light attenuation helps dental clinicians to gain critical information about oral tissue lesions. The imaging power of OCT has potential benefits for improving oral cancer screening. OCT allows clinicians to obtain a quantitative characterization of tissue lesions that can inform clinical decision-making. Further investigation can be done to correlate the calculated attenuation coefficients with the patient diagnoses.

ONE HEALTH

Presenter's Name: Keng, Isabelle
Additional Authors: McKinley G, Olea-Popelka FJ
Abstract Title: Social Prescription Evaluation: Developing a Method for Evaluating Social Prescription as a Means of Supporting Individuals with Lived Experience with Substance Misuse in Whitefish River First Nation Community

Abstract:
Introduction: Colonization silenced indigenous voices in healthcare policies, leading to culturally inappropriate healthcare services and on average, indigenous peoples are disproportionately affected by substance misuse in Canada. This led researchers to develop services, like social prescription programs to help meet this need. However, these programs are new, and an evaluation plan is required to ensure it works in the context of indigenous health and substance misuse. The aim of this project is to create an evaluation plan for the pilot social prescription program taking place in the Whitefish River First Nation community that includes the environmental, animal, and human factors that influenced the observed community public health outcomes.

Methods: Kumu software will be used to create a stakeholder map. Apart from the individuals directly involved in the project additional stakeholders will be identified through literature analysis. A program logic model will be created following the W.G Kellogg (2004) 5 category model. Interviews of staff members involved in the project implementation will be conducted by myself under the supervision of Dr. Gerald McKinley. A qualitative analysis will be completed following the framework method by Gale et al., (2017) with modifications to the first and second steps.

Expected Results: I am expecting to identify factors that encouraged and deterred participant attendance. I am also looking to identify natural and built social environmental, and animal factors that influenced the observed outcomes, and to draw evaluative conclusions about the quality, value, and significance of the program to the community.

Significance: This project will help expand the applications of social prescription programs in Canada. Additionally, the social prescription program being evaluated has a large environmental and animal component, and as such, requires a one health approach to be fully comprehensive.

Presenter's Name: Li, Duo
Additional Authors: Frisbee SJ, Jaganathan S
Abstract Title: A One Health Approach to Localizing and Implementing Sustainable Development Goals in London, Ontario

Abstract: The United Nations' 17 Sustainable Development Goals (SDGs) provide a framework for a sustainable future by recognizing the complex relationships between various topics in human health and the environment. However, many of the totalled 231 SDG indicators were created based on a global scale, and therefore, are not always applicable or available in data within specific cities. To properly use and benefit from the SDG framework in local areas, it is important to adapt the list of indicators to fit the city's unique state and needs. Additionally, the One Health approach is essential in understanding the importance of stakeholder collaboration and interconnections between human health, animal health, and the environment when implementing the SDG framework. Thus, the main goal of this thesis is to make recommendations on localizing the SDG framework with a focus on the city of London, Ontario. To achieve this, data sources that measure progress of London's existing localized indicators will be summarized in a report, different SDG localization methods in other cities will be explored, and various stakeholders involved in the SDG framework will be mapped using the software Kumu. Preliminary findings show that although a list of localized indicators has been created for London, around half of them do not have available or reliable data sources. Without data to measure progress within the indicators, this would call for modification of London's localized indicators or new initiatives that collect the SDG data needed on a local scale. Additionally, none of the localized indicators address animal health, an important pillar of the One Health paradigm. Thus, London will need to consider creating new indicators related to animal health to fill this gap. Ultimately, this project will help further the knowledge of how to localize and implement the SDG framework and provide London with its next steps in measuring SDG progress.

ONE HEALTH

Presenter's Name: Liu, Peter
Additional Authors: He F, Mallikarachchi M, Frisbee SJ
Abstract Title: Cardiovascular disease in neuroendocrine tumours

Abstract: Patients with neuroendocrine tumours (NETs) commonly develop cardiovascular complications that can manifest as carcinoid heart disease (CHD). NET tumours may secrete diverse substances such as catecholamines, serotonin, arachidonic acid metabolites, and other neuroendocrine factors. Chronic exposure to vasoactive NET secretions may cause endothelial cell dysfunction, impair vascular network perfusion, and remodel heart structure or function causing poor clinical outcomes in these patients. Given that NET incidence is increasing worldwide and are now recognized as the fastest growing class of tumours, there is a critical need to understand the underlying mechanistic contributors that lead to CHD or NET development.

In this thesis project, a One Health approach is used to examine the interconnected environmental factors that play a role in animal and human NETs. A narrative literature review was conducted to investigate NETs in animal species, in addition to identifying environmental and socioeconomic factors that may be associated with NETs. Potential stakeholders within and beyond scientific research will be identified and visually mapped using Kumu. A scoping review was conducted to evaluate the existing primary literature about CHD in human NET patients.

A systematic search of the MEDLINE database was performed and 434 studies were extracted. After title and abstract screening, 273 studies were selected for full-text review and data extraction if eligible. Within these studies, 173 were identified as case reports and 94 as cohort studies of various designs. Currently, the cause and progression of CHD in NET patients is poorly understood and researched. Preliminary results show a lack of study designs investigating mechanistic changes underlying CHD before or after treatment. Although NETs have been reported in domestic dogs, cats, horses, and cows, the potential of animal NET research is unrecognized. A One Health perspective should be explored as it is well-suited to address the interconnection between carcinogenic processes in the environment that contribute to human and animal NETs.

ONE HEALTH

Presenter's Name: Tang, Celina
Additional Authors: Darling MR
Abstract Title: One Health Evaluation of Oral Cancer and S100A7 as a Predictor of Malignant Transformation from Oral Potentially Malignant Lesions (OPMLs)

Abstract: With oral cancers, driven by various environmental and socio-economic risk factors, impacting the quality of life of humans and animals alike, prevention and early diagnosis is crucial for effective treatment. Histopathological examination and dysplasia grading has historically been the standard for predicting the risk of oral potentially malignant lesions (OPMLs) undergoing malignant transformation in humans, but it is vital to seek more diagnostic and prognostic tools. Straticyte is one such tool that produces a percent probability risk for malignant transformation of OPMLs and classifies them as high, medium, or low risk by measuring expression of the protein, S100A7. Overexpression of S100A7, in addition to Mcm2, and Ki67, have been found in many cancers and premalignant tumours. It is hypothesized that Straticyte is a more accurate and reliable predictor of malignant transformation of OPMLs than the two- and three-tier dysplasia grading systems. To test this, tissue specimens of OPMLs and healthy controls was stained for the aforementioned proteins. The image analysis program, QuPath, was used to measure and compare the area and stain percentage between malignant and healthy tissues. The percent probability risk and risk classifications that Straticyte produced will be compared to QuPath's outputs and the two- and three-tier dysplasia grades. S100A7 is expected to be overexpressed in OPMLs, and Straticyte is expected to be a more accurate and reliable predicator of malignant transformation of OPMLs than the two- and three-tier dysplasia grading systems. Subsequently, a literature review investigating the role of environmental health and socio-economic risk factors for oral cancer in humans and dogs will be conducted. Key stakeholders involved in oral cancer will be identified, including, but not limited to, tobacco companies and the government. The literature review, protein biomarker research, and mapping of key stakeholders will enable a comprehensive investigation of oral cancer to improve prevention, diagnosis, and treatment of oral cancers in humans and dogs.

