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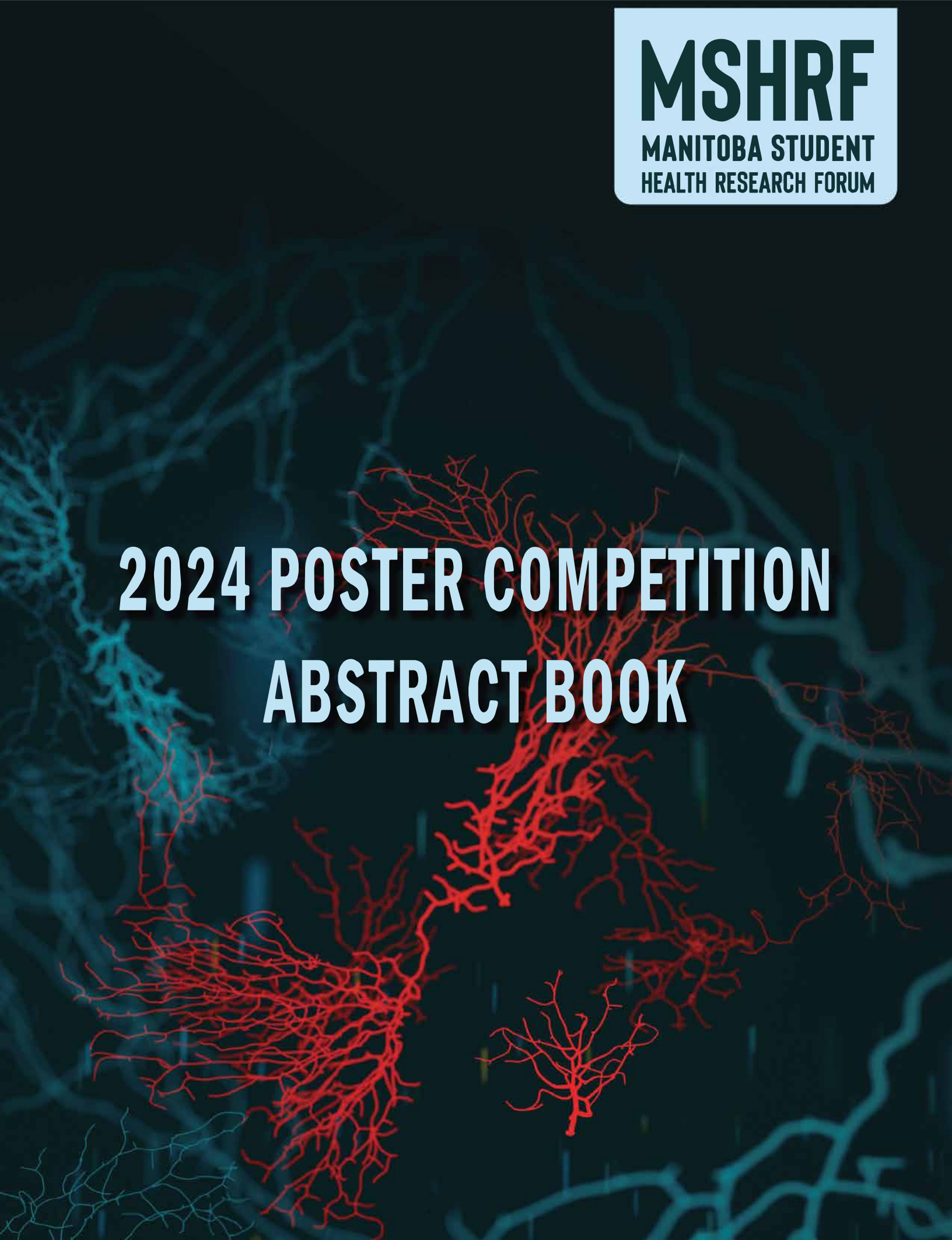
MANITOBA STUDENT HEALTH RESEARCH FORUM

2024 POSTER COMPETITION ABSTRACT BOOK



POSTER #146

Gonorrhea Cluster Detection in Manitoba, Canada: Spatial, Temporal, and Spatiotemporal Analysis

Amin Abed[1], Mahmoud Torabi[1], Zeinab Mashreghi[2]

[1]Community Health Sciences, University of Manitoba, Canada.
[2]Mathematics and Statistics, University of Winnipeg, Canada.

Introduction

In Canada, Gonorrhea infection stands as the second most prevalent sexually transmitted infection, with Manitoba reporting an incidence rate three times greater than the national average in 2018. This study investigates the spatial, temporal, and spatiotemporal patterns of Gonorrhea infection in Manitoba using individual-level laboratory-confirmed administrative data from 2000 to 2016, focusing on the 96 Regional Health Authority Districts (RHADs). This study is the first investigation into detecting spatial, temporal, and spatiotemporal clusters of Gonorrhea infection in Manitoba, emphasizing the novelty of the research.

Methods

Data includes sex, date of birth, diagnosis date, postal code of residence, and scrambled personal health identification numbers. Diagnosed infections are aggregated into 96 RHADs and linked to 2016 Statistics Canada census data to obtain sex and age of population residing in those 96 RHADs. Temporal analysis examines monthly trends using time series decomposition and a seasonal auto-regressive integrated moving average model. Spatial analysis utilizes global Moran's I statistic and Kulldorff's spatial scan statistics to identify high-risk clusters. Spatiotemporal analysis employs Kulldorff's space-time scan statistic within a discrete Poisson model to unveil complex interaction patterns between geographic location and time.

Results

Age and gender trends show younger females are more affected than males. Repeated infections surged in 2016, constituting 16% of total cases. Spatial analysis across 96 Manitoba regional health districts reveals significant positive spatial autocorrelation, indicating clustered infection distribution, notably in northern Manitoba and central Winnipeg. Temporal analysis exposes seasonal spikes in late summer and fall. Spatiotemporal analysis pinpoints high-risk clusters, primarily in northern Manitoba from January 2006 to June 2014, and a secondary cluster in central Winnipeg from June 2004 to November 2012.

Conclusion

The outcomes of this study provide valuable insights for public health and Manitoba Health as they reveal the existence of high-risk clusters of Gonorrhea infections. Policymakers should prioritize age and sex groups that are at higher risk of both contracting and spreading the infection. Furthermore, these high-risk clusters determine where the localized prevention strategies, control measures, and allocation of resources should be focused and strengthened to reduce the burden of the infection.

POSTER #49

Mechanisms of laminar organization of hippocampal excitatory synapses

Mohammed Abrar Basha[1,2], Benyamin Karimi[1,2,5], Tabrez J. Siddiqui[1,2,3,4]

[1]Prairie Neuroscience Research Centre, Kleysen Institute for Advanced Medicine, Health Sciences Centre.
[2]Physiology and Pathophysiology, Max Rady College of Medicine, RFHS, University of Manitoba.
[3]Program in Biomedical Engineering
[4]Children's Hospital Research Institute of Manitoba.
[5]Department of Neurology, Johns Hopkins Medicine.

Introduction

The hippocampus is characterized by well-defined laminae and specific neuronal pathways. This study explores the developmental expression and functional roles of leucine-rich-repeat transmembrane neuronal proteins (LRRTMs), focusing on LRRTM1 and LRRTM2 in distinct hippocampal layers.

Methods

We employed immunohistochemistry to analyse the temporal and spatial expression patterns of LRRTM1 and LRRTM2 within the hippocampal laminae. We used stereotaxic injections to deliver viruses for the targeted deletion of the gene in CA1 region of the mice hippocampus. Subsequent behavioural assays evaluated the cognitive and behavioural roles of these proteins in conditional knock-out mice.

Results

LRRTM1 and LRRTM2 were found to be localized predominantly in the stratum radiatum (SR) and stratum lacunosum moleculare (SLM), respectively. LRRTM2's presence in the SLM was associated with excitatory synapse formation on temporoammonic cortical inputs to CA1. Behavioural analyses revealed that conditional deletion of LRRTM1 in the dorsal CA1 impaired contextual fear memory and social interactions, without affecting social novelty preference. Behavioural analysis of *Lrrtm2-CA1-cKO* mice, specifically females, revealed an anxiety-associated phenotype, highlighting a sex-specific functional impact.

Conclusion

These findings reveal the compartmentalization of LRRTM 1 and 2 proteins in the hippocampus and their significant roles in synaptic architecture and behaviour.

POSTER #156

Male and Female Mice Lacking Periostin Show Varying Responses in Survival Rate and Extracellular Matrix Composition after Myocardial Infarction

Besher M. Abual'anaz[1,2,3], Sara H. Rasul[1,2,3], Sunil G. Rattan[1,2,3], Petra C. Kienesberger[4], Ian M. Dixon[1,2,3]

[1]Institute of Cardiovascular Sciences, Albrechtsen Research Center, Winnipeg, Canada.
[2]Physiology and Pathophysiology, University of Manitoba, Winnipeg, Canada.
[3]Rady Faculty of Health Sciences, Max Rady College of Medicine, Winnipeg, Canada.
[4]Biochemistry and Molecular Biology, Dalhousie University, Saint John, Canada

Introduction

Ischemic heart disease often begins with myocardial infarction (MI), which remains a significant cause of heart failure. Heart failure frequently involves the activation of fibroblasts into myofibroblasts, resulting in an increase in the deposition of extracellular matrix (ECM) proteins. Myofibroblasts secrete periostin (PN), a matricellular protein. Periostin is re-expressed in the adult heart following any pathological injury, typically around day 3 post-MI, to support wound healing by promoting collagen crosslinking. We examined sex-related differences in mice with a periostin knockout (PN KO) phenotype, with particular emphasis on variations in ECM reconfiguration after MI.

Methods

An analysis was conducted on a cohort of 53 wild type (WT) and 95 PN KO mice. The PN KO phenotype was confirmed using quantitative polymerase chain reaction (qPCR), western blotting (WB), and immunohistochemistry (IHC). A subset of genes associated with collagen fibrogenesis, and collagen crosslinking was explored using qPCR.

Results

Following MI, male PN KO mice exhibited a survival rate around 15.4% (±7.95%) after one-week post-MI, in contrast with a significantly higher survival rate around 66.7% (±9.01%) in female PN KO (*$P < 0.001$), as determined by log-rank test. Cardiac rupture was found to be a factor leading to mortality in all cases. Furthermore, examining the infarct scar area one-week post-MI revealed significant overexpression of the Fmod gene, coding for fibromodulin protein, in female PN KO mice compared to male PN KO mice.

Conclusion

The lack of periostin secretion is associated with an increased susceptibility to cardiac rupture in male PN KO mice compared to their female counterparts. While periostin aids in the acute wound healing process after MI in both male and female hearts, males exhibit a more pronounced sensitivity to periostin absence. These findings support the hypothesis that sex-related differences in cardiac wound healing after MI among mice lacking periostin are due to varying levels of fibromodulin expression between females and males.

POSTER #63

The Clinical and Demographic Factors Associated with the Development and Severity of Neonatal Abstinence Syndrome: A Population-Based Cohort Study

Omaymah M. Abulannaz[1,2], Chelsea A. Ruth[3,4], Geert W. 't Jong[1,5], Lauren E. Kelly[1,5]

[1]Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Canada.
[2]Pharmacology, Jordan University of Science and Technology, Jordan.
[3]Pediatrics and Child Health, University of Manitoba, Winnipeg, Canada.
[4]Community Health Sciences, University of Manitoba, Winnipeg, Canada.
[5]Children's Hospital Research Institute of Manitoba, Winnipeg, Canada.

Introduction

Neonatal Abstinence Syndrome (NAS) is a clinical condition that results from the abrupt discontinuation of opioids and/or other substances that were used or misused during pregnancy. Significant variability has been observed in the development and severity of NAS. The aim of this study was to evaluate the association between certain clinical and demographic factors and the development and severity of NAS.

Methods

A cohort of mother-child dyads with children born in Manitoba, Canada, between 1995 and 2021 was identified. Data were obtained from the population data repository housed at the Manitoba Centre for Health Policy. NAS was defined as ICD-9-CM code 779.5 or ICD-10-CM code P96.1 in hospital abstracts. Non-pharmacological interventions are the first-line response to NAS symptoms; if ineffective, pharmacotherapy is administered, starting with morphine and potentially including adjunct therapies such as clonidine or phenobarbital if symptoms persist. NAS is considered severe if pharmacological management is required. In-hospital pharmacy data were available from 1999 to 2012. Logistic regression models were used to assess the association between clinical and demographic factors and the development and severity of NAS.

Results

Of the 381,610 eligible children, 1268 were diagnosed with NAS and 174 required pharmacological treatment. The models included gestational age, birth weight, infant sex, and maternal characteristics. Maternal characteristics included the use of benzodiazepines, selective serotonin reuptake inhibitors, gabapentin, smoking, alcohol use, breastfeeding initiation, and socioeconomic status. All covariates were significantly associated with the development of NAS. The model discriminated well with an Area Under the Curve of 0.87. However, none of the listed variables showed a significant association with NAS severity.

Conclusion

We identified the clinical and demographic factors associated with the development of NAS. Early identification of NAS is crucial to provide timely intervention, improve infant outcomes, prevent complications, and reduce the healthcare costs associated with NAS by preventing the need for prolonged hospital stays. Further research is needed to elucidate the genetic factors contributing to NAS development and severity.

POSTER #47

The role of thioredoxin-interacting protein in corticosterone-induced damage in astrocytes

Sushank Acharya[1], Hua Tan[1], Jun-Feng Wang[1]

[1]Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, MB, Canada.

Introduction

Chronic stress is one of the major risk factors for depression and stress-related disorders. During the stress, HPA axis stimulates adrenal cortex to release glucocorticoids. The high level of glucocorticoid can promote oxidative stress and neuroinflammation. The studies have shown that chronic stress increases thioredoxin-interacting protein (Txnip). Txnip is an endogenous inhibitor of oxidoreductase protein thioredoxin (Trx) and it can bind to NOD-like receptor protein 3 (NLRP3) to activate NLRP3 inflammasome. Besides this, chronic stress has been shown to alter the morphology of astrocytes and induce atrophy in astrocytes along with alteration in gap-junction coupling which could cause astrocytic dysfunction. The objective of this study is to determine if CORT treatment can promote oxidative stress and inflammation in astrocytes.

Methods

Primary mice astrocytes were used in this study. Cells with treated with 1-10 µM CORT for 24 hrs and then protein level of Trx1 and Txnip was determined using immunoblotting assay. Trx activity was measured using eosin-labeled insulin. Reduced thiol levels were measured using Ellman's assay. Protein sulfenylation and protein carbonylation were measured by using dimedone conjugation and biotin hydrazide respectively followed by immunoblotting analysis. NLRP3/Txnip binding was measured by co-immunoprecipitation assay. Caspase-1 activity and IL-1β release were measured by using fluorescent substrate Z-YVAD-AFC and ELISA respectively.

Results

We found that 0.1, 1 and 10 µM increased Txnip protein level while it didn't any effect of Trx1 level. CORT slightly decreased Trx activity in primary astrocyte while it had no effect on reduced thiol level, protein sulfenylation and carbonylation. The co-immunoprecipitation study showed that corticosterone treated astrocytes had significantly higher Txnip/NLRP3 binding as compared to the controls. Furthermore, we found that astrocyte treated with corticosterone increased caspase-1 activity and IL-1β release in media as compared to the control.

Conclusion

The findings suggest that corticosterone treatment increases Txnip level which could promote NLRP3 activity, activate caspase-1 and release IL-1β to promote inflammation while increase in Txnip and decrease in Trx may not be sufficient to exert oxidative stress in astrocytes.

POSTER #35

Determining kinase activation states in chronic lymphocytic leukemia (CLL) through multiplexed mass spectrometry

Havva Afshari[1], Victor Spicer[2], Oleg Krokhin[1,2,3], Sachin Katyal[4,5], René P. Zahedi1,[2,3,5]

[1]Department of Biochemistry and Medical Genetics.
[2]Department of Internal Medicine; [3]Manitoba Centre for Proteomics and Systems Biology.
[4]Department of Pharmacology and Therapeutics.
[5]Paul Albrechtsen Research Institute, CancerCare Manitoba.

Introduction

Protein phosphorylation, mediated by the interplay of kinases and phosphatases, plays a crucial role in regulating various cellular processes, including cell proliferation, apoptosis, and gene regulation. Dysregulation of kinases is considered a hallmark of many cancers but determining kinase activation state is challenging. Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in older adults. First-line treatment for CLL are Bruton's tyrosine kinase (BTK) inhibitors, which are costly, and many patients develop resistance over time. Therefore, there is an urgent need to identify other potential drug targets in CLL. Mass spectrometry (MS)-based *discovery* phosphoproteomics enables the relative quantification of thousands of phosphorylation sites, offering a comprehensive perspective on signaling networks; however, inferring kinase activity from such data remains challenging. Notably, most kinases are regulated through phosphorylation of their activation loop (T-loop), serving as a direct marker for kinase activity that can potentially be measured by *targeted* mass spectrometry.