Presenter's Name: Abo-Amer, Yamen
Additional Authors: Charkrabarti S, Feng B
Abstract Title: The Role of miR-9 in the Protection against Diabetic Renal Fibrosis

Abstract:
Introduction: Diabetic kidney disease (DKD), a major complication of diabetes, is increasingly becoming more of a problem across the globe due to its potential to progress into end-stage renal disease. Previous studies have shown that kidney fibrosis, a major hallmark of DKD, is driven by the TGF- β pathway via the binding of TGF- β1 to TGFBR2. Prior studies have also shown the effectiveness of miRNA-9 (miR-9), an abundant microRNA found in humans, in inhibiting TGFBR2 mRNA and downregulating its expression. The purpose of this study is to assess the renal fibrosis caused by DKD and determine the protective potential of overexpressed miR-9 on diabetic renal fibrosis.

Hypothesis: We hypothesize that the overexpression of miR-9 will reduce TGFBR2 expression levels and improve kidney fibrosis in diabetic nephropathy.

Methods: Transgenic (TG) B6 mice were generated with EC-specific overexpression of miR-9 via a tie-2 promoter. Wildtype (WT) and TG mice were then separated into 2 groups: one group received 5 doses of STZ on consecutive days to induce type I diabetes mellitus. The mice were euthanized after 8 weeks and their kidney tissues were harvested. All 4 groups will undergo qRT-PCR and ELISA to quantify various fibrotic markers, and TGF- β pathway mRNA and protein levels. Furthermore, fibrotic histological features in all the kidneys using Masson's Trichrome will be examined and compared.

Results: It is expected that there will be a significant increase in fibrotic markers and the TGF- β pathway mRNA levels in the WT diabetic group compared to the WT nondiabetic group. It is also expected that there will be a significant increase in fibrotic markers and the TGF- β pathway mRNA levels in the TG non-diabetic group compared to the WG non-diabetic groups. Histological features reflective of nephropathy such as present glomerular fibrosis are expected to be present in only the WT diabetic kidney group.

Discussion: The results of this study will shed further light on the therapeutic potential of microRNAs and pave the way for future research into their usage. This study also has the potential to contribute to the discovery of a new approach in preventing diabetic kidney fibrosis.

Presenter's Name: Bajwa, Gurleen
Additional Authors: Ni R, Peng T
Abstract Title: Forced Overexpression of CHOP Induces Myocardial Necrosis in a Mouse Model

Abstract:
Introduction: Cardiovascular disease (CVD) is the leading cause of mortality worldwide. When left untreated, CVD leads to terminal heart failure due to excessive cardiac cell death. To this day, treatment options remain limited and heavily rely on prevention strategies. A novel treatment approach is to therapeutically inhibit cardiac cell death. To do this, we must first identify a potential molecular target. C/EBP homologous protein (CHOP) is a transcription factor that regulates apoptosis during periods of endoplasmic reticulum (ER) stress. Previous studies verify its role in cardiac apoptosis; however, CHOP function in necrosis is widely unknown. We hypothesized that the overexpression of CHOP can sufficiently induce cardiomyocyte necrosis in an in vivo mouse model.

Methods: To test this hypothesis, we intravenously injected male BALB/c mice with CHOP-containing plasmids. We then performed a chemiluminescent western blot after euthanasia and heart extraction to confirm that there was CHOP overexpression in cardiac tissue. Fluorescence microscopy was performed to Evans blue dye and Hoechst 33342 stain. The total number of physiologically normal and necrotic nuclei was quantified using Image-Pro Plus. Data were analyzed using a two-tailed Student's t-test where significance was determined when $P<0.05$.

Results: Our results demonstrate that CHOP overexpression induces cardiac injury as demonstrated through elevated troponin in mouse serum. We also show that the up-regulation of CHOP increases the total number of necrotic cells in mouse cardiac tissue.

Discussion: These findings show that CHOP plays a role in mediating cardiac necrosis in a three-dimensional biological system. Therefore, CHOP may be a potential therapeutic target for CVD treatment. More specifically, its inhibition may repress cardiomyocyte death in patients with chronic heart failure.

PATHOLOGY

Presenter's Name: Canatan, Aysegul
Additional Authors: Samuels TN
Abstract Title: Epigenetic Biomarkers of Diabetic Cardiomyopathy: A Translational Scoping Review

Abstract:
Objectives: This scoping review aims to identify and summarize epigenetic biomarkers that are associated with diabetic cardiomyopathy (DCM) such as lncRNA, miRNA, cirRNA, acetylation, and methylation. Our scoping review will take a translational approach which will include both animal and human models to compare and contrast potential epigenetic biomarkers. Given that the onset and early progression of DCM is asymptomatic, diagnosis often occurs in later stages. By identifying DCM-specific epigenetic biomarkers, earlier detection of DCM can be facilitated, and RNA-based therapeutics can be developed to improve prognosis of diabetes mellitus (DM).

Methods: A literature search was conducted on the following databases: MEDLINE, EMBASE, and Scopus. Our search includes only primary articles that mention DCM-specific epigenetic biomarkers. All experimental designs that meet our inclusion criteria will be split into three groups: in vivo, in vitro, and human.

Results: Our search provided 188 articles after duplicates were excluded and abstract screening was done. So far, 53 primary articles have met the study criteria. Our preliminary results show that there are currently 32 miRNA, 11 lncRNA, 10 acetylation, 4 cirRNA, and 6 methylation-mentioning articles. In addition, 39 in vivo, 27 in vitro, and 7 human experimental designs were identified. However, we anticipate that these numbers will change once full-text screening and data extraction is completed.

Conclusions: The evidence from this scoping review concludes that certain epigenetic biomarkers are associated with DCM. Therefore, these epigenetic biomarkers can be used in early DCM detection and as potential targets for future RNA-based therapeutics to improve prognosis of DM.