Methods:

CLL and MEK1 cell line samples treated with different stimuli have been analyzed by *discovery* proteomics/ phosphoproteomics to identify potential kinase targets and their kinase t-loop phosphopeptides in CLL. The target list was used to build a parallel reaction monitoring (PRM) method for targeted MS. PRM conditions are optimized to allow maximum multiplicity and sensitivity. We aim to develop a hybrid method combining *targeted* T-loop analysis with machine learning-assisted identification of potential driver kinases from *discovery* phosphoproteomics data.

Preliminary Results:

In the MEC1 CLL cell line (untreated, DMSO, ibrutinib-treated) 12,142 unique phosphopeptides from 2,936 unique proteins were identified, resulting in 9,863 high-confidence phosphorylation sites. This data comprised 28 kinase t-loop phosphopeptide targets that have been used to develop a PRM method that is currently being optimized.

Conclusion:

The low coverage of T-loop phosphopeptides in discovery datasets underlines the need for targeted MS to determine kinase activation states. Once optimized, the utility of our method will be evaluated by treating MEC1 cells with kinase inhibitors based on predicted targets. If successful, kinase activities will be determined in CLL patient samples. Notably, the developed assays can be used to determine the activity of the same kinases in other samples beyond CLL.

POSTER #120

Amniotic Fluid as Mediator of the Maternal Environment and of The Fetal Lung

John Paul Aguilar[1,2], Hesham Mohamed[1], Dina H.D. Mostafa[1,2], Meghan Riddell[3,4,] Christopher D. Pascoe[1,2]

[1]Biology of Breathing, Children's Hospital Research Institute of Manitoba.
[2]Department of Physiology and Pathophysiology, University of Manitoba.
[3]Department of Obstetrics and Gynaecology, University of Alberta, [4]Department of Physiology, University of Alberta

Introduction

Fetal breathing movements are part of normal lung development and permit the exchange of lung fluid with amniotic fluid (AF). Changes in AF composition, including cytokines, could therefore alter lung development and health. AF may bridge the maternal external environment and the developing lungs. Exposure to cigarette smoke (CS) *in-utero* increases risk for chronic lung diseases like asthma. Currently, little is known about if AF cytokine profiles are affected by environmental exposure, specifically CS. The aim of the study is to characterize the cytokine profile of human AF in smokers and non-smokers and determine if airway epithelial cells respond to these changes.

Methods

Matched AF, maternal, and cord blood were collected from patients undergoing a term caesarean delivery. Cotinine was measured using an enzyme-linked immunosorbent assay. Cytokine/chemokine profiles were measured using a 96-plex assay and profiles compared using partial least squares-discriminant analysis and t-tests. Epithelial cells were exposed to AF, and trans-epithelial electrical resistance (TEER) was measured. Data is presented as mean±SD.

Results

Samples were collected from diverse ethnicities (45% Caucasian) with a median age of 35 and gestational age of 38.9 weeks. Cotinine was detected in 27% AF samples at levels 1.38-fold higher than in maternal blood ($p<0.05$). AF cytokine profiles are distinct from maternal and cord blood, characterized by higher abundances of IL-1RA (30-, 118-fold), IL-15 (11-, 16-fold) and IL-6 (67-, 378-fold, relative to cord and maternal blood respectively). Cotinine-positive AF had over 3-fold higher levels of IL-6 and 2-fold lower levels of IL-15 relative to cotinine-negative ($p<0.0001$, $p<0.05$ respectively). Epithelial cells exposed to cotinine-positive AF had TEER measurements that were 22% lower than cotinine negative samples 24-hours after exposure. After 72-hours of exposure, barrier integrity in cotinine-positive samples was 48% lower than cotinine-negative samples.

Conclusion

AF has a distinct cytokine/chemokine profile from maternal and cord blood. CS exposure prenatally alters the cytokine and chemokine profile of AF, increase in pro-inflammatory IL-6, and bioaccumulation of cotinine. This may provide a novel insight into the role AF plays in mediating the external and fetal environments and in understanding its role in the developmental origins of chronic lung disease.

POSTER #122

Incorporating novel audiogram classification strategies to identify genes and pathways involved in subtype components of age-related hearing loss

Samah Ahmed[1], Kenneth I. Vaden Jr[2], Judy R. Dubno[2], Britt Drögemöller[1,3-5]

[1] Biochemistry and Medical Genetics, RFHS, University of Manitoba, Winnipeg, MB, Canada.
[2] Hearing Research Program, Department of Otolaryngology-Head and Neck Surgery, Medical University of South Carolina, SC, Charleston, USA
[3] The Children's Hospital Foundation of Manitoba, Winnipeg, MB, Canada.
[4]CancerCare Manitoba Research Institute, Winnipeg, MB, Canada.
[5]Centre on Aging, Winnipeg, MB, Canada.

Introduction

Age-related hearing loss (ARHL) affects one-third of the population over 65 years. Although genome-wide association studies (GWAS) have uncovered genetic variants underlying ARHL, there are large gaps in our understanding of the genetic factors involved. This may be due to challenges associated with accurately phenotyping large cohorts of older adults with hearing loss. In this study, we used a mathematical model fitted to individual audiograms to estimate the magnitude of subtype components of ARHL.

Method

We have obtained genomic and audiologic data from 26,622 healthy older individuals participating in the Canadian Longitudinal Study on Aging. By adopting a novel approach developed by Vaden et al., we derived metabolic and sensory estimates for each audiogram. GWAS was performed by linear regression, including significant clinical variables and the first ten genetic ancestry-related principal components as covariates. We performed functional enrichment analysis to identify biological pathways underlying hearing loss phenotypes.

Results

We found that metabolic estimates were higher for older compared to younger individuals, with no noticeable difference between males and females. Although sensory estimates were significantly higher in older individuals, the magnitude of the association was a minor, with males showing more sensory hearing loss compared to females. GWAS revealed that rs6453022, a missense variant in ARHGEF28 gene, was significantly associated with the metabolic phenotype (P=2.67x10-9); while rs36062310, a missense variant in KLHDC7B gene, was significantly associated with the sensory phenotype (P=2.37x10-12). Sex-stratified analyses also revealed key differences in the GWAS results. Enrichment analyses revealed differences in the biological pathways underlying the two hearing phenotypes, with the RhoA activity regulation pathway implicated in the metabolic phenotype, and pathways relating to sensory processing of sound by hair cells and the calcium/calmodulin signalling implicated in the sensory phenotype.

Conclusions

In this large-scale genetic study, we have identified differences in the associations observed for two distinct subtype components of age-related hearing loss. The identification of specific processes that are involved in sensory and metabolic hearing loss has improved our understanding of the biological mechanisms underlying different components of ARHL.

POSTER #61

Targeting the phenotypic impairments and molecular deficits of the brain in Rett Syndrome by in vivo studies in a clinically relevant transgenic mouse model

Ghanan Bin Akhtar[1], Khatereh Saei Arezoumand[1], Marjorie Buist[1], and Mojgan Rastegar[1]

[1] Department of Biochemistry and Medical Genetics, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada

Introduction

Epigenetic mechanisms play a crucial role in brain development, particularly through DNA methylation, which influences neuronal maturation. The Methyl CpG Binding Protein (MeCP2) is pivotal in binding methylated DNA in the brain. Mutations in the MECP2 gene, particularly the R255X nonsense mutation, lead to Rett Syndrome (RTT), an X-linked disorder characterized by impaired neurite formation and neuronal maturation. MeCP2 has two isoforms, MeCP2E1 and MeCP2E2, highly expressed in the brain.

Rationale and Hypothesis

Despite predominantly affecting females, most preclinical studies on RTT use male mice. The lack of a cure and incomplete understanding of RTT's mechanisms underscore the need for further research. RTT has been linked to disrupted glucose metabolism, with metformin, an anti-diabetic drug targeting gluconeogenesis, showing promise. Thus, I hypothesize that metformin administration in vivo can ameliorate RTT symptoms and molecular deficits in R255X RTT mice.

Methodology

Wild type and mutant R255X RTT male and female mice were divided into sham, vehicle control, and metformin treatment groups. Daily intraperitoneal injections of metformin were administered over three weeks. Mice were monitored for phenotypic changes, and brain tissues were collected for molecular analysis post-treatment. Additional behavioral studies relevant to RTT symptoms are ongoing.

Results and Conclusion

Both male and female transgenic mice exhibited altered body weight and various abnormal behaviors compared to wild type littermates. Metformin-treated RTT mice showed improved phenotypic criteria compared to controls, suggesting its potential therapeutic benefits. These findings highlight metformin's efficacy and propose it as a promising avenue for future RTT therapies.

POSTER #52

Prenatal Exposure to Gabapentin and the Risk of Autism Spectrum Disorder in Children

Pelumi Samuel Akinola[1], Christine Leong[1,2], Marcus Ng[3], Roxana Dragan[4], Silvia Alessi-Severini[1], Sherif Eltonsy[1,5]

[1]College of Pharmacy, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada.
[2]Department of psychiatry, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada.
[3]College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada.
[4]Manitoba Centre for Health Policy, University of Manitoba, Winnipeg, Manitoba, Canada 5Children's Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada.

Introduction

Gabapentin, commonly used off-label for treating neuropathic pain, has seen an increased usage among pregnant people in recent years. The association between gabapentin exposure during pregnancy and ASD is not known. We aim to assess the risk of ASD in children following prenatal exposure to gabapentin.

Methods

We conducted a retrospective population-based cohort study using data from the Manitoba Centre for Health Policy, which included data from pregnancies in Manitoba from January 1998, to March 2021. We included all live singleton births during this period. The primary exposure time window of interest for ASD was the second and/or third trimester. A secondary analysis included exposure at any time during pregnancy. The comparison group included pregnancies not exposed to gabapentin. We randomly selected one child per mother to account for familial confounding. Using multivariate Cox regression, we reported crude and adjusted hazard ratios (aHRs) and 95% CIs.

Results

The study included 289,794 children, with 51.3% being male. The cohort included 1,029 exposed and 288,765 unexposed children. The mean maternal age was 30 for gabapentin users compared to 28 for non-users. Among the exposed group, 383 (37.2%) were exposed during the second and/or third trimester, and 646 (62.8%) at any time during pregnancy. Out of those exposed, 14 children (2.2%) during the second and/or third trimester and 27 children (2.6%) at any time during pregnancy were diagnosed with ASD. The mean age of children at the time of diagnosis was 3.5 years. The aHRs for ASD after exposure during the second and/or trimester and at any time were 1.58 (95% CI, 0.93-2.68) and 1.70 (95% CI, 1.16-2.49), respectively. In the cohort of one child per mother, the aHRs for ASD after exposure during the second and/or third trimester and at any time were 1.18 (95% CI, 0.52-2.64) and 1.22 (95% CI, 0.67-2.22), respectively.

Conclusion

Gabapentin use at any time during pregnancy was associated with an increased risk of autism in children, but not during late pregnancy. Shared genetic traits within the family might play an important role. Further research on gabapentin safety using large longitudinal databases is warranted.

POSTER #66

Application of MXene nanomaterials for maturation of induced pluripotent stem cells derived cardiomyocytes

Keshav Narayan Alagarsamy[1], Leena Regi Saleth[1], Abhay Srivastava[1], Weiang Yan[1], Alireza Rafireed[1], Niketa Sareen[1], Sanjiv Dhingra[1]

[1]Institute of Cardiovascular Sciences, St. Boniface Hospital Albrechtsen Research Centre, Department of Physiology and Pathophysiology, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, R2H 2A6, Canada

Introduction

Engineered myocardium using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) offers a robust platform for understanding cardiovascular diseases, validating drug efficacy, and investigating regenerative therapies. However, current protocols yield hiPSC-CMs resembling late-stage fetal cardiomyocytes, not adult ones. Various tissue engineering strategies have been explored, with limited success. One promising strategy involves the use of electroconductive nanomaterials to replicate the native myocardium's mechanical and electrical microenvironment. MXene, a novel class of electroconductive nanomaterial, has gained attention due to its unique electrical and physiochemical properties. These characteristics have facilitated its use in a wide range of biomedical applications, such as bioimaging, biosensing, and drug delivery.

Methods

We cultured hiPSC-CMs on both MXene and control matrices and examined cellular phenotypes, ion channel gene expression, and mitochondrial maturation. Techniques employed included multielectrode array, calcium imaging, qRT-PCR, JC-1 staining, and Seahorse XF Analyzer. This assessment aimed to compare the maturation status of iPSC-CMs grown on the MXene matrix with those on the control matrix.

Results

In this study, we devised an innovative MXene matrix, distinguished by its outstanding electroconductivity and biocompatibility, tailored specifically for the maturation of hiPSC-CMs. Compared to cells cultured on a matrix devoid of MXene, hiPSC-CMs grown on the MXene matrix demonstrated a more rapid beating rate, superior calcium kinetics, and an augmented action potential. Additionally, these phenotypic observations were substantiated by enhancements in key ion channel genes associated with maturation. Moreover, hiPSC-CMs cultivated on MXene exhibited heightened mitochondrial maturation, as manifested by an increased mitochondrial membrane potential and an amplified respiratory capacity. Collectively, these results suggest that the MXene matrix fosters a comprehensive enhancement in the cellular metabolism of hiPSC-CMs.

Conclusion

The incorporation of MXene within the matrix has demonstrated its superiority as an electroconductive scaffold for the maturation of iPSC-CMs. By facilitating the development of more mature cardiomyocytes, this innovative matrix can significantly advance effective regenerative therapies and targeted drug treatments in the field of cardiovascular science.

POSTER#148

The Association of Sarcopenia with Chronic Kidney Disease (CKD) and Decline in Kidney Function.

Mackenzie R. Alexiuk[1] & Navdeep Tangri[2,3]

[1]Department of Community Health Sciences, University of Manitoba [2]Department of Internal Medicine, University of Manitoba [3]Chronic Disease Innovation Centre

Introduction

Sarcopenia, defined as the loss of muscle mass, is a growing public health concern, and is an underrecognized problem in adults with Chronic Kidney Disease (CKD). Clinical sarcopenia diagnosis can be achieved via appendicular lean mass index (ALMi, indexed to height by m^2), traditionally obtained through whole-body dual-energy X-ray absorptiometry (DXA) scans, however these are not frequently performed. As a result, large population studies examining the relationship between CKD and sarcopenia are lacking.

Methods

Using databases held at the Manitoba Centre for Health Policy, we identified adults who had at least one
DXA scan linkable to serum creatinine values within 365 days, between 2007 and 2022. Serum creatinine was used to calculate estimated glomerular filtration rate, and estimated ALMi (eALMi) was calculated through central DXA scans via a previously developed algorithm. We constructed Linear, logistic, and Cox proportional hazards models to examine the relationship between CKD, sarcopenia, and adverse clinical outcomes.

Results

Our cohort contained 24,660 individuals (64.4 ± 12.5 years, 84.4% female), with 3,204 individuals (13.0%) having eALMi indicating sarcopenia. 22,648 individuals (91.8%) had eGFR $\geq$ 60, and 2,012 (8.2%) had eGFR < 60. After adjustment for age, sex, estimated central mass index, and comorbid conditions, the presence of eGFR < 60 was associated with higher odds of sarcopenia (OR: 1.39; 95% CI: 1.16–1.67). In individuals with two DXA scans (n=2,985), eGFR < 60 at baseline was associated with a larger decline in eALMi compared to individuals with preserved eGFR (OR: 1.61; 95% CI: 1.05–2.45). Baseline sarcopenia and declining eALMi were also associated with adverse clinical outcomes including hospitalization and emergency room visits, home care use, long-term care use, and all-cause mortality.

Conclusions

Our results show that CKD is associated with sarcopenia and leads to more rapid declines in appendicular lean mass over time. Furthermore, we demonstrate that eALMi as derived from routine central DXA scanning is associated with long-term downstream clinical outcomes. These findings highlight the importance preservation of muscle mass in individuals with CKD.

POSTER #123

The LMO2 Oncogene Establishes Autocrine FTL3 Signalling In T-Cell Acute Lymphoblastic Leukemia

Ovini Amarasinghe[1,2], Jesslyn Saw[3], David J Curtis[3] and Cedric S Tremblay[1,2,3]

[1]Department of Immunology, University of Manitoba, Canada.
[2]Paul Albrechtsen Research Institute CancerCare Manitoba, Canada.
[3]Australian Centre for Blood Diseases (ACBD), Monash University, Australia.

Introduction

FLT3 is a receptor tyrosine kinase expressed by hematopoietic progenitors that plays a crucial role for stem cells and the immune system. FLT3 is frequently overexpressed or mutated in high-risk subtypes of acute leukemia, including T-cell acute lymphoblastic leukemia (T-ALL). Although overexpression and aberrant activation of FLT3 have been shown to promote drug resistance and relapse, how aberrant FLT3 expression and activation are regulated in T-ALL remains undefined. To address this, we will 1) assess whether FLT3 and its ligand (FLT3-L) are transcriptional targets of LMO2 in T-ALL, and 2) define how FLT3 signaling is aberrantly activated in T-ALL.

Methods

We have utilized ChIP-seq, transcriptomics and flow cytometry, combined with co-culture assays and utilized ELISA to assess how FLT3 signaling is regulated in our *Lmo2*-driven (*Lmo2*^{Tg}) mouse model of T-ALL.