Presenter's Name: Dhupar, Narisa
Additional Authors: Dhupar N, Khan ZA
Abstract Title: The impact on CXCL12/CXCR4 signaling in target organ dysfunction in diabetes

Abstract:
Introduction: Diabetes is a metabolic disease characterized by hyperglycemia. The secondary complications associated with diabetes are of major concern for diabetics, and they usually stem from the effects of sustained hyperglycemia on the vasculature of select organs such as the heart and kidney. Normally, in hypoxic conditions leading to tissue injury or stress, the CXCL12 chemokine's association with its receptor, CXCR4, is involved in the homing of stem and progenitor cells in the bone marrow and controls their mobilization into peripheral blood and tissues. Thus, reduced CXCL12 expression in organs damaged by diabetes could account for the lack of CXCR4-positive stem and progenitor cell migration to these tissues to be able to assist in their repair. Here, we aim to investigate the expression and activity of the CXCL12/CXCR4 axis, as well as hypoxia inducible factor 1 subunit alpha (Hif1a), in diabetic complications. We hypothesize that CXCL12 expression is reduced in the tissues that are damaged by diabetes compared to healthy tissues, resulting in the disruption of the CXCL12/CXCR4 signaling axis in the damaged tissues. Also, Hif1a expression will be increased in the tissues that are damaged by diabetes compared to healthy tissues.

Methods: Heart and kidney tissue were extracted from both control mice and streptozotocin-induced diabetic mice. RNA was then isolated from these tissues in order to perform qPCR to determine the expression and regulation of CXCL12, CXCR4, and Hif1a. Immunostaining and imaging will also be done to visualize CXCL12-expressing cells in these diabetic and control tissues.

Results: Surprisingly, our preliminary data showed that CXCL12, CXCR4, and Hif1a all had increased expression in diabetic kidney tissues compared to control kidneys, while CXCR4 and Hif1a also showed increased expression in diabetic heart tissues compared to control hearts. CXCL12 showed a slight increase of expression in diabetic heart tissues compared to control heats. Upon staining and imaging, we expect to see more CXCL12 and CXCR4 from CXCL12-expressing cells in the tissues from diabetic mice.

Discussion: The results from this study will have therapeutic value for diabetic patients. Increasing CXCL12 levels in dysfunctional organs caused by diabetes may be able to increase the mobilization of CXCR4-positive stem and progenitor cells to these organs to potentially repair them and mitigate the negative consequences associated with diabetes.

PATHOLOGY

Presenter's Name: Gholami, Hasti
Additional Authors: Jawhri MA, Hong MM, Morin A, Cameron L, Castellani CA
Abstract Title: The Effect of Asthma and Cardiovascular Drug Exposures on Mitochondrial Function

Abstract:
Introduction: The mitochondrion is a membrane-bound organelle that plays a crucial role in adenosine triphosphate (ATP) production for cellular energy. Oxidative capacity changes in mitochondria can lead to mitochondrial dysfunction and cause insufficient cellular energy production, decreasing ATP production and increasing reactive oxygen species (ROS) production. Mitochondrial DNA copy number (mtDNA-CN) is a biomarker for mitochondrial function and decreases in mtDNA-CN have been associated with chronic inflammatory diseases, such as asthma and cardiovascular disease. Modifications to mitochondrial function can be mediated by chemical therapeutics, though research on this topic is limited. My study aims to examine the mechanisms mediating the effect of asthma and cardiovascular disease drugs on mitochondrial function.

Methods: This project utilizes CCRF-CEM and THP1 cell lines to assess the effects of asthma and cardiovascular drugs in vitro, respectively. The study assesses the effect of the drugs dexamethasone and formoterol for asthma, and simvastatin and ezetimibe for cardiovascular disease. DNA was extracted from each treatment assay and mtDNA-CN was quantified using qPCR. Citrate synthase (CS) and lactate dehydrogenase (LDH) assays were used to assess mitochondrial function.

Results: A decrease in mtDNA-CN and mitochondrial function was detected in the CRM-CEM cell line upon exposure to increasing dexamethasone concentrations (0.1-0.9μM). An increase in mtDNA-CN was detected in the CRM-CEM cell line upon exposure to increasing formoterol concentrations (0.01-0.05Mm). The CS and LDH assays are expected to show an increase in mitochondrial function in formoterol treated CCRM-CEM cells. An increase in mtDNA-CN and mitochondrial function following simvastatin, and ezetimibe treatment on THP1cells is expected in standalone. A net increase in mtDNA-CN and mitochondrial function is expected when both dexamethasone and formoterol, and simvastatin and ezetimibe are given in combination.

Discussion: The results of this study reveal the effects of asthma and cardiovascular disease drugs on mitochondrial function and will contribute to refining treatment plans for patients to optimize patient health. Future studies should look at the mechanistic sex differences in mitochondrial function within chronic inflammatory settings by examining the effect of estrogen in addition to these asthma and cardiovascular drugs on mitochondrial function.

Presenter's Name: Jasani, Arish
Additional Authors: Rutledge A, Stevic I, Bhayana V
Abstract Title: Drug interference studies on clinical chemistry tests at London Health Sciences Centre

Abstract:
Introduction: Clinical chemistry laboratory tests work on many different principles, including spectrophotometric and colorimetric designs. During analysis, there is potential for assay interference if the patient's biological specimen contains anything endogenous or exogenous (such as a drug) that can absorb at the assay wavelengths. These drugs tend to absorb light in a similar spectrum to the absorption spectrum of these laboratory tests and may lead to erroneous diagnosis of diseases. Metronidazole and methylene blue absorbing wavelengths around 340 nm and 550 – 700 nm respectively have been previously seen to interfere with some chemical tests from different manufacturers using a similar absorption spectrum.

Methods: For each potential interfering drug, assays that used a wavelength around the absorbance peak of the drug were selected to test for interference. A pool of biological specimens from different patients containing the analyte of interest at desired (clinically relevant) concentration ranges is made to be tested by a clinical chemistry test. The pooled analyte sample is aliquoted and spiked with a higher and a lower concentration of the selected potentially interfering drug separately, while same volume of water is used to spike the control aliquots. After performing these tests, the difference in concentration between experimental and control are reviewed against the total allowable error to identify if interference has occurred.

Results: There was no interference observed with Roche chemical assays when metronidazole was present in the samples. With methylene blue, we observed interference with the urine amphetamine screen and urinalysis tests. Methylene blue did not interfere with any of the plasma/serum assays tested, except for the lipemic index.

Discussion: This study provides reassurance that metronidazole is not interfering with the chemistry assays used at our site. For methylene blue, now that affected tests have been identified, we will investigate measures to limit release of erroneous results in patients treated with this medication. Our findings will also be beneficial for other laboratories using chemical tests from the same manufacturer.