Results

ChIP-seq data revealed that both *FLT3* and *FLT3-L* are transcriptional targets of LMO2, thereby leading to aberrant expression of these genes in T-cell progenitor populations responsible for progression and relapse in our *Lmo2*^{Tg} model of T-ALL. ELISA confirmed that preleukemic *Lmo2*^{Tg} T-cell progenitors secrete 10-fold more FLT3-L than normal T-cell progenitors. Importantly, our data suggest *Lmo2* induces an autocrine FLT3 signalling in the cells responsible for driving T-ALL progression and relapse.

Conclusion

Both FLT3 and FLT3-L are transcriptionally regulated by the initiating oncogene *LMO2*, thereby establishing an aberrant *LMO2*-induced autocrine FLT3 signalling loop in T-ALL.

POSTER #147

Developing PET/MR Methods for Brain Imaging

Melissa Anderson[1], Melanie Martin[2]

[1,2]Biomedical Engineering, University of Manitoba, Winnipeg, Canada.

Introduction

Positron Emission Topography (PET) uses a radiotracer, a chemical compound used by the body with at least one atom changed to a radioactive atom. The radioactive atom decays and releases a positron (anti-electron). The electron and anti-electron, annihilate producing two photons which PET detects to map the location of the radiotracers. PET provides images of the function of tissue.

Magnetic Resonance Imaging (MRI) can be the anatomical imaging method for PET. It has three main advantages. The contrast in MRI can be varied to provide needed information. No additional radiation is given to the subject. With recent advances, the PET scanner can be put into the bore of the magnet allowing for simultaneous PET-MR imaging, which allows imaging during movement, e.g., beating heart. My research will be looking at the attenuation and scatter correction algorithms. I will be optimizing image processing methods for improved PET quantification.

Methods

Phantoms are being used for quantitative studies. Phantoms are imaged with either PET, MRI, or PET/MR.

Results

Images can be obtained, and preliminary analysis can be performed.

Conclusion

With a reliable set of imaging systems, studies can begin to perform attenuation and scatter correction.

POSTER #162

TXNDC12 Regulates Breast Cancer Cell Migration

Shamima N. Anonna[1], Mousumi Majumder[1]

[1]Department of Biology, Brandon University, Manitoba, Canada

Introduction

Thioredoxin Domain Containing Protein 12 (TXNDC12) plays a multifaceted role in redox regulation, oxidative stress, and transcriptional control. Despite its established significance in different types of cancer, the involvement of TXNDC12 in Breast Cancer (BC) remains poorly understood. Two small oncogenic endogenous RNAs, miRNA-526b and miRNA-655, contribute to regulating BC tumour growth and metastasis. Overexpression of these miRNAs in poorly metastatic BC cells (MCF7 and SKBR3) has been associated with elevated migration, proliferation, spheroid formation, invasion, and epithelial-mesenchymal transition (EMT). However, the precise mechanisms of these miRNAs regulating the tumor microenvironment (TME) remain elusive. To address this gap, our laboratory conducted a high-throughput cell secretome analysis comparing cell-free secretion of miRNA-overexpressing and miRNA-low tumor cells and identified TXNDC12 as one of the upregulated markers. Current research investigates the intricate interplay between TXNDC12 and oncogenic microRNAs in shaping the TME and driving BC progression.

Methods

We analysed mRNA and protein expressions of TXNDC12 across various BC cell lines, including MCF7, MCF7-526b, MCF7-655, MDAMB231, HST, T47D, SKBR3-MOCK, and SKBR3-526b. To elucidate the functional roles of TXNDC12 in BC, we employed small interfering RNA (siRNA) to knock down (KD) its expression in the highly aggressive triple-negative tumor cell line MDAMB231. In all the cases, scrambled siRNA served as control. Subsequently, we conducted assays to assess cell proliferation, scratch wound healing assay, and transwell migration assay, providing insights into the impact of TXNDC12 modulation on BC cellular phenotypes. We also measured EMT markers (Vimentin, E-cadherin, N-cadherin, and Twist) after TXNDC12 manipulation.

Results

mRNA and protein expressions resulted in high expression of TXNDC12 in miRNA-high aggressive BC cell lines. siRNA silenced up to 80-90% of TXNDC12 expression and significantly reduced the cell migration rate compared to the scramble KD, indicating it is involved in cell migration. We observed a significant alteration in mesenchymal marker expression, which supports migration data.

Conclusion

TXNDC12 could significantly influence EMT, migration, and proliferation in BC. By unravelling the crosstalk between TXNDC12, miRNAs, and the TME, our research paves the way for novel therapeutic strategies to mitigate BC progression.

POSTER #3

Microglia ablation Mediates motor impairement in the Lateral cerebellar nuclei

Azam Asemi-Rad[1], Farshid Ghiyamihoor[1], Giacomo Consalez[2], Hassan Marzban[1]

[1]Department of Human Anatomy and Cell Science, The Children's Hospital Research Institute of Manitoba (CHRIM), Max Rady College of Medicine, Rady Faculty of Health science, University of Manitoba, Winnipeg, MB, Canada
[2]Division of Neuroscience, San Raffaele Scientific Institute, and San Raffaele University, Milan, Italy

Introduction

Cerebellar microglia have some unique properties compared to microglia in the cerebrum. Due to their important role in brain development and homeostasis, microglia are involved in various pathological conditions affecting the CNS, but little is known about their role in motor function. Cerebellar nuclei (CN) constitute the sole cerebellar output and play a central role in cerebellar circuits. Accumulating evidence implicates a pivotal role of CN connectivity in neurological diseases, including ataxia. However, because of the compact topography and close functional connection between CN and the cerebellar cortex, identifying cerebellar deficits exclusively linked to CN is challenging. In this study, we evaluated the role of microglial ablation in lateral CN in motor impairment and ataxia sign.

Methods

We performed stereotaxic surgery to inject adeno-associated virus (AAV) into the lateral CN of CX3cr1 Cre+ mice, followed by intraperitoneal injection of diphtheria toxin (DT) to induce microglial ablation. The mice were then observed for three weeks. Motor coordination was assessed using rotarod and beam walking tests conducted three weeks post-DT injection. Immunolabeling with anti-IBA1, anti-P2Y12, and anti-GFP antibodies was carried out on cerebellar sections. Subsequently, we repeated the procedure on CX3cr1 Cre + mice and conducted the same behavioral tests and immunolabeling three days post-DT injection.

Results

Double staining with anti-IBA1 and anti-GFP antibodies confirmed GFP expression and a reduction in IBA1-positive cells at the site of AAV injection in the lateral cerebellar nuclei (CN) of CX3cr1 Cre + mice compared to CX3cr1 Cre - mice. Analysis of motor coordination using the rotarod test revealed a significant difference in latency to fall before and after AAV/DT injection in the CX3cr1 Cre + group three weeks post-DT injection. Furthermore, the elapsed time and number of steps in the beam walking test were significantly higher in AAV/DT-injected CX3cr1 Cre + mice compared to controls three weeks post-DT injection. However, behavioral tests conducted three days post-DT injection showed no significant differences between CX3cr1 Cre + AAV/DT mice and CX3cr1 Cre - mice.

Conclusion

We demonstrate that long term ablation of microglia in lateral CN plays a major role in ataxic phenotype development.

POSTER #60

Multi-Ancestry GWAS of set-point viral load (spVL) in ~14,000 people of diverse ancestries living with HIV-1.

Abraham Awada[1,2], Paul J McLaren[1,2]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba
[2]National HIV and Retrovirology Laboratory at the JC Wilt Infectious Diseases Research Centre, National Microbiology Laboratories, Public Health Agency of Canada, Winnipeg, MB, Canada

HIV-1 spVL is as an indicator of disease progression, and, since it is a direct correlate of transmission potential, its reduction is a critical aspect of disease eradication. Previous genome-wide association studies (GWAS) of spVL have been generally conducted in small, single ancestry cohorts, limiting power for discovery. For this project, I will perform a GWAS in a combined analysis of ~14,000 people living with HIV-1 of diverse ancestries to identify genetic associations not previously observed. A large scale analysis such as the one proposed will increase detection power of genetic variants shared across ancestry groups and provide further insight into mechanisms of host control of HIV replication. Genome-wide genetic and clinical data have been obtained through the International Collaboration for the Genomics of HIV including 13,996 individuals and 16,010,173 genetic variants. Quality control and statistical analysis of the genotype data has been performed using PLINKv1.9. Imputation of the genotype data is being performed using the TOPMed Imputation Server, followed by association testing per cohort, and meta-analysis across cohorts. Following the completion of the GWAS, we will apply statistical fine-mapping approaches to identify potentially causal variants for further testing in laboratory models. Additionally, transcriptome-wide association study (TWAS) using predicted gene expression levels will be performed with PrediXcan to identify genes with expression levels correlated to spVL to further understand the biological mechanisms of spVL control. Finally, linkage disequilibrium score regression (LDSR) tests will be performed with webtools such as LD Hub to identify disease phenotypes with genetic overlap to HIV-1 spVL. Overall, the results of this study will identify previously undetected genetic variants that confer resistance to HIV-1 susceptibility, and will identify shared genetic variants with other immune and inflammatory diseases.

POSTER #95

Invariant Natural Killer T cell response to *in vitro* anti-LAG-3 and/or anti-PD-1 checkpoint marker blockades in HIV infection

Allison L Balasko[1], Monika M Kowatsch[1], Julie Lajoie[1,2], Keith R Fowke[1,2,3,4]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg MB. [2]Department of Medical Microbiology, University of Nairobi, Nairobi Kenya, [3]Department of Community Health Sciences, University of Manitoba, Winnipeg MB, [4]Partners for Health and Development in Africa, Kenya.

Introduction

Invariant Natural Killer T (iNKT) cells are innate lymphocytes critical in combatting viral infection. In people living with HIV (PLWH), iNKT cells are dysfunctional; our lab has shown that expression of lymphocyte activation gene 3 (LAG-3), an inhibitory checkpoint marker, is increased on iNKT cells, correlating with decreased cell function. Another checkpoint, PD-1, was shown to be increased on iNKT cells in HIV infection, correlating to decreased function. We hypothesize that blocking LAG-3 and/or PD-1 via immunotherapeutic blockades will restore *in vitro* iNKT function.

Methods

Utilizing peripheral blood mononuclear cells from PLWH (n=35) and HIV-uninfected controls (n=30), efficacy of anti-LAG-3 and anti-PD-1 blockades to enhance iNKT function was assessed via a 10-day *in vitro* iNKT stimulation assay, using iNKT cell-specific α-GalCer glycolipid antigen. Primary outcomes include (1) proportion of HLA-DR activation marker expression, and (2) enhanced proliferation, reported as proportional $\log_2$ fold-change.

Results

Elevated iNKT activation in PLWH group was seen by significantly increased proportion of iNKT cells expressing HLA-DR, wherein the LAG-3 alone, PD-1 alone, and dual PD-1+LAG-3 blockade conditions induced an increased mean expression of 60% (p=0.011), 62.6% (p=0.003) and 65.1% (p=0.0002), respectively, compared to the no blockade control (45.2%). In the HIV-uninfected control group, HLA-DR was significantly upregulated in the dual PD-1+LAG-3 condition (68.1%; p=0.008) compared to no blockade control (52.9%). Enhanced proliferation in the PLWH group was observed in PD-1 alone and dual PD-1+LAG-3 blockade conditions, with a mean of 5.7 (p=0.028) and 5.7 (p=0.036) $\log_2$ fold-change, compared to the no blockade control (4.1). In the control group, enhanced proliferation was observed in the PD-1 alone and dual PD-1+LAG-3 blockade conditions, with a mean of 6.1 (p=0.014) and 5.7 (p=0.036) proportional $\log_2$ fold-change, compared to the no blockade control (4.5).

Conclusion

This study provides proof-of-concept for targeting LAG-3 and PD-1 to reverse iNKT cell exhaustion in PLWH by enhancing iNKT cell activation and proliferative ability of iNKT cells. These findings are significant as anti-PD-1 and/or anti-LAG-3 immune checkpoint inhibitors may be used in PLWH to potentially ameliorate immune responses to various opportunistic infections, as well as boost viral control in a functional HIV cure approach.

POSTER #114

Alternative splicing-dependent interaction between presynaptic neurexin-1β and postsynaptic mGluR5

Kaden R. Baskerville[1,2], Sarah J. Cameron[1], Henry A. Dunn[1,2]

[1]Division of Neurodegenerative Disorders, St. Boniface Hospital Albrechtsen Research Centre, Winnipeg, Manitoba, Canada.

[2]Department of Pharmacology & Therapeutics, University of Manitoba, Winnipeg, Manitoba, Canada.

Introduction

G protein-coupled receptors (GPCR) regulate every aspect of physiology and are the target of ~34% of therapeutics. Recently, the accepted pharmacological principles of GPCRs have been critically altered by the discovery of trans-synaptic complexes involving synaptic adhesion molecules and GPCRs: specifically, a subset of metabotropic glutamate receptors (mGluRs). This paradigm-shifting discovery suggests other subsets of mGluR drug targets may also be modulated trans-synaptically: representing new avenues for drug design. The high sequence homology between all 8 mGluRs led us to investigate if the other mGluRs are modulated by different synaptic adhesion molecules. Of all mGluRs, mGluR5 stands out as the most encouraging target for neurological diseases, representing an attractive target to search for new trans-synaptic modulators.

Methods

Potential binding partners were identified via literature analysis of published IP: mGluR5 LC-MS/MS data. Verification of the interactions was done via trans-cellular co-immunoprecipitation experiments between two discrete HEK293 cell populations that were separately transfected.

Results

Published mass spectrometry data revealed that neurexin-1 binds mGluR5. The subsequent co-immunoprecipitation experiments we conducted demonstrated that neurexin-1 alternative splice variants (AS4- and AS4+), known for their integral roles in synaptic density, excitatory/inhibitory synaptic differentiation, glutamatergic excitability and organization, and autism-like behavior also trans-cellularly bind to mGluR5. Interestingly, the alternative splice variant neurexin-1β (AS4-) preferentially interacted mGluR5 as compared to neurexin-1β (AS4+). Furthermore, the ectodomain of neurexin-1β (AS4-) was sufficient to bind mGluR5, demonstrating that this interaction occurs extracellularly *in trans.*

Conclusion

This research demonstrates a novel mGluR-adhesion molecule interaction. Experiments are currently underway to determine if mGluR5 pharmacology is modulated by neurexin-1β. Interestingly, both mGluR5 and neurexin-1β mutations are associated with neurological disease etiology and treatment strategies. This research could lead to the development of novel mGluR5 targeted pharmaceuticals for patients harboring deficits in neurexin-1.

POSTER #65

Synthesis of boron-containing drug candidates and application as potential PARP inhibitors.

Ahmet Burak Berk[1], Geoffrey K Tranmer[1, 2]

[1] Rady Faculty of Health Science, College of Pharmacy, University of Manitoba, Winnipeg, MB R3E 0T5, Canada.
[2] Department of Chemistry, Faculty of Science, University of Manitoba, Winnipeg, MB R3E 0T5, Canada

Introduction

Poly (ADP-Ribose) Polymerase (PARP) are enzymes that consists of 18 family members and NAD^+ is used as a substrate by the enzyme. PARP is involved in many cellular activities including DNA repair and apoptosis. The inhibition of PARP is utilized for therapeutic purposes such as anticancer therapy. These inhibitors can be used as sensitizers to increase the efficacy of anticancer treatments such as chemotherapy drugs and radiation therapy. Especially, PARP inhibitors are preferred as single-agent drugs in BRCA-deficient breast/ovarian/prostate cancer patients. On the other hand, designing new drugs possessing boron-containing heterocyclic rigid structures will allow them to improve additional drug-enzyme interactions based on forming tertiary complexes between boronic acids and responsible amino acids by serine and threonine residues due to the capability of their Lewis acidity. This study aims to develop a new synthesis method and create a library for the compounds based on benzoxazaborine and benzadiazaborine scaffold, which might be utilized as PARP inhibitors.

Methods

Conventional and microwave synthesis methods are used in this study. Although liquid-liquid extraction is enough to receive some pure products, solid-phase extraction is also utilized for some product purification. The compounds will be confirmed by proton and carbon-13 nuclear magnetic resonance spectroscopy (^{1}H-NMR and ^{13}C-NMR) and ^{19}F NMR will be used to confirm the presence of fluoride or its absence.

Results:

Compounds have been synthesized successfully and clear NMR spectra are received. The range of yield for compounds is between 40% to 95%.

Conclusion:

2-aminophenyl boronic acid is reacted with acetic anhydride, acetonitrile, and benzoyl chlorides, and a new library of boron-containing heterocycles is created in good yield. We will continue to synthesize some molecules and will carry out the enzymatic PARP inhibition assay.

POSTER #129

Examining Structural Changes using Voxel-Based Morphometry and Cortical Thickness Analysis in Patients with Posttraumatic Stress Disorder following Cognitive Processing Therapy

Annika Bernacki[1,2], Tiffany A. Kolesar[1,2], Iman Beheshti[1,2], Ronak Patel[3], Ji Hyun Ko[1,2]

[1]Department of Human Anatomy and Cell Sciences, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada.

[2]PrairieNeuro Research Centre, Kleysen Institute for Advanced Medicine, Health Science Centre, Winnipeg, MB, Canada.

[3]Department of Clinical Health Psychology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada.

Introduction

Posttraumatic stress disorder (PTSD) is a debilitating psychiatric condition occurring following exposure to a traumatic event. Structural neuroimaging studies have highlighted neuroanatomical differences in brain regions such as the hippocampus, amygdala, and medial prefrontal cortex in individuals with PTSD. Cognitive Processing Therapy (CPT) is an evidence-based treatment for PTSD that focuses on reframing the thoughts that occur following traumatic exposure. Although CPT has shown success in decreasing clinical symptom severity, its underlying neural mechanisms remain unclear. We hypothesize that CPT would increase grey matter volume (GMV) and cortical thickness (CT) in the brain regions that are relevant to PTSD symptoms.

Methods

Sixty-seven participants, including patients with PTSD (n = 40) and healthy controls (n = 27), underwent MRI scans at baseline and follow-up, 12 weeks later. The PTSD group completed a 12-week CPT treatment, while the controls did not undergo any treatment. All participants completed the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5) before and after CPT treatment. To investigate the effects of successful CPT treatment, participants who did not respond to treatment were excluded from analyses. The T1-weighted MRI data were analyzed by voxel-based morphometry (VBM) and cortical thickness (CT) routine embedded in Computational Anatomy Toolbox 12 (CAT12). CT analyses were performed using 72 cortical parcellations. Imaging-based results (GMV and CT) results were correlated with CAPS-5 scores.

Results

CAPS-5 scores were found to decrease significantly following CPT treatment in the PTSD group. VBM analysis revealed a positive interaction between group and time, revealing increased GMV in the dorsolateral prefrontal cortex (DLPFC) of patients with PTSD post-CPT.Interestingly, in the increased GMV in the DLPFC was correlated with decreased score in a subsection of the CAPS-5 (criterion B) which accounts for intrusive symptoms and memories. No significant CT results were found.

Conclusion

Our findings suggest that CPT leads to neuroanatomical changes in regions associated with cognitive control and emotional regulation, notably the DLPFC. Changes in DLPFC GMV may underlie CPT-driven symptom reduction in PTSD and may also act as a critical biomarker for monitoring treatment effectiveness. Our findings also highlight the importance of investigating neural mechanisms underlying psychotherapeutic interventions.

POSTER #83

Investigating the white adipose tissue phenotype in the male and female Phb1-C69A knock-in mice.

Kate Bernier[1], Suresh Mishra[1,2]

[1]Department of Physiology and Pathophysiology, Rady Faculty of Health Sciences, University of Manitoba.
[2]Department of Internal Medicine, Max Rady College of Medicine, University of Manitoba

Introduction

White adipose tissue (WAT) is the primary site in the body for storing triglycerides. WAT distribution and function vary based on sex, and accordingly, disorders in which WAT plays a role, including obesity, type two diabetes, and metabolic syndrome, have sex differences in risk factors and etiologies. Prohibitin 1 (PHB1) is a protein involved in adipocyte-intrinsic activities sensitive to sex hormone signaling. PHB1 has a bidirectional relationship with estrogens and androgens, partly mediated by tyrosine phosphorylation. Another post-translational modification site in PHB1 is Cys69. It is the only cysteine residue in the amino acid sequence of the Phb1 gene, and it is essential in targeting PHB1 to plasma membranes in adipocytes. To investigate the role of Cys69 at a systemic level, we developed a knock-in mouse model replacing Cys69 with an alanine in Phb1. These are known as the Phb1-KiC69A (or Phb1-Ki) mice. Based on what is known about PHB1, we hypothesized that these mice would display sex differences in their WAT phenotype. This study's objectives were to define changes in the WAT phenotype in the male and female Phb1-Ki mice compared to wild-type mice, and to determine whether any differences displayed sex-specificity.

Methods

Visceral (VAT) and subcutaneous (SAT) adipose tissue was collected from wild-type and Phb1-Ki male and female mice. They were compared based on histology, gene and protein expression (RT-PCR and Western immunoblotting), and PHB1 subcellular localization. Primary cells from VAT and SAT were collected and differentiated into adipocytes, and lipolysis and lipid uptake were measured. The serum concentrations of three adipokines involved in metabolic regulation were analyzed.

Results

Phb1-Ki mice have sex- and depot-specific changes compared to wild-type mice in lipid droplet size, tissue mass, PHB1 subcellular localization, and adipocyte protein expression levels. Resistin levels are also decreased in both sexes of Phb1-Ki mice compared to wild-type. Adipocytes from VAT and SAT show no changes in lipogenesis or lipolysis in primary cultures between sex and genotype.

Conclusion

The Phb1 Cys69 residue affects the WAT phenotype in a sex-specific manner in mice. However, this does not interrupt triglyceride synthesis or release in adipocytes in vitro.

POSTER #43

Novel knock-in mouse models of prohibitin-1 revealed its role in sex-related differences in kidney biology.

Yeshika Bhatia[1], Niloofar Beheshti Dehkordi[1], Katherine Bernier[1], Kinnari Shah[1], Suresh Mishra[1,2]

[1]Department of Physiology & Pathophysiology.
[2]Department of Internal Medicine, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada

Introduction:

Prohibitin-1 (PHB1) is an evolutionarily conserved pleiotropic protein. Recent findings in our laboratory from transgenic mouse models of PHB1 (PHB1-Tg) and a phospho-mutant form of $PHB1^{Y114F}$ (m-PHB1-Tg) have revealed its role in interrelated sex differences in adipose and immune functions in physiology and pathophysiology. This involves two interconnected post-translational modifications of PHB1 (i.e., the palmitoylation at Cys^{69} site and the phosphorylation at Tyr^{114} site) previously identified in our laboratory. However, it is not known whether PHB1 has a role in sex-related differences in other cell, tissue, and organ types.

Methods:

To gain new insights and to further explore sexually dimorphic pleiotropic attributes of PHB1 at the systemic level, we developed *Phb1*^{C69A} and *Phb1*^{Y14F} knock-in mouse models separately (lacking the Cys^{69} palmitoylation site and the Tyr^{114} phosphorylation site in PHB1) using state-of-the art CRISPR/Cas9 technology. Here, we report initial phenotypic characterization of the *Phb1* knock-in mouse models using a combination of research tools and techniques.

Results:

The *Phb1*^{C69A} and *Phb1*^{Y114F} mice displayed both similarities and dissimilarities in sex-related differences in their immunometabolic phenotypes. Interestingly, sex-related differences in their kidney size were apparent in *Phb1* knock-in mice when compared with age- and sex-matched wild-type mice. The male *Phb1*^{Y114F} mice had significantly larger kidney than the male *Phb1*^{C69A} mice and wild-type mice, as well as their female counterparts. Further analysis of kidney from the knock-in mice showed structural differences in glomeruli and tubules correlating their size differences. Moreover, analyses of kidney lysates by immunoblotting revealed sex-specific differences in the levels of K48- and K63-polyubiquitinated proteins suggesting sex-related differences in ubiquitin-proteasome system in *Phb1* knock-in mice, which may have contributed to sexually dimorphic kidney phenotype.

Conclusion:

Initial phenotypic characterization of the *Phb1* knock-in mouse models further established our discovery of PHB1 as a pleiotropic mediator of sex-related differences adipose and immune functions and revealed a role of PHB1 in sex-related differences in kidney biology. As sex differences are known to exist in the structure, physiology, pathophysiology, and in age-related decline in kidney function, the *Phb1* knock-in mouse models have created new opportunities and research directions to advance our understanding in this field.

POSTER #125

Omega-3 fatty acids modify monocyte glucose metabolism through mitochondrial bioenergetic rewiring.

Michael J. Byun[1], Roni Armon[1,2], Tamaris Souza[3], Hope D. Anderson[1,2], Ayesha Saleem[3,4], Samantha D. Pauls[1,2,4]

[1]College of Pharmacy, University of Manitoba, Winnipeg, Canada.
[2]Canadian Centre for Agri-Food Research in Health and Medicine, St Boniface Hospital Research Centre, Winnipeg, Canada.
[3]Faculty of Kinesiology and Recreation Management, University of Manitoba, Winnipeg, Canada.
[4]Children's Hospital Research Institute of Manitoba, Winnipeg, Canada.

Background

Chronic inflammation is a driving factor in metabolic diseases like obesity and type 2 diabetes. Enhanced glucose metabolism, including via oxidative phosphorylation, may contribute to heightened immune activation. A recent clinical trial showed that supplementation with the omega-3 fatty acid α-linolenic acid (ALA) reduced oxidative phosphorylation rates in circulating monocytes. However, the mechanism(s) remains unknown.

Objective

Therefore, our objective was to replicate the findings in a cell culture model to explore the molecular mechanism.

Methods

THP-1 monocytes were treated for 48h with 10-40 µM of fatty acid, with a bolus dose at 24h. The Seahorse XFe24 and Oroboros O2k Oxygraph instruments were used to approximate catabolic rates (including oxidative phosphorylation and glycolysis) in the presence of glucose as the metabolic substrate. We also examined mitochondrial reactive oxygen species (ROS) levels using the fluorescent indicator mitoSOX, measured by flow cytometry. Pro-inflammatory cytokine (IL-1β) level was measured by ELISA. Finally, gene expression was assessed by reverse-transcription quantitative polymerase chain reaction (RT-qPCR).

Results

ALA significantly reduced mitochondrial ATP production by ~26% and increased glycolytic ATP production by ~50% in the presence of glucose. Unexpectedly, another omega-3 fatty acid, docosahexaenoic acid (DHA) had similar effects. ALA had no effect on ROS while DHA enhanced ROS Both ALA and DHA treatment downregulated IL-1β levels compared to vehicle. We also identified pyruvate dehydrogenase kinase 4 (PDK4), an enzyme that inhibits the conversion of pyruvate to acetyl-CoA, as a possible mechanistic candidate. It was significantly upregulated by ALA and DHA by 4- and 13-fold, respectively.

Conclusion

Overall, ALA and DHA both upregulated PDK4 and dampened oxidative phosphorylation rates in our cell culture model. This was accompanied by suppressed pro-inflammatory cytokine production. However, DHA specifically enhanced ROS, a sign of mitochondrial stress. This is an important step towards understanding how omega-3 fatty acids may be useful as part of an intervention strategy to prevent or treat chronic metabolic diseases relevant to children and youth.

POSTER #86

Investigating the contribution of the zebrafish rostral migratory stream to neural repair

Aurelien Caron[1], Benjamin W. Lindsey[1,2]

[1]University of Manitoba, Department of Human Anatomy and Cell Science., [2]Children's Hospital Research Institute of Manitoba (CHRIM).

Introduction

The zebrafish model is renowned for its remarkable regenerative ability. Following injury to the brain, neural stem cells (NSCs) increase proliferation and differentiation to replace lost neurons. However, brain injuries are often performed near the stem cell niche, thus we have little understanding of how distal NSCs might contribute to long-distance brain repair. To address this question, we capitalize on the presence of a Rostral Migratory Stream (RMS)-like structure in the adult zebrafish brain, that like mammals, continuously sends new neurons to the olfactory bulbs (OB) from the forebrain stem cell niche. We hypothesized that the RMS can be recruited for OB regeneration.

Methods:

We developed an OB injury model and used a thymidine analogue to measure the recruitment of progenitors to the injury site. However, because zebrafish also contain NSCs locally in the OB, in addition to the RMS, a method to distinguish the contribution of both populations to repair was necessary. To overcome this challenge, we developed a labeling approach combining cerebroventricular microinjection (CVMI) and thymidine analogue tracing to specifically label NSCs arising from the RMS.

Results:

Results to date show that following OB injury, local immune cells are recruited to the injury site, followed by an increase in NSC proliferation in the forebrain. By 7- and 14-days post-injury an increase in neural progenitors is observed at the injury site, many of which differentiate into neurons, demonstrating that cellular migration of NSCs along the RMS can be stimulated by distal injury. Additionally, we were able to successfully track progenitors derived uniquely from the RMS and not local NSCs in the bulbs using our CVMI/thymidine method.

Conclusion:

The zebrafish RMS is, much like its mammalian counterpart, a plastic structure that can be recruited for OB repair. This is a first step towards understanding long-distance recruitment of neural progenitors for brain regeneration.

POSTER #40

Investigating RIPK3 Inhibition as a Therapeutic Target for Respiratory Syncytial Virus Infection

Júlia Cerato[1,2], Maria Serda[1], Wenming Duan[3], Theo Moraes[3], Kevin Coombs[1], and Barbara Porto[1,2]

[1] Department of Medical Microbiology and Infectious Diseases, Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada.

[2] Biology of Breathing Group, Children's Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada.

[3] Program of Translational Medicine, The Hospital of Sick Children, Toronto, Ontario, Canada.

Introduction

Respiratory syncytial virus (RSV) is the leading cause of hospitalization due to pediatric viral respiratory tract infection, responsible for 200,000 infant deaths worldwide. There are no effective antiviral therapies or approved vaccines for infants. Therefore, studying drug repurposing could lead to a novel therapeutic approach against RSV infection. RSV infection induces necroptosis through the activation of receptor-interacting protein kinase 3 (RIPK3), enhancing disease pathogenesis. Pharmacological inhibition or genetic deficiency of RIPK3 decreases RSV viral load and lung inflammation in mice. Dabrafenib is an FDA-approved anticancer drug that selectively inhibits RIPK3. Thus, we hypothesized that dabrafenib has antiviral effects against RSV by inhibiting RIPK3-mediated necroptosis.

Methods

MTT assay was performed to evaluate the effect of increasing concentrations of dabrafenib for different time points on A549 cell viability. A549 cells were infected with RSV-GFP and treated with dabrafenib either simultaneously, prophylactically, or therapeutically. Lactate dehydrogenase (LDH) release was measured as a marker of lytic cell death. Infection rate and fluorescence intensity were quantified by immunofluorescence. Proteomic analyses were conducted using quantitative mass spectrometry. RSV-GFP infectious progeny release was assessed through a lysis plate titration assay. We quantified RSV-GFP replication by immunofluorescence and qPCR in human primary nasal epithelial cells (HNECs) treated with dabrafenib.

Results

Dabrafenib did not alter A549 cell viability at all concentrations tested. The drug showed a dose-dependent inhibition of RSV infection, with an IC_{50} at 40.17µM, and protected A549 cells from RSV-induced lytic cell death. All treatments significantly decreased RSV infection rate and fluorescence intensity. Furthermore, therapeutic treatment with dabrafenib significantly reduced the release of RSV infectious progeny. Dabrafenib also reduced 80% of RSV viral load in HNECs. Dabrafenib profoundly altered the A549 cell proteome, inducing the upregulation of antiviral proteins.

Conclusions

Dabrafenib treatment significantly impairs RSV replication *in vitro*, suggesting that RIPK3 is necessary for viral replication.

POSTER #82

Role of scleraxis in angiotensin II-induced cardiac fibrosis

Sikta Chattopadhyaya [1,2]; Raghu Sundaresan Nagalingam [3]; D. Allison Ledingham [1]; Michael P. Czubryt[1,2]

[1]Institute of Cardiovascular Sciences, St. Boniface Hospital Albrechtsen Research Centre.
[2]Department of Physiology and Pathophysiology, Rady Faculty of Health Sciences, University of Manitoba.
[3]BC Children's Hospital Research Institute, Simon Fraser University.

Introduction

Cardiac fibrosis involves the activation and conversion of fibroblasts to myofibroblasts in response to pathophysiological stresses, resulting in structural and functional remodeling of the heart leading to heart failure and death. Although it affects millions of people worldwide, currently no medications are available against cardiac fibrosis. Similar to the pro-fibrotic growth factor TGF-β, angiotensin II (AngII) contributes to cardiac fibrosis, however AngII inhibitors are not used against fibrosis despite growing evidence of efficacy. AngII induces the nuclear translocation of Myocardin Related Transcription Factor-A (MRTF-A) which acts with Serum Response Factor (SRF) to play a major role in the conversion of fibroblasts to myofibroblasts, similar to the transcription factor scleraxis. Scleraxis has been shown by our lab to induce fibrosis by transactivating various pro-fibrotic gene promoters. Here we investigated whether AngII works through MRTF-A/SRF/scleraxis pathway to induce cardiac fibrosis.

Results

AngII delivery through osmotic mini-pumps in C57Bl/6 mice significantly increased blood pressure and cardiac hypertrophy compared to saline control group. In the AngII group, cardiac fibrosis was observed with enhanced mRNA expression of pro-fibrotic genes, Col1a1, Col3a1, periostin, and EDA-fibronectin. Interestingly there was a significant increase in scleraxis as well, suggesting its involvement in AngII-mediated fibrosis. Rat cardiac fibroblasts treated with AngII showed a significant increase in scleraxis expression, and scleraxis knockdown significantly attenuated AngII-induced collagen expression, confirming a requirement for scleraxis in the AngII-mediated fibrosis pathway. NIH3T3 cells transfected with MRTF-A and SRF together showed a high level of induction of scleraxis expression. Luciferase assays showed that MRTF-A and SRF act together to regulate scleraxis expression by binding to and transactivating the scleraxis promoter.