PATHOLOGY

Presenter's Name: Kim, Matthew
Additional Authors: Kiser P
Abstract Title: Impact of life long western diet consumption on late-term placentae

Abstract:
Introduction: With the increasing rates of high fat diet consumption in North America and around the world, it is important to understand how these diets can potentially affect the proper placental formation and function. The placental area that is known to be directly affected by high fat consumption is the labyrinth area which is involved in nutrient and gas exchange between the fetus and the mother. Previous lab findings from Takashi in 2018 had found a decrease in the placental labyrinth area from maternal guinea pigs at day 40 gestation due to the high fat western diet. Our lab will be looking at the labyrinth area from western diet guinea pigs at day 60 to see if this reduction continued or remained the same. We hypothesize that the western diet will continuously cause a reduction in the placental labyrinth area from day 40 to 60, and that phenotypic changes on fetal guinea pigs from western diet mothers compared to control are expected to be seen.

Methods: Immunofluorescence staining will be done on the placental labyrinth areas at day 60 gestation from maternal guinea pigs on western and control diet. Two primary antibodies, anti-vimentin and anti-cytokeratin will be used along with two fluorescent secondary antibodies, AF 647 and AF 568, to visualize the change in the placental labyrinth area. To track any phenotypic changes that may occur on the fetal guinea pigs, every offspring from mothers on western and control diets will be measured in terms of their weight and length, which will be recorded on an excel spreadsheet.

Results: Our results showed an increase in fetal weight from western diet mothers possibly due to placental villi hypermaturation (PVH) that can occur within a hypoxic placenta. We also identified an increase in placental weight in guinea pigs on western diet contributed by necrotic events of the placenta such as fibrin deposition and edema. An expected outcome still unknown is whether the western diet continues to decrease maternal placental labyrinth area from day 40 to 60 gestation.

Discussion: By inferencing results found so far, we expect that the high fat western diet will continue to have negative effects on the placental tissue by causing hypoxia and necrosis. This placental degeneration also contributes to PVH, directly affecting guinea pig development. Once the change in labyrinth area have been analyzed, our lab can compare the placental effects of the western diet from day 40 to 60 gestation.

Presenter's Name: Lad, Mrinal
Additional Authors: Hsia C, Chin-Yee B, Hedley B, Chin-Yee I
Abstract Title: A survey of current clinical practice in the management of Monoclonal B-cell Lymphocytosis (MBL): Are we over-investigating?

Abstract:
Introduction: Monoclonal B-cell lymphocytosis (MBL) is an indolent, hematologic condition presumed to precede all cases of chronic lymphocytic leukemia (CLL). MBL is defined by an excessive monoclonal B-cell population in the blood, but most MBL patients remain asymptomatic and otherwise healthy. Although MBL is common in older adults, only the high-count MBL subtype is of clinical significance because it can progress to CLL at a rate of 1-2% annually. Recently, studies have provided favourable evidence towards using the CLL-International Prognostic Index (CLL-IPI) to risk-stratify all patients with MBL. However, this risk-stratification process requires costly molecular testing to conduct such as flow cytometry so it is important to consider when the appropriate time is to administer testing and what is the right test to administer. The purpose of the current study is to determine if current clinical practices are over-investigating this indolent condition.

Methods: A retrospective chart review will be conducted using health records from the Hematology Clinic at London Health Sciences Centre. Clinical variables and laboratory parameters will be recorded and compared between CLL and MBL patients to understand patient characteristics of those diagnosed with MBL and to compare rates of testing between the two patient populations.

Results: It is expected that CLL-IPI scores will change clinical management between MBL patients as those placed in a higher risk group will receive significantly more testing compared to patients placed in a lower risk group. Similarly, it is expected MBL patients will have similar rates of testing to early-stage CLL patients.

Discussion: The results of this novel descriptive study will provide an accurate description of the standard of care for patients with MBL and may contribute to reconsiderations in testing procedures. Consequently, it will help improve healthcare quality and resource utilization for patients with MBL.

PATHOLOGY

Presenter's Name: Lau, Ethan
Additional Authors: Fung M, Armstrong JJ, Liu H, Hutnik CML
Abstract Title: Investigating Netarsudil's inhibition of myofibroblast activity using a novel microfluidics model

Abstract:
Introduction: Glaucoma is a progressive ocular disease that often requires surgical management. Whereas the last decade has seen considerable advancement in minimally invasive glaucoma surgical techniques, there has been virtually no innovation in wound healing modulation and surgical scar prevention. Fibroblasts are one of the key elements of the scarring response and transform into myofibroblasts through TGF-β1 signalling triggered by surgery. The main cause of surgical failure is from post-surgical scarring that reduces permeability of aqueous humor flow of the sustained drainage tract, leading to an increase in intra-ocular pressure (IOP). A pathway downstream of TGF-β1 known as Rho-associated protein kinase (ROCK), may contribute to fibroblast trans-differentiation to myofibroblasts.

Methods: Human Tenon's capsule fibroblasts (HTCFs) were isolated from glaucoma patients. HTCFs were established, propagated, and maintained in culture. MTT and LDH assays were used to evaluate the cell metabolic activity and necrosis of HTCFs with the ROCK inhibitor, Netarsudil. Western blot was used to quantify alpha-SMA, which is an indication of fibroblast trans-differentiation to myofibroblasts. The morphological effects of Netarsudil on HTCFs were assessed by immunofluorescence microscopy.

Results: For the MTT assay, the TGF-β1 group induced an increase in cellular metabolic activity in HTCFs when compared to the TGF-β1 + Netarsudil group. The change in absorbance in LDH assay was similar across all groups. Cells treated with TGF-β1 showed an increase in signal intensity of alpha-SMA compared to vehicle control by Western blot, and in the presence of Netarsudil, the signal intensity of alpha-SMA decreased. Similarly, using confocal microscopy, the fluorescent intensity of alpha-SMA was lower in the TGF-β1 + Netarsudil group compared to TGF-β1 alone.

Discussion: The development of novel strategies to modulate the scarring response may improve surgical success rates, leading to better patient outcomes. Using Netarsudil in a novel way to prevent post-operative fibrosis can reduce the extra time and cost associated with more surgeries caused by scarring after the initial surgery. Since Netarsudil is already approved in the USA, it has the potential to bring benefits to patients much sooner.