Conclusion

AngII activates a MRTF-A/SRF/scleraxis pathway for inducing cardiac fibrosis, therefore targeting scleraxis may be an effective anti-fibrotic strategy.

POSTER #57

Muscarinic acetylcholine type-1 receptor antagonism activates TRPM3 enhancing mitochondrial function for sensory neuron regeneration.

Sanjana Chauhan[1,2], Shiva Shariati-Ievari[1,3], Michel Aliani[1,3], and Paul Fernyhough[1,2]

[1]Division of Neurodegenerative Disorders, St. Boniface Hospital Albrechtsen Research Centre, University of Manitoba, Winnipeg, Canada.

[2]Department of Pharmacology and Therapeutics, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada.

[3]Department of Food and Human Nutritional Sciences, Faculty of Agricultural and Food Sciences, University of Manitoba, Winnipeg, Canada.

Diabetic peripheral neuropathy (DPN) comprises a dying-back axonal degeneration leading to sensory loss and neuropathic pain. We are developing strategies to drive regeneration of sensory neurons by blocking the muscarinic acetylcholine type 1 receptor (M_1R). M_1R antagonism induces a slow increase of intracellular Ca^{2+} that stimulates mitochondrial function and neurite outgrowth. The transient receptor potential channel 3 (TRPM3) is a potential source of Ca^{2+} when phosphatidylinositol 4,5-bisphosphate (PIP_2) levels are low. Hence, we hypothesized that M_1R antagonism caused blockade of G protein signaling leading to PIP_2 levels rising and activation of TRPM3. Adult dorsal root ganglion (DRG) sensory neurons derived from control or type 1 diabetic rats were used to test this hypothesis. TRPM3 agonists elevated mitochondrial function and augmented neurite outgrowth and this effect was abolished by TRPM3 inhibitors blocking Ca^{2+} influx. Moreover, shRNA mediated TRPM3 knockdown blocked TRPM3 agonist stimulation. Pirenzepine, a selective M_1R antagonist, induced neurite outgrowth which was suppressed by TRPM3 knockdown. Untargeted metabolomics revealed a marked increase in galactose and pyruvate metabolism induced by TRPM3 agonists, demonstrating TRPM3 activation stimulated neuronal bioenergetics. These novel results reveal that TRPM3 channels mediate the stimulatory effect of M_1R antagonism on mitochondrial function and neurite growth. These findings support ongoing clinical trials with M_1R antagonists in persons with peripheral neuropathies and DPN.

POSTER #164

Targeting Non-Genetic Kinase-Dependent Signaling Pathways to Prevent Medulloblastoma Tumor Relapse

Ujala Chawla[1], Emma Martell[1,2], Helgi Kuzmychova[1], Harshal Senthil[1], Esha Kaul[3], Chitra Venugopal[4], Sheila K. Singh[4,5], Tanveer Sharif[1,2]

[1]Department of Pathology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada.
[2]Department of Human Anatomy & Cell Science, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada.
[3]Faculty of Science, University of Manitoba, Winnipeg; MB, Canada.
[4]Department of Biochemistry, MacMaster University, Hamilton. [5]Department of Surgery, McMaster University, Hamilton, Ontario, Canada

Introduction

Brain tumors are the leading cause of pediatric cancer death, and medulloblastoma (MB) is one of the most common malignancies, contributing to around 20-25% of all pediatric brain tumors. Proteogenomic profiling categorizes MB into four subgroups: Wingless (WNT), Sonic Hedgehog (SHH), Group 4 (G4), and Group 3 (G3). G3 MB, which contributes to a quarter of all cases, presents a significant challenge due to its high metastatic potential and poor prognosis, with only a 50% 5-year survival rate. Standard treatment involves surgery, radiation, and chemotherapy. However, about 30% of MB patients experience recurrence, often recurrent tumors are insensitive to current treatments, leading to fatalities. Genomic analyses show that recurrent tumors maintain the genetic characteristic of the original subgroup, suggesting that non-genomic cellular signaling changes are key drivers of tumor recurrence. Kinases, which regulate cellular signaling, play a crucial role. By altering protein phosphorylation, kinases can affect cellular functions like transcription, migration, proliferation, and survival. Therefore, understanding the cellular signaling adaptations in recurrent tumors is crucial. We hypothesize that alterations in signaling mechanisms contribute to tumor recurrence in MB.

Methods

Various well-characterized MB cells and orthotopic intracerebellar xenograft mouse models were treated with a clinically relevant chemoradiotherapy (CRT) regimen. Proteome Profiler Human Phospho-Kinase Array Kit and Immunohistochemistry identified changes in kinase activation. Therapy-resistant cells were treated with kinase inhibitors to assess sensitivity, stemness, and metastatic characteristics through cell viability, tumorsphere, and migration assays.

Results

Phospho-Kinase Array Kit and Immunohistochemistry revealed that there were increased levels of phosphorylated-Src (p-Src) and its related downstream mediators in G3 MB CRT-resistant (CRT-Res) cells but not in SHH MB CRT-Res cells. G3 MB CRT-Res cells were highly susceptible to the p-Src inhibition compared to untreated cells; inhibiting p-Src impaired stemness capacity and self-renewal of stem cells in G3 MB CRT-Res cells. Additionally, p-Src inhibition hindered the migratory potential of CRT-Res G3 MB cells in transwell migration assays.

Conclusion

These findings reveal distinctive signaling mechanisms that could contribute to tumor recurrence in G3 MB, while also highlighting the therapeutic potential of targeting the Src signaling pathway to combat CRT resistance in recurrent G3 MB.

POSTER #135

Safety of cannabinoids used for medical purposes in children: A systematic review and meta-analysis.

Manik Chhabra[1], Mohamed Ben-Eltriki[1], Holly Mansell[2], Richard J Huntsman, MD[3], Yaron Finkelstein[4], Lauren E Kelly,[1,5]

[1]Department of Pharmacology and Therapeutics, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada.,

[2]College of Pharmacy, University of Saskatchewan, Saskatoon, Canada.

[3]Department of Pediatrics, IWK Health Centre, Halifax, Nova Scotia, Canada.

[4]Divisions of Emergency Medicine and Clinical Pharmacology and Toxicology, Hospital for Sick Children, University of Toronto, Toronto, Canada.,

[5]Children's Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada

Introduction

Cannabinoids are increasingly used for medical purposes in children. Evidence of cannabinoid's safety in this context is sparse, creating a need for reliable information to close this knowledge gap. We conducted a systematic review and meta-analysis of randomized controlled trials (RCTs) to study the safety of cannabinoids used for medical purposes in children.

Methods

MEDLINE, Embase, PsycINFO, and the Cochrane Library were searched. Two reviewers independently performed the title and abstract, full-text review, data extraction, and quality assessment. Data extraction included for characteristics of included studies, study participants, interventions, withdrawals, and adverse events. Adverse event data was analyzed using a "metabin" package in R studio. The primary outcome was the incidence of withdrawals, withdrawals due to adverse events, overall adverse events, and serious adverse events in the cannabinoid and control arm groups. The secondary outcomes were the incidence of specific serious adverse events and adverse events based on organ system involvement.

Results

Of 39,341 citations, 23 RCTs were included; 14 trials were in children only. Interventions included purified CBD (47.8%, n=11), nabilone (17.3%, n=4), tetrahydrocannabinol (17.3%, n=3), cannabis herbal extract (17.3%, n=3), and dexanabinol (8.6%, n=2). The most common indications were epilepsy (39.1%, n=9) and chemotherapy-induced nausea and vomiting (30.4%, n=7). Compared with the control group, cannabinoids were associated with an overall increased risk ratio (RR) of adverse events (RR:1.09, 95%CI: 1.02-1.16; I251%, 13 trials), withdrawals due to adverse events (RR:2.99, 95%CI: 1.69-5.29, I20%, 14 trials), and serious adverse events (RR:1.77, 95%CI: 1.13-2.79, I262%, 11 trials). Cannabinoid-related adverse events with higher RR were diarrhea (RR:1.94, 95% CI 1.37-2.77; I241%, 10 trials), increased serum levels of AST (RR:5.69, 95%CI 1.74-18.64; I20%, 5 trials), ALT (RR:5.74, 95%CI 2.26-14.57; I20%, 6 trials), and somnolence (RR:2.23, 95%CI: 1.77-2.82; I223%, 14 trials)

Conclusion

Cannabinoids used for medical purposes in children in RCTs are associated with an increased risk of adverse events. Long-term safety studies, including those exploring cannabinoid-related drug interactions and tools that improve adverse event reporting, are needed.

POSTER #99

The Influence of Geometric Model Selection on Estimates of Axon Diameters in the Corpus Callosum of a Mouse

Madison Chisholm[1], Bibek Dhakal[2], Emma Friesen[3], Sheryl Herrera[4], Morgan Mecredi[4], John Gore[2], Mark Does[2], Melanie Martin[4]

[1]University of Winnipeg, Department of Biology.
[2]Vanderbilt University Institute of Imaging Science (VUIIS).

[3]The University of Winnipeg, Department of Chemistry, [4]The University of Winnipeg, Department of Physics.

Introduction

Axon diameters can be estimated using temporal diffusion spectroscopy (TDS). To investigate smaller diameter axons, Oscillating Gradients in a Spin Echo (OGSE) pulse sequence at higher frequencies can replace Pulsed Gradient Spin Echo pulse sequences (PGSE) to achieve shorter diffusion times. The most common geometric model used to estimate axonal dimensions assumes axons are parallel cylinders. However, previous research has demonstrated that this geometric model tends to overestimate the intra-axonal diameters. TDS can be used to infer cell sizes assuming other geometries such as spheres.

Methods

In this project we compare results obtained by acquiring TDS in the genu substructure of the corpus callosum in a mouse model. These were analyzed using the ActiveAx Model comparing cylindrical and spherical geometries to test the importance of geometric model selection for estimating the diameters of smaller axons. Imaging was performed using a 15.2 T Bruker BioSpec MRI System equipped with a triaxial gradient system with maximum gradient strength of 1000 mT/m. The signals (means ± standard deviations) were then extracted from ROIs and fitted to the ActiveAx model, modeled either as cylinders or spheres.

Results

The mean effective axon diameter inferences for the signal fitting to the cylindrical model is 1.9 ± 0.2µm. The mean effective axon diameter inferences for the signal fitting to the spherical model is 2.1 ± 0.3µm.

Conclusion

The results of the current project suggest both cylindrical and spherical geometrical models of axons used with TDS and OGSE pulse sequences infer similar axon diameters in the mouse corpus callosum. Validation of experimental results will be obtained using electron microscopy. Future work focused on optimizing imaging parameters towards better uncertainties is required. The authors thank NSERC for funding.

POSTER #163

Mycoplasma genitalium infection in a cohort of adolescents and young women of Mombasa, Kenya.

Angela R. Copete[1], Irene Martin[2], David C. Alexander [3], Shelley W. Peterson [2], Lyle R. McKinnon [1], Ruth S. Mwatelah [1], Marissa Becker [4], Sharmistha Mishra [5], Mariana del C. Herrera[1], Yoav Keynan [1,4], Zulma V. Rueda [1]

[1] Department of Medical Microbiology and Infectious Diseases, University of Manitoba.
[2] Streptococcus and STI Unit, National Microbiology Laboratory, Public Health Agency of Canada.
[3] Cadham Provincial Laboratory, Province of Manitoba-Canada.
[4] Department of Community Health Sciences, University of Manitoba., [5] Institute of Health Policy, Management and Evaluation, University of Toronto.

Introduction

This study aimed to determine the clinical and analytical performance of a commercial M. genitalium assay and to estimate its prevalence in the female urinary tract among adolescents and young women.

Methods

We selected 169 midstream urine samples from participants who reported symptoms consistent with sexually transmitted infections (STIs) and matched 183 samples from asymptomatic participants based on age and exposure to STIs. Samples were stored at -80°C until processing. Two assays were used for M. genitalium detection: the Anyplex™ STI (Seegene), a multiplex real-time PCR that detects seven STI pathogens, and a TaqMan™ Microbe Detection Assay (ThermoFisher), a real-time PCR that detects M. genitalium. Positive samples for M.gen were sent to the National Microbiology Laboratory for antimicrobial genotyping.

Results

Among 169 midstream urine samples from symptomatic participants, the Anyplex™test detected three (1.7%) positive samples for M. genitalium. The TaqMan™ qPCR detected nine (5.3%), including those detected by the Anyplex® test. It was found that the 24 preliminary tested samples from asymptomatic participants did not contain M. gen. Two strains had mutations in the 23SrRNA gene that predict azithromycin resistance, and one of these strains also had mutations in the parC gene that predict moxifloxacin resistance. The analytical limit of detection of TaqMan Microbe assay was confirmed from 0.5 copies/µl, and the detection was linear up to 10.000 copies/µl of the M. genitalium genome. Among females with positive results for M. genitalium, there were no coinfections with Chlamydia trachomatis or Neisseria gonorrhoeae.

Conclusions

The prevalence of M. genitalium in this cohort is lower than in previous studies in other Kenyan and African cohorts. The TaqMan™ Microbe Detection Assay performed better than Anyplex™ STI to detect M. genitalium.

POSTER #70

Non-optimal bacteria species induce neutrophil-driven inflammation and epithelial barrier disruption in the female genital tract.

Marina Costa Fujishima[1], Paul Lopez[1], Morgan Taverner[2], Oluwaseun Ajibola[1], Adam Burgener[2,3,5] and Thomas Murooka[1,2]

[1]University of Manitoba, Rady Faculty of Health Sciences, Department of Immunology. [2]University of Manitoba, Rady Faculty of Health Sciences, Department of Medical Microbiology and Infectious Disease [3]University of Manitoba, Rady Faculty of Health Sciences, Department of Obstetrics and Gynecology [4]National HIV and Retrovirology Labs, JC Wilt Infectious Disease Research Centre, Public Health Agency of Canada, Winnipeg, Canada.

[5]Center for Global Health and Diseases, Case Western Reserve University.

Introduction

The mucosal surface of a healthy female genital tract (FGT) is comprised of physicochemical, immunological and microbial components that serve as a rapid, first line of defense against infections. Alterations in any of these components have been associated with higher HIV acquisition risk. Analysis from >700 women from the CAPRISA004 cohort show that women with a non-*Lactobacillus* dominant (non-optimal) vaginal microbiome were at significantly higher risk of sexual HIV acquisition, and that this strongly correlated with vaginal epithelial barrier disruption, inflammation and neutrophil accumulation. However, how FGT barrier function is impacted by changes in the vaginal microbiota, and a mechanistic understanding of mucosal neutrophils in this process, remains unclear. Here, we utilized microscopy and proteomic approaches to better define the interplay between vaginal microbial species, epithelial barrier function and neutrophil activation *in vivo*.

Methods

Balb/c mice were intravaginally inoculated with either PBS, *L. crispatus* (optimal), *Mobiluncus mulieris* or *Gardnerella vaginalis* (non-optimal). Cervicovaginal lavage (CVL) was collected on day 0, 2, 4, and 7 to confirm bacteria colonization through 16S rRNA sequencing and assess protein expression profile through mass spectrometry. Vaginal tissues were collected for immunohistochemical analysis. To determine neutrophils contribution to barrier disruption, barrier integrity was assessed in the presence or absence of neutrophils by intravaginally inoculating mice with lucifer yellow (0.45Da) and harvesting tissues to measure dye penetration into the epithelium by immunohistochemistry.

Results

Immunohistochemistry analysis demonstrate a larger neutrophil influx within the vaginal epithelium and the submucosa of mice inoculated with *M. mulieris* and *G. vaginalis* but not *L. crispatus.* High levels of inflammatory cytokines and neutrophil-related factors were also identified by proteomic analysis of collected CVL samples. Additionally, permeability assay revealed a significant loss of barrier integrity in mice inoculated with *M. mulieris* and *G. vaginalis*. However, upon depletion of neutrophils *in vivo*, vaginal barrier integrity was restored even in the presence of these non-optimal bacteria.

Conclusion

Our study demonstrates that the presence of non-optimal bacteria species in the FGT results in genital inflammation, high neutrophil activation and increases barrier breakdown. Excitingly, we show that neutrophils response to these non-optimal bacteria species directly impacts FGT barrier function *in vivo* and ongoing work will determine whether these changes in barrier function directly can enhance HIV acquisition.

POSTER #81

Loss of Circadian Period Gene Increases Cyp7A1 and Bile Acid Mediated Cardiac Cell Death Following Ischemia-Reperfusion in Cardiac Myocytes

Molly Crandall[1], Ashish H Shah[2], Inna Rabinovich-Nikitin[1], Lorrie Kirshenbaum[1,3]

[1] Department of Physiology and Pathophysiology, The Institute of Cardiovascular Sciences, St. Boniface Hospital Albrechtsen Research Centre, Winnipeg, Manitoba, Canada.

[2] Department of Internal Medicine, Section of Cardiology, St Boniface Hospital, Winnipeg, Manitoba, Canada.

[3] Department of Pharmacology and Therapeutics, Rady College of Medicine, Max Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada.

Introduction

Circadian rhythm serves as a potent regulator of lipid metabolism and bile acid (BA) synthesis, controlling nutrient availability in a time-of-day dependent manner. Digestion and absorption of nutrients occurs via BAs whose synthesis occurs through the enzymatic oxidation of cholesterol by the rate limiting enzyme, cholesterol 7alpha-hydroxylase (Cyp7A1). Studies have shown that expression of Cyp7A1 oscillates in a distinct circadian manner with peak expression occurring during the dark cycle, preceding circadian modulated triglyceride, and lipid-synthesis. Circadian misalignment contributes to several pathologies including obesity, type 2 diabetes, and cardiovascular disease. Interestingly, loss of circadian negative regulators, such as *Period 1/2*, causes abnormally high levels of Cyp7A1. Production of BAs is a major route of cholesterol elimination; however, excess BA production can be cytotoxic. Therefore, individuals with disrupted circadian rhythms, namely shift workers, may be at greater risk of cardiovascular disease from abnormal levels of Cyp7A1.

Methods/Results

Herein, we reveal for the first time, that genetic loss of Per gene produces inappropriate levels of circulating Cyp7A1, causing increased production of cytotoxic BAs and cardiac cell death. Notably, germ-line knockout of *Per1* and *Per2* in mice subjected to ischemia-reperfusion (IR) injury promotes increased cardiac expression of the Cyp7A1 protein. Cardiomyocytes treated with primary bile salt by-products of Cyp7A1, such as lithocholic acid, chenodeoxycholic acid, and deoxycholic acid, induces varying degrees of cell death, with lithocholic acid having the greatest impact on cell viability.

Conclusion

Our findings reveal a novel signaling axis that functionally connects circadian regulated Cyp7A1-bile salt production to cardiac injury following myocardial infarction.

POSTER #89

Barriers and facilitators to using a novel virtual reality treatment for phantom leg pain.

Megan Crooks[1], Renee El-Gabalawy[2,3], Lily Pankratz[1], Michael Smith[4], Elizabeth Hammond[5], Patrick Gross[6]

[1]Department of Psychology, University of Manitoba.
[2]Departments of Clinical Health Psychology and Anesthesiology, Perioperative and Pain Medicine, University of Manitoba.
[3]Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba.
[4]National Research Council., [5]College of Rehabilitation Sciences, Rady Faculty of Health Sciences, University of Manitoba., [6]Winnipeg Regional Health Authority.

Introduction

Despite the high prevalence rate of phantom limb pain (PLP) amongst people with amputations, there are not currently any interventions to address it that are both efficacious and feasible. For example, while there is promising evidence to suggest virtual reality (VR) and graded motor imagery (GMI) can reduce PLP, there are many barriers preventing their clinical implementation. The novel integration of GMI and VR offers the unique potential to address their individual shortcomings, improving the clinical utility of PLP treatment. Accordingly, the present multidisciplinary research team developed a head mounted virtual GMI program for people with lower limb amputations. The current research aimed to develop the VR prototype by evaluating barriers and facilitators in the population targeted for eventual implementation.

Methods

Twelve people with unilateral lower limb amputations recruited from outpatient physiotherapist and prosthetic clinics piloted the VR program in a single 2.5-hour session and identified potential facilitators and motivators to its use via semi-structured interviews. Field notes were also recorded to improve the feasibility of the VR program and provide context to the qualitative analysis.

Results

Mixed thematic analysis suggested that primary motivators to using the virtual GMI program include perceived benefit, ease of access, rehabilitative efficiency, and gamification. Meanwhile, primary barriers to its use included technological literacy, lack of resources, and the psychological/physical limitations of lower limb amputation. The identified barriers and facilitators are mapped onto the Consolidated Framework for Implementation Research (CFIR) to further elucidate how the VR program may be iteratively approved.

Conclusions

Future development will improve the practicality of the program by prioritizing embedded tutorials on VR operation, GMI psychoeducation, and a more simplified, accessible, and customizable user interface that addresses the diverse needs of people with lower limb amputations. Future research will assess the feasibility of the VR program in the postoperative setting immediately following lower limb amputation and assess its potential to mitigate PLP onset through a randomized controlled trial.

POSTER #106

L-Rham: gathering the evidence for a new treatment option for tubo-ovarian, high-grade serous cancer patients.

Sara K. Crooks[1], Megan C. Rodriguez[1], Christine A. Doucette[2], Mark W. Nachtigal[1,3,4].

[1]Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MB, Canada.
[2]Physiology and Pathophysiology, University of Manitoba, Winnipeg, MB, Canada.
[3]Obstetrics, Gynecology and Reproductive Sciences, University of Manitoba, Winnipeg, MB, Canada.
[4]Paul Albrechtsen Research Institute, CancerCare Manitoba, Winnipeg, MB, Canada

Introduction

The most commonly diagnosed and lethal subtype of ovarian cancer is tubo-ovarian, high-grade serous carcinoma (HGSC). While the majority of patients respond well to initial treatment with surgery and chemotherapy, many develop a chemotherapy-resistant recurrence of the disease. Therefore, finding new drugs that can kill HGSC using unique mechanisms is crucial in order to improve patient lives and outcomes. My research project focuses on a new drug called L-Rham. Our lab has already proved L-Rham is able to kill chemotherapy-resistant HGSC cells *in vitro* and *in vivo*. In order to progress to clinical trials, we must show the mechanism as to how L-Rham kill cells.

Methods

ROS production and mitochondrial depolarization were measured in L-Rham-treated CaOV3 HGSC cells. Alterations in cellular oxygen consumption were determined using a Seahorse XF Analyzer. Mitophagy was inferred by analysis of proteomic data and visualized using electron microscopy.

Results

We determined that L-Rham induces ROS, mitochondrial depolarization and decreased mitochondrial respiration in HGSC cells. I hypothesized that L-Rham initiates ROS-induced loss of mitochondrial function via mitophagy, and preliminary data using electron microscopy appear to confirm this. Ongoing experiments are examining L-Rham's effects on glycolysis and the TCA cycle, necroptosis as an alternative cell death pathway, and evaluating altered protein expression/localization associated with mitophagy.

Conclusion

Collectively, my project will result in a new understanding for how L-Rham kills chemotherapy-resistant HGSC cells. Eventually, this research will allow L-Rham to progress to clinical trials.

POSTER #100

Genomic analysis of antifungal resistance in *Candida parapsilosis* in Canada

Domenica G. De Luca[1,2], David Alexander[2,3], Tanis C. Dingle[4], Philippe Dufresne[5], Jeff Fuller[6], Greg J. German[7,8], David Haldane[9], Linda Hoang[10], Lei Jiao[11], Julianne Kus[12], Kathy Malejczyk[13], Caroline Sheitoyan-Pesant[14], Markus Stein[3], Morag Graham[1,2], Gary Van Domselaar[1,2], Amrita Bharat[1,2]

[1]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB [2]Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, MB [3]Shared Health Manitoba, Winnipeg, MB [4]Alberta Precision Laboratories – Public Health Laboratory, Calgary, AB [5]Laboratoire de santé publique du Québec, Sainte-Anne-de-Bellevue, QC [6]London Health Sciences Centre, London, ON [7]Health PEI, Charlottetown, PEI [8]University of Toronto Department of Laboratory Medicine and Pathobiology, Toronto, ON [9]QEII Health Science Centre, Halifax, NS [10]BC Centre for Disease Control, Vancouver, BC [11]Public Health Microbiology Laboratory, St. John's, NL [12]Public Health Ontario, Toronto, ON [13]Roy Romanow Provincial Laboratory, Regina, SK [14]Centre hospitalier universitaire Dr-Georges-L.-Dumont, Moncton, NB.

Introduction

Azole resistance in *Candida parapsilosis* has recently emerged, and most reports have found that the main mechanism of resistance arises from substitutions in the azole target, *ERG11*. Here, we used whole genome sequencing to elucidate the genomic epidemiology and to identify genetic determinants of resistance in fluconazole-resistant *C. parapsilosis* isolates.

Methods

The ten provincial public health laboratories in Canada provided 95 clinical *C. parapsilosis* isolates. Azole-resistant *C. parapsilosis* isolates from invasive infections were prioritized in the collection. We conducted phylogenomic analysis based on single nucleotide variants (SNVs) in the core genome for 21 fluconazole-resistant (MIC ≥ 8 ug/mL), 2 susceptible-dose dependent (MIC = 4 ug/mL), and 72 fluconazole-susceptible (MIC ≤ 2 ug/mL) *C. parapsilosis* isolates. We also examined variants in genes associated with azole resistance in *C. parapsilosis*, such as *CDR1*, *ERG11*, *MDR1*, *MRR1*, *TAC1*, and *UPC2*.

Results

Phylogenomic analysis revealed 12 clusters of genetically related isolates. Fluconazole-resistant *C. parapsilosis* isolates were associated with seven of the 12 clusters (cluster 1, n = 2; cluster 5, n = 7; cluster 7, n = 1; cluster 8, n = 1; cluster 9, n = 1; cluster 11, n = 2; and cluster 12, n = 6) and differed by five to 2577 SNVs. One fluconazole-resistant *C. parapsilosis* isolate did not cluster. Some fluconazole-resistant *C. parapsilosis* isolates were found in clusters with at least one other fluconazole-resistant isolate from the same healthcare institution. *ERG11* variants, Y132F (n = 2), Y132/Y132F (n = 1), K143/K143R (n = 1) and G458S (n = 1) were identified in five fluconazole-resistant *C. parapsilosis* isolates. Potential novel resistance variants present exclusively in azole-resistant *C. parapsilosis* isolates were found in *CDR1*, *MDR1*, *MRR1*, *TAC1*, and *UPC2*.

Conclusions

These findings demonstrate the diversity of fluconazole-resistant *C. parapsilosis* isolates circulating in Canada. Since most fluconazole-resistant *C. parapsilosis* isolates in this collection contained a wildtype *ERG11* gene, other mechanisms, such as transcription factors, efflux pumps or copy number variations, may mediate azole resistance.

POSTER #165

Concordance of taxonomic classifications for rare enteric bacterial pathogens from whole genome sequencing and 16S rRNA sequence analysis

Julie Donahue[1, 2], Sara Christianson[1], Celine Nadon[1, 2]

[1]National Microbiology Lab, Public Health Agency of Canada, Winnipeg.
[2]University of Manitoba, Department of Medical Microbiology and Infectious Disease, Winnipeg.

Introduction

Canada's National Microbiology Laboratory operates a reference service for the identification of enteric bacterial pathogens using whole genome sequencing (WGS), which is crucial for guiding patient care, surveillance, and informing public health decision-making. However, current methods are limited to the most frequently occurring bacterial pathogens. This project aims to enhance WGS-based identification methods and propose taxonomic classifications for broader pathogen identification, to ultimately improve patient care and public health outcomes.

Methods

Bacterial isolates, derived from clinical specimens submitted to the NML from 1997-2023, underwent genomic DNA extraction and Illumina MiSeq WGS. WGS data was analyzed using RefSeqMasher. Pathogen identifications were assigned according to the Genome Taxonomy Database and the List of Prokaryotic Names with Standing in Nomenclature. 16S rRNA sequences were analyzed using BLASTn with the Reference RNA Sequence database. Concordance of taxonomic classifications of the WGS and the traditional gold-standard approach, 16S rRNA sequence analysis, was assessed.

Results

Among 374 taxonomic classifications, 113 isolates (30.2%) demonstrated concordance between WGS and 16S rRNA sequence analysis, while 154 (41.2%) did not. Additionally, 39 (10.4%) samples could not be taxonomically classified via WGS and 68 (18.2%) samples could not be taxonomically classified via 16S sequence analysis. Inability to taxonomically classify certain isolates stemmed from computational limitations or contamination in the sequence data. Discordance arose from four primary factors. Firstly, WGS offered a higher level of taxonomic resolution (25.3%) than 16S sequence analysis, indicating more thorough classification. Additionally, while classifications were consistent at the genus level, disparities surfaced at the species level (33.2%), or taxonomic agreement was restricted to the family level or below (31.2%). Furthermore, the taxonomic classification for 10.4% of samples had not been validly published according to the International Code of Nomenclature of Prokaryotes, thus rendering them non-valid.

Conclusion

The notable low concordance between taxonomic classifications derived from WGS and those from 16S rRNA sequence analysis aligns with expectations, highlighting inherent discrepancies between the methodologies. These finding suggest potential taxonomic implications favouring contemporary WGS identification techniques over traditional approaches. Modernizing reference and diagnostic tests for enteric pathogens that contribute to illness in Canada is key to understanding disease and improving public health surveillance strategies.

POSTER #152

Constructing A Care Cascade Framework for Syphilis: A Strategy to Identify Gaps in Care

Amanda Everton[1], Souradet Shaw[1]

[1]Department of Community Health Sciences, University of Manitoba, Canada

Introduction

Syphilis infections have resurged in Canada where, from 2018 to 2020, cases of infectious syphilis and congenital syphilis increased by 109% and 599% respectively. A 2020 report recognized that knowledge is missing on the 'cascade of care' for syphilis in Canada. A 'cascade of care' is a tool used to identify gaps in care pathways and to inform and monitor interventions. As no standardized syphilis care cascade currently exists, this study aims to develop one.

Methods

Using best practice guidelines, we will develop an indicator-based framework for syphilis management that identifies (1) care pathways at key stages of prevention, diagnosis, treatment, and follow-up; (2) outcome indicators (clinical targets) to measure care effectiveness; and (3) process indicators to identify gaps and barriers to improving care. The framework will be developed by an expert group of researchers, service providers, and decision makers, using focus groups and a modified nominal group technique to reach consensus. Indicators will be broadly defined for use in large-scale national evaluations, and then further adapted for use at a local level, specific for Manitoba.

Results

This study will produce a standardized cascade framework tool based on established best care practices, that will identify (1) primary through tertiary care activities shown to effectively manage syphilis, and at each care stage; (2) process indicators to assess adherence (e.g., whether providers can implement best practices as planned); and (3) key fields of a data surveillance system needed to monitor program effectiveness. Framework application will generate comprehensive knowledge about changes in structures and processes needed to improve syphilis management. This study will produce (1) a syphilis cascade framework to be assessed for future national implementation (2) guidance on how to adapt this framework for use locally or for various sub-populations; and (3) a framework adapted for use specifically in Manitoba.

Conclusion

A standardised syphilis care cascade framework will provide a valuable tool for health officials to effectively address the ongoing syphilis epidemic. Including researchers, service providers, and decision makers in the development process facilitates effective design and facilitates the next steps of implementation and routine utilization of this framework.

POSTER #55

COVID-19 Vaccine Uptake Rates and Determinants among Pregnant Population

Faiza Farooq[1], Jamie Falk[1], Marcelo Urquia[2], Sherif Eltonsy[1,3]

[1]College of Pharmacy, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada.
[2]Manitoba Centre for Health Policy, Winnipeg, Canada.
[3]Children's Hospital Research Institute of Manitoba, Winnipeg, Canada.

Introduction

Pregnant women tend to be more apprehensive about vaccines compared to the general population, and disparities exist in the acceptance of vaccines among them based on individual characteristics. Additionally, the immunization rates vary considerably within pregnant population, especially among immigrants, and those belonging to ethnic minorities in comparison to the host population of a country. Historically, the highest number—over 1.3 million—new immigrants settled as permanent residents from 2016-2021, which includes the COVID-19 pandemic period, and COVID-19 vaccination rates might have been impacted by the demographic origin of the pregnant population. This research project aims in examining the COVID-19 vaccine uptake rates among pregnant women compared to the general population and its potential determinants, including demographic background, socioeconomic status, and clinical conditions.

Methods

Administrative health data from the Manitoba Center for Health Policy (MCHP) data repository was used, and a population-based retrospective cohort study, from December 2020 to April 2023, is being conducted. The incidence rates of vaccines are compared between pregnant and nonpregnant women, and immigrant vs non-immigrant expectant mothers. The data will be stratified by immigration characteristics, neighbourhood income quintiles (higher vs. lower) and clinical characteristics (hypertension, diabetes, mood and anxiety disorders, schizophrenia, personality disorders, chronic pain, asthma, and cardiovascular diseases) along with other variables like age, parity, and area of residence (rural vs urban). Multivariable logistic regression models will be used to ascertain the association of vaccine uptake with the variables of interest. All analysis is performed using SAS software (SAS 9.4).

Results

The project analysis is currently underway. The results of the descriptive and regression models will be presented.

Conclusion

This research project aims to provide essential real-world evidence that can inform Canadian and provincial health authorities about previously unknown variables, like their immigration status, which might have affected COVID-19 vaccine uptake rates. The research study findings will highlight a key aspect of vaccine disparities, with a focus on marginalized population group.

POSTER #27

Characterizing Plasma Biomarkers in Creutzfeldt-Jakob Disease

Olivia Ferreira[1,2] and Stephanie Booth[1,2]

[1]Medical Microbiology and Infectious Diseases, University of Manitoba.

[2]National Microbiology Laboratory, Public Health Agency of Canada.

Introduction

Creutzfeldt-Jakob disease (CJD) is a rare, fatal neurodegenerative disorder characterized by the misfolding and aggregation of the host prion protein. CJD progresses through a clinically silent incubation phase marked by the accumulation of misfolded prion protein in the brain, plateauing before symptom onset. Clinical manifestations of CJD include cognitive impairment, dementia, ataxia, psychiatric symptoms, and myoclonus. With no effective treatment available, prognosis monitoring is challenging due to rapid deterioration, typically resulting in death within six months of symptom onset.