Presenter's Name: Lee, Connar
Additional Authors: Lu H, Zhang ZX
Abstract Title: The Role of RIPK3 and GLUD1 during TLR3-mediated Necroptosis in Mouse Microvascular Endothelial Cells

Abstract: Necroptosis is defined as a type of regulated cell death triggered by disruptions of homeostasis, and critically relies on MLKL, RIPK3, and sometimes RIPK1. TLR3 signaling has been shown to induce downstream cell death mechanisms such as necroptosis, however, this pathway has not been completely defined. Interestingly, TLR3-mediated necroptosis is known to cause mitochondrial dysfunction. During necroptosis, mitochondria can enhance the formation of necrosomes through reactive oxygen species (ROS) formation. GLUD1 is a mitochondrial enzyme which previous studies have shown that RIPK3 can activate under TNFR1-mediated necroptosis. However, this relationship has not been studied for TLR3-mediated necroptosis. Therefore, it is hypothesized that RIPK3 promotes mitochondrial dysfunction by activating GLUD1 during TLR3-mediated necroptosis. Western Blot analysis will be used to quantify the expression of RIPK3 and GLUD1 in relation to TLR3 stimulation. Cell death assays will be utilized to determine the treatment effect on cell viability. Mitochondrial morphology will be tracked through MitoTracker and JC-1 dyes. Mitochondrial function will be tracked through ATP determination and MitoProbe transition kits. Increased expression of RIPK3 and GLUD1 is expected in relation to TLR3 stimulation. Increased cellular survival during TLR3-mediated necroptosis is expected with GLUD1 inhibition. Premature graft failure emerged as the greatest challenge in transplantation—resulting from diverse cell death mechanisms that promote inflammation, increases organ dysfunction, and blocks immune tolerance. Clinical treatment strategies to control cell death and inflammation have not yet been focussed on. Through this study, fundamental pathways of necroptosis are researched and can provide potential clinical targets to limit inflammation and prolong graft survival.

PATHOLOGY

Presenter's Name: Lewis, Natalie
Additional Authors: Greasley A, Abu Omar AA, Zheng X
Abstract Title: The Role of Circular RNA in Colon Cancer Cells in Response to Chemotherapeutics

Abstract:
Introduction: Colon Cancer is a disease with both high incidence and mortality rates. It is commonly treated with the chemotherapeutic drug 5-Fluorouracil (5-FU), among other interventions. However, the development of resistance to 5-FU has emerged as a major problem interfering with the ability to successfully treat this disease. Circular RNAs (circRNAs), a type of non-coding RNA, have been increasingly studied in the context of cancers, and some have been shown to play a role in drug resistance. CircRNA PNN (circPNN), has previously been shown to be upregulated in colon cancer patients, however its involvement in chemotherapy-induced cell death remains unknown. This study aims to demonstrate the effect of circPNN on the response of colon cancer cells to chemotherapeutic treatment with 5-FU.

Methods: To understand how circPNN expression changes in response to chemotherapy, the expression profile of circPNN is being determined using quantitative real-time PCR (q-PCR). We treated HT29 colon cancer cells with increasing doses of 5FU, and circPNN expression will be detected at different timepoints after treatment. To explore the effect of circPNN on the response of colon cancer cells to treatment with 5-FU, we designed small interfering RNAs (siRNAs) to knockdown circPNN. We transfected HT29 cells with the siRNAs and cell death upon treatment with 5-FU will be compared between normal colon cancer cells and circPNN-knockdown cells. Cell death will be detected using an MTT assay, an LDH assay, and dynamically with an Incucyte system.

Results: Preliminary results indicate that circPNN is upregulated by 5-FU. We expect that knocking down this circRNA will enhance the sensitivity of colon cancer cells to this chemotherapeutic agent. Increased cell death in response to treatment is expected to be seen in the colon cancer cells that have been transfected with the siRNA designed to knock down circPNN.

Discussion: This study will elucidate the impact of circPNN on the sensitivity of colon cancer cells to the anti-cancer drug 5-FU. It may provide a foundation for future investigations of methods to improve the chemotherapeutic treatment of colon cancer. Additionally, the results from this study will add to the limited knowledge of the functions of circRNA in drug resistance.

PATHOLOGY

Presenter's Name: Lin, Sherman
Additional Authors: Samsoondar JP, Keow S, Pokharel BB, Tan D, Martinez-Acevedo J, Pham M, Wu NJ, Misra T, Lam VHK, Sansano I, Cecchini MJ
Abstract Title: Digital Quantification of Tumor Cellularity as a Novel Prognostic Feature in Lung Adenocarcinoma

Abstract:
Introduction: Lung cancer is staged based on the size of the tumor and involvement of other structures. This staging may be a surrogate measure for the number of cells present in the tumor. The recently updated grading system for lung adenocarcinoma assesses the presence of high risk architectural patterns, which tend to have more complex cellular growth. Counting individual tumor cells is impractical for a pathologist using a conventional light microscope. Image analysis tools applied to digital slides can be utilized to automate the quantification of lung adenocarcinoma. We hypothesize that tumor cellularity can be used as a novel prognostic tool in lung cancer that integrates quantification of high risk architectural patterns.

Methods: Digital slides (n=102) from the Cancer Genome Atlas (TCGA) lung adenocarcinoma (LUAD) dataset were obtained and analyzed in QuPath. Representative areas of tumor were annotated and reviewed by a thoracic pathologist, the annotations were used as training data for a random trees based object classifier that utilized detected cell features to identify and quantify tumor cells across entire slides. This was normalized with the surface area of the tumor present on the slide to provide a measure of tumor density. The overall total cellularity was calculated by combining the size of the grossly measured tumor with the tumor density. Major histologic patterns in representative panels were determined by a thoracic pathologist and were compared with the tumor density of the tile. The overall and progression free survival was compared between groups of high and low tumor cellularity.

Results: High-grade histologic patterns had a significantly greater tumor density compared with other patterns of lung adenocarcinoma. A trend between survival and cellularity was identified and a cut-off of 5.5 x 1010 cells was found to predict outcome. Cases with a low cellularity had an improved progression free survival (HR 0.21; 95% CI 0.096-0.47) and overall survival (HR 0.25; 95% CI 0.088-0.7) compared with cases that had higher cellularity.

Discussion: Tumor cellularity represents a novel prognostic tool in lung cancer that takes into account both the size and composition of the tumor. Use of advanced image analysis tools allows for the automation of this task in a simplified and efficient manner. Future work will seek to validate these findings in additional larger datasets to refine the classification of tumors by cellularity.