Early diagnosis of CJD is hindered by the absence of diagnostic techniques before symptom onset, occurring late in the disease course. Preexisting biomarker studies predominantly focus on surrogate markers associated with neuronal loss and inflammation, with neurofilament light emerging as a potential indicator of axonal degeneration. Research into plasma biomarkers for CJD diagnosis is in its infancy, limited by small sample sizes and a lack of longitudinal data. Technological advancements offer promising avenues for investigating biomarkers in easily accessible biological fluids, such as plasma, which may enable routine monitoring in those who are at risk of inheriting CJD.

This project aims to identify plasma biomarkers distinguishing CJD from other neurodegenerative disorders with high diagnostic accuracy. Objectives include assessing the prognostic value of diagnostic biomarkers using clinical data and stratifying biomarker diagnostic ability across human prion disease subtypes. Additionally, identifying plasma biomarkers may facilitate monitoring of individuals at risk of inheriting prion diseases during asymptomatic and early disease stages, particularly where invasive procedures like lumbar punctures are not yet warranted.

Methods

Using Meso Scale Discovery (MSD) electrochemiluminescence technology, exploratory biomarker panels will be performed on archived patient plasma samples obtained through CJD diagnostic testing. MSD multiplex technology can assess up to 10 analytes per well while requiring significantly less sample volume compared to traditional ELISA, thereby conserving the limited volume of archival samples while maximizing the biomarkers investigated. Exploratory biomarkers include markers of neurodegeneration and neuroinflammation, including neurofilament light. Statistical analyses comparing the biomarker profiles between CJD and non-CJD cohorts, the diagnostic performance of each biomarker, demographic correlations, and survival correlations will be performed following data collection.

Results

Forthcoming.

Conclusions

Forthcoming.

POSTER #38

Monkeypox Virus (MPXV) Surveillance in Endemic and Non-Endemic Regions of East Africa at a One Health Animal-Human Interface

Christina Frederick[1], Kristian Forbes[2], Jason Kindrachuk[1]

[1]University of Manitoba, Department of Medical Microbiology, and Infectious Disease (MMID), *Winnipeg, Canada*.
[2]Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas, United States of America.

Introduction

There has been an increasing frequency of emergence events for zoonotic diseases over the past decades. Animals carry ~60% of viruses globally, including those with zoonotic potential. Rodents, one of the most geographically widespread and abundant mammals, have been recognized as essential links for viral spread between animals and humans.

Mpox (formerly monkeypox), the zoonotic disease caused by the *Poxviridae* family member monkeypox virus (MPXV), is endemic in multiple sub-Saharan African countries, including an ongoing historic outbreak in the Democratic Republic of the Congo and a global epidemic in 2022. Unfortunately, surveillance activities are impacted by resource limitations in endemic regions, reducing proper investigation of the virus' behavior, spread, and prevalence in the affected areas. While the specific animal reservoir(s) for MPXV is unknown, rodents are highly suspected, including squirrel species. The geographic ranges of suspected reservoir hosts extend beyond endemic regions with known human cases into East Africa, including Kenya.

The One Health approach interlinks animal, human, and environmental sciences, which involves multiple disciplinary experts collaborating, sharing knowledge, and monitoring global threats in understudied areas such as the Global South.

Methodology

We will assess the circulation of MPXV in East African Rodentia. We aim to capture 200 mammals annually using Sherman traps. Traps will be installed close to communities and rural areas. Once captured, blood and serum samples will be collected, inactivated, and used for serology testing, such as enzyme-linked immunosorbent assay (ELISA), to determine the presence of MPXV IgG antibodies.

In addition, animals that have visible wounds or lesions will be swabbed. If there is any sign of illness, they will be euthanized, and tissue samples will be collected. PCR will assess active viral infection with specific primers for MPV. Cell culture and viral titration will be calculated to evaluate virological properties such as infectivity rate and environmental persistence.

Conclusion

The importance of this project is to increase MPXV surveillance and early detection in East African countries. This will help facilitate a better understanding of the role of animal-human interactions in zoonosis and protect African communities when they tend to their recreational needs.

POSTER #145

Characterizing Early Neurons in the Mouse Cerebellar Nuclear Transitory Zone

Farshid Ghiyamihoor[1,2], Azam A Rad[1,2], Hassan Marzban[1,2]

[1] University of Manitoba, Human Anatomy and Cell Science, Winnipeg, Canada.

[2]The Children's Hospital Research Institute of Manitoba (CHRIM), University of Manitoba, Winnipeg, Canada.

Introduction

Understanding the development of cerebellar nuclei (CN) has the potential to answer various question when we study neurodevelopmental disorders such as autism spectrum disorder (ASD). Conventionally, glutamatergic excitatory and GABAergic inhibitory CN neurons originate respectively from the rhombic lip and ventricular zone post-embryonic day 9 (E9). Our study reveals a novel cell subset in the nuclear transitory zone (NTZ) preceding E9. The objective of this study is to characterize the developmental origin, the phenotype, and axonal projection of the early subset of neurons in the mouse cerebellar nuclei.

Methods

We employed a combination of thymidine analogue EdU-based birth-dating analysis, in utero viral vector injection, western blotting, immunofluorescence labeling, and confocal imaging to investigate the developmental trajectory of the early subset of neurons in the NTZ of $Snca^{GFP}$ transgenic mouse. This is confirmed by *ex vivo* AAV-mediated neuronal tracing. Additionally, fluorescence-activated cell sorting (FACS) is used to isolate our target neurons from SncaGFP transgenic embryo, which is followed by cell fractionation assay to find the subcellular location of the specific proteins in the neuron.

Results

This early subset of cells expresses α-synuclein protein (SNCA, encoded by the *Snca* gene), and emerge in the NTZ as early as E8.75. These cells most probably originate from the mesencephalon as a potential germinal zone. Moreover, colocalization of SNCA in these cells with neuronal and glutamatergic markers hints at their nature as excitatory neurons. Also, cytosolic location of SNCA protein hints to its role in the in the processes such as metabolism, signaling, and cytoskeletal organization.

Conclusion

In conclusion, our study suggests that a subset of glutamatergic neurons emerges in the cerebellar nuclei of mice as early as E8.75. Unraveling the origin and developmental timeline of these early neurons could significantly contribute to advancing our understanding of neurodevelopmental disorders such as ASD.

POSTER #12

Sex-specific Immune Responses to COVID-19 Vaccination in an Immunocompromised Population

Shanelle N. Gingras[1,2], Samantha E. Kirby[2], Carmen Lopez [2], Jessica Ahmed[2], Samantha J. Krosta[2], Erika H. Labossiere[2], Terry B. Ball[1,2], Ruey C. Su[1,2], Lyle R. McKinnon[1], Catherine M. Card[1,2], Joe Bueti[3,4,5], Sandra A. Kiazyk[1,2], Paul J. McLarenJ[1,2]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, MB, Canada.
[2]National HIV and Retrovirology Laboratory, JC Wilt Infectious Diseases Research Centre, National Microbiology. Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada.
[3]Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada.
[4]Section of Nephrology, Department of Internal Medicine, University of Manitoba, MB, Canada.
[5]Health Sciences Centre, Winnipeg, MB, Canada.

Introduction

Vaccination has been critical in stemming the COVID-19 pandemic; however, the effectiveness of vaccination in immunocompromised populations remains an important area of research. Studies have shown end-stage renal disease (ESRD) patients have muted memory B cell formation and reduced humoral responses to COVID-19 vaccination; however, early innate immune responses have yet to be characterized.

Methods

We have collected blood before (BD1) and 1-4 days post dose 1 (PD1) of BNT162b2 vaccination in ESRD patients (n= 39 BD1, 35 PD1) and healthy controls (34 BD1, 15 PD1) for quantification of 20 plasma cytokines using the Meso Scale Discovery platform. Detailed enrolment and follow-up questionnaires capturing demographic, medical and COVID-19 infection information was collected from participants. Samples were also collected for RNA sequencing, cellular, and antibody responses at multiple timepoints post vaccination.

Results

We observed ESRD patients to have an elevated ($p<0.01$) baseline (BD1) inflammatory cytokine profile (IFN- , IL-1 , IL-6, TNF- , eotaxin, IP-10, MCP-1, MIP1- , MIP1-) compared to healthy controls. When stratifying by sex, female ESRD patients had a higher concentration of key inflammatory cytokines than males at baseline, which appears to drive the inflammatory profile in the full sample. This trend is also found when considering clinical biomarkers (MIP1- , MIP1-) for ESRD, with a higher concentration found in female ESRD patients. Despite baseline inflammation, ESRD patients were able to mount cytokine responses to vaccination similar to those of healthy controls, with females in both populations more reactogenic to vaccination than males. Specifically, IL-2, IL-10, and IP-10 were more significant in ESRD females ($0.001p<0.0001$) compared to ESRD males ($0.01<p<0.001$), and IL-6, IFN- , IL-13, eotaxin, were exclusive to females ($0.05<p<0.01$). Similar findings were present in the healthy population.

Conclusion

ESRD patients elicit similar cytokine responses to COVID-19 vaccination compared to healthy controls despite baseline inflammation. Female participants have higher concentration of inflammatory cytokines in response to ESRD or to COVID-19 vaccination. Further analyses will assess how these data impact long term vaccine responses in ESRD patients.

POSTER #11

The serological detection of mpox: A retrospective analysis in the Canadian Prairies

Elise Gork[1], Mikayla Hunter[2], Jason Kindrachuk[1]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba.
[2]Department of Community Health Sciences, University of Manitoba.

Introduction

The 2022 global mpox epidemic resulted in broad geographic expansion of the disease across non-endemic regions with cases concentrated within dense sexual networks. This included overrepresentation in men and those that identify as gay, bisexual, and other men who have sex with men (GBMSM). Additionally, there is little known about the seroprevalence in gender diverse people. The altered clinical presentation may have led to misdiagnoses and lack of self-recognition of symptoms, with subsequent underreporting of cases. Furthermore, stigma associated with a diagnosis may have discouraged individuals from seeking healthcare. This study will examine prior exposure to mpox within GBMSM communities in Manitoba, Saskatchewan and Alberta.

Methods

A monkeypox virus-specific ELISA will be developed to enable differentiation of vaccinated and naturally infected individuals. Individuals who reside in the Canadian Prairies, who are over 18 years of age, have not had a known mpox infection, who contemplated receiving the mpox vaccine, and who self-identify as GBMSM, or as being potentially eligible (including gender diverse individuals and trans women) for the vaccine can provide a dried blood spot sample. Recruitment will occur through community-based organizations that serve 2SLGBTQIA+ individuals.

Results

This study will quantify mpox exposures in the Canadian Prairies. Additionally, an epidemiological analysis will follow to investigate how age, sex, gender, sexual orientation, and geographical location are associated with mpox infection.

Conclusion

These results will provide insight into mpox and its transmission within the Prairie provinces. As well, the identification of factors associated with infection may guide future prevention strategies.

POSTER #131

Regulation of TCR signaling strength shapes clonal expansion of HIV latently infected CD4+ T cells

Riley Greenslade[1], Nnamdi Ikeogu[1], Wan Koh[2], Oluwaseun Ajibola[1], Xinyun Liu[1], Mario Ostrowski[2], Thomas Murooka[1,3]

[1]University of Manitoba, Rady Faculty of Health Sciences, Department of Immunology, Winnipeg, MB.
[2]University of Toronto, Department of Immunology, Toronto, ON.
[3]University of Manitoba, Rady Faculty of Health Sciences, Department of Medical Microbiology and Infectious Diseases, Winnipeg, MB.

Introduction

Successful antiretroviral therapy (ART) reduces mortality rates by suppressing HIV replication to undetectable levels in people living with HIV (PLWH). However, ART is not curative due to the establishment of the HIV reservoir that persists despite prolonged ART. Long-lived T cells are an important HIV reservoir population and a large proportion of the reservoir is composed of $CD4^+$ T cell clones which are established early after infection. Our studies show that cognate dendritic cell:T cell interactions drive clonal expansion of latent T cell subsets supported by the presence of IL-7 and CD28 costimulation. Low antigenic stimulation drives proliferation without virus reactivation in latent T cell subsets indicating that the *magnitude* of T cell receptor (TCR) stimulation is a key regulator of proliferation and survival mechanisms that maintain the HIV reservoir. However, it remains unknown how antigen stimulation regulates expansion of HIV-infected cells under ART suppression.

Methods

We describe a new dual-fluorescent HIV latency reporter to visualize latently infected T cells and to examine cell-cell contacts that promote their proliferation over time. We combine our reporter system with human $CD4^+$ T cell clones in co-culture studies with a panel of altered peptide ligands (APLs) and checkpoint inhibitors to directly examine the relationship between TCR signaling strength and proliferative responses by latent T cells using flow cytometry.

Results

Initial results show varied activation and proliferative outcomes of $CD4^+$ T cell clones in response to stimulation with APLs of different binding affinities. Current studies are implementing APLs in HIV infection studies to uncover how TCR signaling strength affects proliferation in latently infected cells. Latently infected cells are enriched in checkpoint molecules such as PD-1 which increases in a dose-dependent manner to antigen stimulation. PD-1 blockade decreased the proportion and absolute cell count of the latently infected HIV reservoir while increasing the proportion of the productively infected cells under ART suppression.

Conclusion

Our data argue that a critical balance between stimulatory and inhibitory pathways shape which T cell subsets clonally expand under ART suppression. These studies have implications on stimulatory signals that can be therapeutically targeted to reduce the HIV reservoir size in PLWH.

POSTER #155

Challenging the Regenerative Potential of Neural Stem Cells in the Zebrafish Forebrain using a Repeated Injury Model

Sanjana Grover[1] and Benjamin W. Lindsey[1,2]

[1]Department of Human Anatomy and Cell Science, Rady Faculty of Health Sciences, University of Manitoba., [2]Children's Hospital Research Institute of Manitoba.

Introduction

Neural Stem Cells (NSCs) play a central role in neural repair after brain injury. While mammals struggle with glial scarring, zebrafish, a teleost fish, stands out as an exceptional regenerative model displaying NSC regeneration in the adult forebrain post-injury. Remarkably, upon injury activated NSCs generate newborn neurons to replace lost lineages. A pro-regenerative environment, marked by an acute immune response and upregulation of genes such as *gata3* and *cxcr5* further facilitates timely repair. Despite this capacity for neurorepair, whether these NSCs can maintain a sustained neuronal output in response to successive instances of injury remains unknown. This study investigates the impact of a long-term injury environment on adult zebrafish forebrain NSCs, assessing their neuronal output and alteration in the factors contributing to its pro-regenerative environment.

Methods

To induce a prolonged injury state, a repeated brain injury paradigm was established and validated. Using a 30G syringe, brain injuries were inflicted through the nares at weekly intervals 1-4 times, resulting in a lesion in the dorsal pallium of the forebrain hemisphere. The injury model was validated using H&E staining. Wildtype zebrafish were subjected to repeated injuries (1-4X), followed by EdU intraperitoneal injections and HuC/D immunostaining, where NSC differentiation into neurons was assessed by $EdU^{+}HuC/D^{+}$ co-labelling. To observe how resident microglia are modulated with successive injuries, 4C4 immunostaining was employed. Semi-quantitative analysis for gene expression of *gata3* and *cxcr5* was also performed using endpoint PCR.

Results

H&E staining at 1 day post injury (dpi) revealed a widening lesion, increased blood clotting and vacuole count with each successive injury, confirming sustained injury pathology. These features notably decreased by 7dpi. Pilot data indicates a decreased neuronal output with multiple injuries. In contrast, an increase in microglial recruitment to the injury site indicated an enhanced immune response with successive injuries. Finally, endpoint PCR results displayed downregulation of *gata3* and *cxcr5* with increased injuries.

Conclusion

Findings from this study will unveil whether a regenerative limit exists for adult zebrafish forebrain NSCs and how this is coupled with the changes in immune response and expression of regenerative genes in the injury microenvironment.

POSTER #133

Are current Legionnaires disease diagnostic practices capturing the epidemiology of clinically relevant Legionella? A scoping review.

Ryan Ha[*1], Ashley Heilmann[*1], David Alexander[1,2], Sylvain A. Lother[3], Christine Turenne[2], Yoav Keynan[1,3,4**], Zulma Vanessa Rueda[1,4,5**]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Manitoba, Canada.

[2]Shared Health, Winnipeg, Manitoba, Canada.

[3]Department of Internal Medicine, University of Manitoba, Winnipeg, Manitoba, Canada.

[4] Department of Community Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada.

[5] Universidad Pontificia Bolivariana, Medellín, Colombia.

Introduction

Legionella is an underdiagnosed and underreported etiology of pneumonia believed to be caused almost exclusively by *Legionella pneumophila* serogroup 1 (LPSG1). The diagnosis is mostly done by a urinary antigen test, only capable of diagnosing LPSG1. We aimed to explore the frequency of *Legionella* infections in individuals diagnosed with pneumonia and the performance of different diagnostic methods for detecting *Legionella* infections.