Presenter's Name: Lui, Ryan
Additional Authors: Roes M, Dick FA
Abstract Title: The Role of TBX18 on Resistance Development to Enzalutamide in Prostate Cancer

Abstract: Neuroendocrine prostate cancer (NEPC) is a lethal subtype of prostate cancer that is characterized by low or absent androgen receptor (AR) expression, independence of AR signalling, and gain of a neuroendocrine phenotype. Transdifferentiation from prostate adenocarcinoma to NEPC confers resistance towards next-generation androgen receptor therapies such as enzalutamide (EZ). Our lab has demonstrated that RB-p53 deficiency increases the propensity for LNCaP cells to acquire resistance to EZ, indicating underlying genetic mechanisms that misregulate stemness and cell differentiation pathways. Subsequently, a CRISPR knockout screen was performed on LNCaP cells to determine if the deletion of other genes would also confer EZ resistance. Amongst other genes, TBX18, a transcriptional repressor, was overrepresented. This study aims to investigate the role of TBX18 on EZ resistance in prostate cancer and to elucidate the mechanism of transdifferentiation to NEPC. We hypothesize that a gene knockout of the transcription factor, TBX18, will result in an increased propensity to acquire resistance to EZ in prostate cancer. We performed a CRISPR-Cas9 knockout of TBX18 in a LNCaP cell line and have achieved a population of LNCaP cells with decreased expression of TBX18, confirmed by western blot. Presently, we are examining immediate EZ resistance using an alamarBlue cell viability assay and developed EZ resistance over time through a colony forming assay. The molecular basis of EZ resistance will be investigated by comparing gene expression changes between TBX18 knockout cells and control cells. qPCR will be used to detect expression of known genes that regulate stem cell plasticity such as SOX2 and genes that are known biomarkers of neuroendocrine tumours such as SYP, CHGA, and NSE. Our acquired population of cells likely contains a mixture of partial and full TBX18 knockout cells. Thus, we also seek to isolate and clonally expand a population of cells with full knockout of TBX18 through a limiting dilution assay, which will give greater validity to our findings. Ultimately, the development of resistance to AR inhibitors is a major cause of morbidity and mortality in prostate cancer patients. This study may lead to the discovery of a new biomarker to detect NEPC and identify target pathways for the development of novel therapeutics.

Presenter's Name: Quadri, Ahmed
Additional Authors: Quadri AH, McClennan A, Hoffman LA
Abstract Title: Regional Differences in the Morphology of the Gastrocnemius Muscle of Duchenne Muscular Dystrophic Mice

Abstract:
Introduction: Duchenne Muscular Dystrophy (DMD) is a X-linked recessive disorder that is characterized by progressive muscle weakness and degeneration. It occurs in individuals that have a mutation in the dystrophin gene which results in a non-functional dystrophin protein being expressed in myocytes. While researchers are focused on the development of dystrophin-gene replacement therapies to treat DMD, few studies aim at correcting the damaged microenvironment of DMD-affected muscles. To find novel therapies that reconstruct the microenvironment of DMD-affected muscles and fosters muscle repair and regeneration, a better understanding of the microenvironment of myocytes was needed. In this study, we attempted to determine whether regional differences in the morphology of the upper, middle, and lower regions of the gastrocnemius muscle of mdx/utrn+/- mice exists.

Methods: Sections of the upper, middle, and lower regions of the gastrocnemius muscle of female mdx/utrn+/- mice and healthy controls were stained using Hematoxylin and Eosin (H&E). The entire upper, middle, and lower regions of the gastrocnemius muscle of mdx/utrn+/- mice and healthy controls was then imaged. ImageJ was used to map out and determine the amount of necrotic, regenerative, and healthy muscle tissue present in different regions of the gastrocnemius muscle. Necrotic, regenerative, and healthy muscle regions were found by looking at the position of the nuclei of each myocyte. A two-tailed T-test was then performed to investigate whether regional differences in the morphology of the gastrocnemius muscle between the mdx/utrn+/- mice and healthy controls existed.

Results: Currently, no data has been collected. Issues with H&E staining and sectioning of tissues have contributed to the delays in the study. However, these issues have since been resolved and the use of the whole-imaging scanner to image the slides have streamlined the imaging stage of this study. Right now, we are in the data-collection stage.

Discussion: By investigating the regional differences in the morphology of the gastrocnemius of DMD mice and healthy controls, it has given us a better view of the morphological changes that occurs to the entire muscle of DMD patients, as well as a broader understanding of the defects found in the microenvironment of the myocytes. The will help in finding novel strategies that correct for these defects in future studies.

PATHOLOGY

Presenter's Name: Rehman, Iqra
Additional Authors: Rehman I, Khan ZA
Abstract Title: PPARG Expression and Function in Non-Adipose Tissue

Abstract:
Introduction: Patients with type 1 and type 2 diabetes have demonstrated changes within the bone marrow caused by enhanced adipogenesis; as a result the bone marrow is housed with fewer stem cells and an increased number of adipocytes. The changes exhibited provide a novel mechanism for the cause of impaired endogenous repair that is seen in diabetes. Peroxisome proliferator receptor gamma (PPARG), an essential transcription factor in adipogenesis, is the principal target of inhibition in efforts to restore stem cell levels in the bone marrow. As demonstrated in past studies, deletion of PPARG in adipose tissue and total body deletion result in different phenotypes. This suggests that identifying PPARG and target gene expression within non-adipose tissues will allow one to predict the consequences when PPARG is inhibited. In the present study, we aimed to determine which of the following tissues: heart, kidney, lung, and retina expresses PPARG. We hypothesized PPARG expression will be widespread and different tissues types will exhibit variation in target gene expression.

Methods: To test this hypothesis, we isolated total RNA from kidney, heart, lung, and retina tissue of C57BL/6 mice. RNA retrieved was then subjected to cDNA synthesis followed by qPCR; we used qPCR to qualitatively determine the expression of Pparg and target genes Adipoq and Tmem143. Finally, tissue sections of the heart, kidney, lung, and retina of C57BL/6 mice underwent antigen retrieval staining followed by fluorescent imaging.

Results: Our results demonstrate that PPARG expression is positive in the kidney and lung, but not in the heart or retina. The expression of the target genes, TMEM143 and ADIPOQ, is positive in the heart, kidney, and lung. Tissues have been stained and fluorescent imaging is currently ongoing.

Discussion: These results demonstrate PPARG expression is widespread in non-adipose tissues, and this has implications in predicting the effects of PPARG inhibition. Determining how adipogenesis can be safely inhibited may pave the way for new possibilities of reestablishing endogenous repair and disease management in diabetes.

PATHOLOGY

Presenter's Name: Santaguida, Vincent
Additional Authors: Abdelbaset AE, Rieder M
Abstract Title: Optimization of Cortisol Extraction from Human Hair

Abstract: Cortisol is a glucocorticoid hormone that plays an important role in both normal physiology and numerous diseases. Cortisol has traditionally been measured using blood, urine and saliva samples, however due to the daily fluctuations in cortisol levels, these measurements tend to be inconsistent depending on when the samples are obtained. As such, the measurement of cortisol from human hair has been used clinically due to its ability to obtain consistent and retrospective measurements.

However, current methods for extracting cortisol from hair only extract about 50% of the total cortisol in the hair, and these partial extractions may reduce the accuracy of this method. As such, this study aims to optimize the extraction procedure for hair cortisol measurement and analyze the effects of various changes to the extraction process. First, we will compare the efficiency of milling and mincing the hair. Then, we will compare the effect of doubling the time of the extraction, repeating the extraction twice, and a 4-step extraction using methanol and acetone, to the standard procedure. We do not expect there to be a difference in the amount of cortisol extracted when milling and mincing the hair. However, we expect longer and double extractions to extract more cortisol than the standard procedure, and the 4-step methanol-acetone extraction to extract the most cortisol. The ability to extract all, or close to all of the cortisol in hair would allow for the generation of more precise values and standard reference ranges for hair cortisol. Ultimately, this could be beneficial for the diagnosis and treatment of many cortisol related diseases.

Presenter's Name: Skeba, Danielle
Additional Authors: Asfaha S, Shin AE, Cecchini MJ
Abstract Title: Digital Characterization of Bowel Damage in Chemical- and Bacterial-Induced Experimental Colitis in Mice

Abstract: Crohn's disease and ulcerative colitis comprise inflammatory bowel disease (IBD), an idiopathic disease with genetic and environmental influences. IBD is becoming increasingly prevalent in the global population. To facilitate mechanistic studies of IBD, the colitis phenotype is often induced in mice using orally administered dextran sodium sulfate (DSS). Other models of colitis are achieved with the use of oxazolone, TNBS, Citrobacter rodentium and doxorubicin. Currently, researchers working with histologic colitis samples from these models manually identify distinguishable features of each model and quantify areas of disease activity. Manual analysis introduces inter- and intra-observer variability, as well as considerable time invested. More powerful, reliable, and refined analytical capability may be achieved using digital pathology tools. This study utilizes QuPath, an open-source whole-slide image analysis program, in conjunction with CytoMAP, a built-in MatLab tool for tissue spatial analysis, to compare and characterize the different models of colitis in mice. This method, leveraging both the cell detection and classification features of QuPath, as well as the streamlined clustering analysis pipeline of CytoMap, will extract features from each of the models of colitis.
The findings of this study may contribute to the creation of a more efficient and reliable method for identifying colitis in mice. In addition, this study will contribute to research into the emerging field of digital pathology as a tool in research and potentially the clinical assessment of IBD.

Presenter's Name: Suthakaran, Abitha
Additional Authors: Chin-Yee B, Chin-Yee I, Hsia C
Abstract Title: T Cell Clonality -- Are we identifying pathology or incidental clones? A quality improvement project

Abstract: T cell clonality testing is used to identify clonal populations of T cells in patients by looking at gene rearrangements in the T cell receptor (TCR). While T cell clonality is a clinical feature of many lymphoproliferative disorders such as cutaneous T cell lymphoma (CTCL) and T cell large granular lymphocytic leukemia (T-LGL), clonal T cell populations have also been identified in healthy elderly individuals. Therefore, clonality does not imply malignancy or pathology. This raises the question of whether T cell clonality assessment is being overutilized to investigate incidental clonal populations, and whether these incidental clonal populations are being misattributed to disease. The goal of this project is to survey the use of T cell clonality testing to figure out the impact on patient management and to improve test utilization. We hypothesize that a large population of patients undergoing T cell clonality assessment are not diagnosed with a lymphoproliferative disorder, as well as that a significant portion of T cell clones identified through testing are incidental or of undetermined significance. A retrospective descriptive study will be conducted at London Health Sciences Centre using records of patients who underwent T cell clonality testing between January 1st, 2011 and September 30th, 2021 and were over the age of 18 at the time of testing. Descriptive statistics will be used to analyze data collected on patients' demographics, medical history, laboratory tests (e.g. hematology), and post-test management to characterize the patient population for T cell clonality testing and devise an algorithm to guide physicians on when to order this test. We expect to observe significant clinical differences between patients with incidental clones and those with clones associated with lymphoproliferative disease. We also expect to determine that a large portion of tested patients have incidental clonal populations. These findings will help improve clinic resource management as well as patient outcomes by preventing unnecessary testing.

PATHOLOGY

Presenter's Name: Teplitsky, Jack
Additional Authors: Vinokurtseva A, Liu H, Hutnik C
Abstract Title: Modulating wound healing in glaucoma surgery: ALK5 inhibition to counteract subconjunctival scarring

Abstract: Excessive ocular scarring is a common postoperative complication of glaucoma surgery. Currently, the chemotherapeutic agent mitomycin C (MMC) is the gold standard used to manage resulting fibrosis despite its cytotoxicity and unreliable efficacy. Our lab has investigated the drug SB-431542 (SB) as a safer alternative to MMC for reducing ocular fibrosis. Previously, we demonstrated SB's ability to reduce collagen contraction in 3D in vitro tissue model of human Tenon's capsule fibroblasts (HTCFs). To further understand the mechanism underlying anti-fibrotic effects of SB, in the current study, we investigate SB's effects on cell metabolism, cytotoxicity and expression of pro-fibrotic proteins MMP9 and ACTA2 in myofibroblasts. We hypothesize SB will exhibit significantly lower cytotoxicity in HTCFs than MMC while displaying comparable anti-fibrotic efficacy.

HTCFs derived from patients undergoing trabeculectomy were pre-treated with SB (20 nM) for 5, 10 or 20 min or MMC (0.2 mg/mL) for 1, 2 or 4 min followed by incubation with TGFβ (2ng/ml) for 48 hrs. Myofibroblasts underwent MTT and LDH assays to characterize cell metabolic rate and cytotoxicity. Total protein was extracted from treated myofibroblasts and protein expression of ACTA2 and MMP9 was measured using immunoblotting.

TGFβ treatment alone induced an increase in ACTA2 and MMP9 protein expression compared to the vehicle control in HTCFs. Treatment of TGFβ-treated myofibroblasts with SB resulted in a downward trend of ACTA2 and MMP9 expression. Similarly, the MMC treatment resulted in reduced ACTA2 and MMP9 expression relative to positive control. 5-minute SB treatment and 4-minute MMC treatment demonstrated a statistically significant reduction of MMP9 and ACTA2 expression relative to the positive control. SB treatment was associated with mildly elevated cell metabolic activity and reduced LDH release compared to MMC treatment.

SB has shown comparable efficacy to MMC in reducing expression of pro-fibrotic proteins ACTA2 and MMP9, while being less cytotoxic in TGFβ-induced myofibroblasts. In the context of SB being previously shown effective to counteract fibroblast-mediated collagen contraction in 3D in vitro tissue-mimetic, these findings suggest that SB could be a promising new alternative to MMC for management of postoperative ocular fibrosis.

Presenter's Name: Thayaparan, Lorelei
Additional Authors: Thayaparan L, Gannavarapu S, Newman S, Rupar T
Abstract Title: Understanding Innate Immune Function Using Bone Marrow Derived Macrophages Collected From MLD Mice

Abstract:
Introduction: Metachromatic leukodystrophy (MLD) is a lysosomal storage disease caused by arylsulfatase A (ARSA) deficiency resulting in inadequate sulfatide degradation. Disease progression occurs as the accumulation of sulfatide in myelin and cells of internal organs contribute to myelin sheath damage, subsequent motor/cognitive impairment, and a heightened inflammatory response. While previous studies have observed a rise in proinflammatory cytokines in the cerebrospinal fluid of MLD patients, our understanding of the impact on innate immunity is limited. Moving forward, our laboratory will be looking at one of the most significant innate immune functions, phagocytosis, to determine whether or not altered lysosomal function has an impact on host phagocytes.

Methods: We will be collecting bone marrow derived macrophages from WT and MLD mice, and then measuring phagocytosis through the Fc-gamma signaling pathway using E. coli bioparticles as a target (Vybrant Phagocytosis Assay Kit, V-6694). We will collect results in triplicate at 3, 5, 10, 20, and 60 minutes; to stop phagocytosis at these time points, we will use ice cold phosphate buffered saline (PBS) and 4% paraformaldehyde (PFA). The results will then be deciphered in two ways: (i) using a plate reader and plotting the rate of phagocytosis comparing the two cell lines, (ii) staining the cells with F4/80 antibody and DAPI and proceeding with imaging.

Results: A difference in the rate or overall intake of particles between the cells of WT and MLD mice would imply an abnormality in phagocytosis in MLD.

Discussion: Current treatment approaches are primarily based on preventative care and minimizing disease-related symptoms. This research could further our understanding of the pathophysiology of MLD, paving the way for development of targeted therapeutics and/or a newborn screening method.

PATHOLOGY

Presenter's Name: Tse, Brennan
Additional Authors: Vanin Moreno S, Hardy D, Arany E
Abstract Title: Omega 3 supplementation's influence on fj9-tetrahydrocannabinol exposure-induced fetal pancreatic growth restriction

Abstract: The recent rise in popularity of cannabis use has driven the need for a more complete understanding of its implications. Recent reports suggest up to 7% of pregnant women use cannabis. Our lab and others previously showed in a rat model that exposure to fj9-tetrahydrocannabinol (fj9-THC) in utero caused fetal growth restrictions and altered pancreas development, which caused glucose intolerance, decreased pancreatic islet density and β-cell mass, and peripheral insulin resistance at 5 months after birth. Previously, our laboratory showed that similar fetal defects caused by gestational diabetes were rescued by the supplementation of dietary olive oil via activation of PPARs. Therefore, we hypothesize that the dietary supplementation of the PPAR ligands omega-3 fatty acids will prevent fetal and pancreatic growth restrictions caused by maternal fj9-THC exposure. To test this, a pregnant rat model was used. Subjects were given daily intraperitoneal injections of either 3 mg/kg fj9-THC or vehicle from gestational day 6 until birth. Those who received the treatment were fed a chow diet that was or was not supplemented with omega-3 fatty acids. We collected pancreata at 3 weeks of age and at 3 months when glucose tolerance was tested to investigate if glucose impairment was detected earlier than our previous research demonstrated. Preliminary data showed no significant glucose impairment at 3 months in any of the treatment or control groups. Next another group at 5 months will be tested. Currently, immunohistochemistry is being conducted to analyze endocrine morphological changes by examining pancreatic islet density and α/β-cell mass. A decrease in β-cell mass and pancreatic islet density in the fj9-THC group is expected. These changes will be eventually rescued by the addition of omega-3 fatty acids. The results of this study will further drive cannabis research and bring awareness to the potential dangers of cannabis use in pregnancy.

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Abstract Title: DLC1β Overexpression on Modulating Apoptosis During Cardiac Ischaemia-reperfusion Injury in Heart Transplantation

Abstract:
Background: Cardiac ischaemia-reperfusion injury (IRI) is a common complication of heart transplantation and is characterized by deregulated angiogenesis, increased hypertrophy, and increased apoptosis. Recently, our lab has identified a molecule known as DLC1β to be highly and exclusively expressed in the heart and was found to be downregulated in heart grafts after IRI in transplanted recipients. Therefore, our lab further investigated the effects of DLC1β during IRI and found that hearts grafts overexpressing DLC1β prevented cardiomyocyte apoptosis and increased cell survival after transplantation. With these results, our lab is working to understand the mechanism in which DLC1β modulates apoptosis during IRI and is currently investigating whether DLC1β has a role in the Bcl-2/Bax apoptotic pathway specifically.

Methods: For our experiments, we cultured H9c2 rat cardiomyocytes and transfected them with a DLC1β overexpressing plasmid. To replicate IRI, we introduced a hypoxic environment by either using a GENbag, or by adding a cellular respiration inhibitor, Antimycin A, to the cultured cells. After a certain time has passed, we removed the hypoxic condition and introduced fresh culture medium to allow the cells to be reperfused. We then used flow cytometry to analyze the level of apoptosis after IRI and used western blot and reverse transcription quantitative real-time polymerase chain reaction to measure protein and mRNA levels respectively of various apoptotic markers in the Bcl-2/Bax pathway of DLC1β transfected cells.

Results: Our results show that DLC1β transfected cells had lower levels of apoptosis than non-transfected cells during regular cell culture conditions. However, more tests need to be done to determine if DLC1β overexpression prevents apoptosis during IRI. We expect that overexpression of DLC1β will reduce the level of apoptotic cells seen after IRI and will be correlated with an upregulation of anti-apoptotic markers and a downregulation of pro-apoptotic markers in the Bcl-2/Bax pathway.

Discussion: If our results are as expected, it will indicate that DLC1β is cardioprotective and could potentially modulate apoptosis through the Bcl-2/Bax pathway during cardiac IRI. Understanding the mechanistic role of DLC1β during IRI may provide a novel therapeutic target for preventing or alleviating the effects of this complication during heart transplantation, and ultimately increase the rate of transplant success and survival.

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