Methods

We conducted a scoping review to answer the following questions: 1) "Does nucleic acid testing (NAT) increase the detection of non-pneumophila serogroup 1 Legionella compared to non-NAT?"; and 2) "Does immunocompromisation increase the frequency of pneumonia caused by non-pneumophila serogroup 1 Legionella compared to non-immunocompromised individuals with Legionnaires' Disease?". Articles were obtained from PubMed, PubMed Central, Cochrane Register of Controlled Trials, Clinicaltrials.org, and LegionellaDB reporting mixed diagnostic methods (both NAT and non-NAT) for pneumonia and a breakdown of species and serogroups found. Studies were eligible if they fulfilled the following criteria: 1) original research that reports data that can be used to calculate incidence, frequency, or prevalence of *Legionella* with a species or serogroup analysis; 2) Outbreaks detailed on LegionellaDB; 3) Comparative quantitation using multiple detection methods; 4) Use of at least one NAT- and one non-NAT-based technique for diagnosis; 5) At least 5 cases of Legionnaires' disease (LD) in the patient group

Results

Of the 3449 articles found using our search criteria, 30 were included in our review. We found that *Legionella* diagnostics neglected non-*pneumophila* serogroup 1 *Legionella* by over-relying on the urinary antigen test. The most common species of *Legionella* were found to be *L. pneumophila*, *L. longbeachae*, and unspeciated *Legionella* appearing in 1.4%, 0.895%, and 0.627% of pneumonia cases. Meanwhile, half of the *Legionella* infections in the studies are caused by unidentified *Legionella* species or unknown serogroups of *L. pneumophila*. NAT-based techniques were found to be more effective at detecting *Legionella* than non-NAT-based techniques.

Conclusion

Identification and detection of non-*pneumophila* serogroup 1 *Legionella* is severely lacking and broader spectrum diagnostics need to be adopted, as the LD etiology is not as rare as previously thought.

POSTER #78

Safe and Inclusive Schools? Institutional Attitudes Towards 2SLGBTQ+ Youth in Manitoba's Funded Independent Christian Schools

S. Chad Hayes,[1] Deborah McPhail,[2] Robert Lorway[3]

[1]M.Sc. student, Department of Community Health Sciences, University of Manitoba.
[2]Department of Community Health Sciences, University of Manitoba.
[3]Department of Community Health Sciences, University of Manitoba.

Introduction

Due to societal discrimination and marginalization, 2SLGBTQ+ youth are at substantial risk of negative health outcomes including depression, suicide, drug and alcohol use, and various forms of abuse. This effect can be mitigated or eliminated if these youth are supported and affirmed in their identities. In 2012, Manitoba passed the *Safe and Inclusive Schools Act*, requiring schools to develop policies that respect *Manitoba Human Rights Code* prohibitions against discrimination due to gender identity or sexual orientation. Approximately 15,000 Manitoba youth attend provincially funded independent schools, which are predominantly Christian. Some expressions of Christianity have shown a consistent pattern of discrimination towards 2SLGTBQ+ individuals. Alleged discrimination based on sexual orientation in a funded independent Christian school has prompted at least one Human Rights Commission complaint in Manitoba.

Methods

A list of funded independent schools was obtained from the Manitoba Ministry of Education and Early Childhood Learning. Schools selected for inclusion in this review (n=38) were Christian (Catholic and Protestant) schools that provided education for at least one grade from 6 to 12. Websites, student handbooks, and statements of faith of included schools were reviewed for content about gender, sexuality, and marriage. This data was analysed for institutional attitudes and policies concerning 2SLGBTQ+ people.

Results

Most schools avoided explicit statements of discrimination towards 2SLGBTQ+ people in publicly available policy documents. However, five schools (18%) published overtly homophobic and transphobic beliefs about gender and sexuality that are clearly discriminatory towards 2SLGBTQ+ people. Two schools openly challenged laws such as the *Safe and Inclusive Schools Act*, stating that "in matters of disagreement between Scripture and government, we believe we must obey God."

Conclusion

Of all schools in this analysis, evangelical Protestant schools were the only ones to publicly publish explicitly anti-2SLGBTQ+ statements. While this does not mean that discrimination is absent in other schools, it does indicate that further governmental action is necessary to prevent negative health outcomes in 2SLGBTQ+ students. More research is needed in this area to understand the experiences of 2SLGBTQ+ students and employees in Manitoba's funded independent schools and the ways in which these anti-2SLGBTQ+ attitudes are manifested.

POSTER #75

Treponema pallidum in Manitoba: Phylogenetic Analysis from Clinical Swabs using Long-Read Sequencing

Adam Hedley[1,2], Ayooluwa Bolaji[3], David Alexander[1,2], Kerry Dust[2], Paul Van Caeseele[1,2], Lyle Mckinnon[1], Souradet Shaw[1], Jared Bullard[1,2,3], Derek Stein[1,2]

[1]University of Manitoba, Winnipeg, Canada.
[2]Cadham Provincial Lab, Winnipeg, Canada.
[3]Public Health Agency of Canada, Winnipeg, Canada.

Introduction

Over the past decade, Manitoba has been experiencing unprecedented rates of syphilis, caused by *Treponema pallidum* subsp *pallidum*. Beyond exceeding the national average, the province was also first to experience the demographic shift from the MSM community to heterosexual groups and women. Adding to the concern is the re-emergence of congenital syphilis, with over 400 infants exposed in the last 2 years and 150 confirmed cases from 2015 to 2022.

To our knowledge *T. pallidum* whole genome sequencing (WGS) attempts have not been successful in Manitoba. Long-read WGS of clinical swabs would aid public health in epidemiological investigations and surveillance. It could also provide novel insights into genome structure around repetitive regions. In this study we aimed to investigate the phylogenetic diversity of *T. pallidum* in the province.

Methods

To identify *T. pallidum* positive samples within mucocutaneous swabs in transport media, we will utilize a screening PCR approach. The challenge of low bacterial loads in syphilis infections, where host DNA dominates, will be addressed through a selective multiple displacement amplification using primers specific to the bacteria. Using modified Oxford Nanopore recommendations, library prep will be optimized to allow for sample multiplexing and sequencing on a Nanopore GridION device.

Results

Samples with higher bacterial loads were successfully sequenced to produce near complete (>99% coverage) genomes. Utilizing Nanopores adaptive sampling enrichment feature improved genome coverage in samples with low copy numbers and provided increased depth among all samples. Phylogenetic analysis reveals these samples cluster closely with previously published sequences belonging to the SS-14 strain.

Conclusion

Through the application of Nanopore sequencing, selective whole genome amplification and adaptive enrichment we were able to generate near complete genomes directly from clinical swabs. The results of our phylogenetic analysis were similar to a previous study using samples from B.C. and Alberta where the SS-14 strain was dominant among the sequenced samples. Further analysis of these genomes can contribute to our understanding of *T. pallidum* infections in Manitoba.

Transmission dynamics and genotypic diversity of *Mycobacterium tuberculosis* in Colombian persons deprived of liberty.

Heilmann A[1], Herrera M[1], Hurtado U[2], Alvarez N[2], Realpe T[2,3], Marin D[3], Lopez L[3], Arroyave L[4], Arbelaez MP5, Keynan Y1,[6,7,] Rueda Z[1,3]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Canada., [2]Corporacion para Investigaciones Biologicas (CIB), Medellin, Colombia., [3]School of Medicine, Universidad Pontificia Bolivariana (UPB) Medellin, Colombia., [4]National Collaborating Centre for Infectious Diseases (NCCID), University of Manitoba, Winnipeg, Canada., [5]National School of Public Health, Universidad de Antioquia, Medellin, Colombia., [6]Department of Community Health Sciences, University of Manitoba, Winnipeg, Canada., [7]Department of Community Health Sciences, University of Manitoba, Winnipeg, Canada.

Introduction

Between 2011 and 2017 in Central America, tuberculosis case notifications quadrupled in persons deprived of liberty (PDL)[1]. The changing understanding of the continuum of *Mycobacterium tuberculosis* spectrum underscores the importance of molecular epidemiology to elucidate transmission in highly overcrowded settings. Our study aims to show the genotypic diversity of *Mycobacterium tuberculosis* strains present in Colombian prisons and examine disease clusters and transmission dynamics of *M. tuberculosis*, and compare the results obtained by whole genome sequencing (WGS) to those obtained by 24-mycobacterial interspersed repetitive unit (MIRU) typing.

Methods

Sixty-four PDL incarcerated in two male and two female Colombian prisons were followed up for 2 years (monthly during anti-tuberculosis treatment, bimonthly for six months, and quarterly during the second year) from 2010-2012. Among them, 132 *M. tuberculosis* strains were obtained, and 49 isolates were determined to be in clusters based on MIRU typing. Demographic information was collected from participants. Strains were sequenced with Illumina MiSeq, and phylogeny and transmission clusters were determined using a single nucleotide variant calling method, the SNVPhyl pipeline, in conjunction with epidemiological data.

Results

Investigating tuberculosis transmission in the prison with WGS revealed five transmission clusters, with 4 of these spanning multiple cell blocks. One re-infection and three mixed infections were also identified, with one mixed infection resulting in a single individual being part of two separate transmission clusters. The high burden of tuberculosis in this setting and the high level of genetic similarity between strains impeded the identification of specific transmission events. MIRU typing identified 10 transmission clusters, and only one cluster matched those identified by WGS. MIRU typing also identified 1 re-infection and 5 mixed infections, with 2 of the mixed infections being incorrectly identified single strain infections.

Conclusions

Our data shows that there is active tuberculosis transmission within these prisons, and that MIRU typing is insufficient in a high burden setting. In the future, whole genome sequencing should be considered as a tool for tuberculosis surveillance and identifying transmission within prisons when developing infection control and prevention strategies.

POSTER #26

Transcriptomic analysis of primary human cells infected with adenoviruses expressing different E1A isoforms.

Rafe Helwer[1] and Peter Pelka[1, 2]

[1]Department of Microbiology, University of Manitoba, 45 Chancellor's Circle, Buller Building Room 427, Winnipeg, MB R3T 2N2, Canada.

[2]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, 45 Chancellor's Circle, Buller Building Room 427, Winnipeg, MB R3T 2N2, Canada.

Adenovirus, like other oncogenic viruses, is studied for its ability to infect and transform cells and as a tool for identifying critical oncogenes. The size constraints of the viral genome and rapid viral evolution forces efficient targeting of critical cellular regulatory pathways. Therefore, the cellular pathways targeted by viruses are an ideal way of identifying oncogenes and critical cellular regulators. It has long been known that the adenovirus E1A protein can transform cells in culture though the disruption of a wide array of growth regulators. Although there have been previous studies on the differences between E1A 13S and 12S, the two largest and earliest expressed isoforms, their global effect on human gene expression is less understood. To that end, we performed RNA-sequencing on primary human lung fibroblast cells (IMR-90s) infected with wild type adenovirus and three adenovirus mutants, the first expressing only the E1A 13S isoform, the second expressing predominantly E1A 12S, and the last with three mutations in 13S that prevent it from binding to the retinoblastoma protein. We subsequently performed differential expression analysis, principal component analysis, clustering, and gene set enrichment analysis on the data. Our results show that the mutant E1A 13S-derived protein induces higher cytokine transcript expression and that the 12S isoform has lower transcripts of heat shock response and cell cycle proteins compared to 13S.

POSTER #160

Characterizing the Neuronal Role of CK2 using *Drosophila melanogaster*

Yina Her[1,2], Adedayo Oladejo[1,2], Alondra Griffiths[1,2], Danielle Pascual[1,2], Harsimran Kaur[1,2], Catherine Bland[1,2], Paul Marcogliese[1,2]

[1]Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MB, Canada.
[2]Children's Hospital Research Institute of Manitoba, Winnipeg, MB, Canada.

Introduction

De novo variants in either *CSNK2A1* or *CSNK2B* cause neurodevelopmental disorders with overlapping features and variable symptoms. These two genes encode subunits of the Casein Kinase 2 (CK2) complex. CK2 is enriched in the central nervous system and is believed to be constitutively active. There are currently in vivo models in flies to study CK2 in the adult central nervous system. Moreover, the variants, particularly missense, found in *CSNK2A1* or *CSNK2B* have yet to be functionally assessed in vivo.

Objective

There are two objectives of this study. First, we will assess the role of CK2 in neurons and glia in development and the adult organism. Secondly, we will generate CK2 disease-associated variants and assess their function in flies.

Methods

CK2 (CkIIα and CkIIβ) are knocked down in *Drosophila* neurons by RNAi using the *nSyb*-GAL4 driver for initiating knockdown during development and elav-GAL4^{GS} for adult specific knock down. *Drosophila* behavioural assessment was performed by negative geotaxis. Seizure-induction was performed by bang-sensitivity assay. Variant transgenic flies were generated via site-direct mutagenesis, followed by Sanger verification, and embryo injection commercially.

Results

We found that neuronal knock-down of either CkIIα or CkIIβ in neurons with nSyb-GAL4 resulted in lethality where some escapers had wing defects. No obvious phenotype was observed with *elav*-GAL4. We successfully generated 16 variants in *CSNK2A1* or *CSNK2B* to generate transgenic flies. Tissue-specific overexpression using nub-GAL4 and ey-GAL4 showed defects in eyes and wings in the variants.

Conclusion

We have been able to establish that neuronal CK2 is critical for the development of *Drosophila melanogaster*. Future studies will examine the adult-specific role of CK2 in neurons. Our variant functional testing is revealing a wide variety of variant impact.

POSTER #30

Non-Invasive Transcutaneous Auricular Vagus Nerve Stimulation (Nitavns) Induces Spatial-Specific Regulation of Granulocytes, Macrophages, And Their Associated Inflammatory Markers in A Preclinical Model of Ulcerative Colitis

Fatemeh Hesampour [1], Ali Veysel Özden [2, 3,] Charles N Bernstein [4, 5,] Jean-Eric Ghia [1, 4, 5, 6]

[1]Immunology, University of Manitoba, Winnipeg, Canada.
[2]Bahcesehir University., [3]Vagustim, Istanbul, Türkiye.
[4]Internal Medicine., [5]Inflammatory Bowel Disease Clinical & Research Centre, University of Manitoba.
[6]Children's Hospital Research Institute of Manitoba, Winnipeg, Canada.

Introduction

In ulcerative colitis (UC), there is a noted reduction in vagus nerve activity alongside an increase in granulocytes and macrophages. Non-invasive transcutaneous auricular vagus nerve stimulation (NitaVNS) has demonstrated protective anti-inflammatory effects in UC-like colitic mice. However, the specific cellular pathways involved remain unclear.

Methods

To assess NitaVNS's impact on granulocytes, macrophages, and inflammatory markers in UC-like colitis, male C57BL/6 mice were utilized. NitaVNS (10 min/day) was initiated one day prior to inducing colitis with 5% dextran sulfate sodium (DSS) for five days in water (control mice received regular water). NitaVNS continued until the end of the study, with sham mice receiving no stimulation. Disease activity index (DAI), distal macroscopic scores, and expression of myeloperoxidase (MPO), nitric oxide synthase (Nos2), matrix metalloproteinase (MMP)-2 and 9, and macrophage inflammatory protein (Mip) 1α and β were evaluated in the distal colon. The frequencies of granulocytes (Ly6G) and macrophages (F4/80) were assessed in the cecum, proximal, medial, and distal colon, spleen, and mesenteric lymph nodes (MLNs) using flow cytometry.

Results

NitaVNS significantly improved DAI and macroscopic scores and decreased colonic expression of MPO, Nos2, MMP-2 and 9, and Mip1α and 1β in colitic mice compared to sham-stimulated colitic mice, with no alterations observed in non-colitic mice. While NitaVNS did not alter the frequency of Ly6G+ cells in the cecum, medial and distal colon, and MLNs, it reduced their positivity in the proximal colon and spleen of colitic mice, with no changes in non-colitic mice. NitaVNS decreased the frequency of F4/80+ cells in the cecum and proximal and medial colon but did not affect the distal colon, spleen, and MLNs.

Conclusion

NitaVNS demonstrates the potential to exert both systemic and local anti-inflammatory effects in colitic mice, suggesting its promise as a novel therapeutic intervention.

POSTER #18

Astrocytes Originated from Neural Precursors Drive Regenerative Remodeling of Pathologic CSPGs in Spinal Cord Injury

Seyed Mojtaba Hosseini[1, 2], Shiva Nemati[1, 2], Soheila Karimi-Abdolrezaee[1,2,3]

[1]Department of Physiology and Pathophysiology, Spinal Cord Research Centre, Rady Faculty of Health Sciences, University of Manitoba Winnipeg, Manitoba, Canada.

[2] Manitoba Multiple Sclerosis Research Center, Winnipeg, Manitoba, Canada.

[3] Children Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada.

Progressive neurodegeneration after spinal cord injury (SCI) causes permanent neurological dysfunctions. Detrimental changes after SCI including upregulation of inhibitory chondroitin sulfate proteoglycans (CSPGs) and impaired neuroglia network restrict the endogenous repair process. Neural precursor cells (NPCs) have the innate capacity to replace damaged neurons after SCI. However, the hostile milieu of damaged spinal cord hinders neurogenesis of endogenous or transplanted NPCs and instead drives their astrogenesis. Astrocytes are critical glial cells for CNS injury and repair as they can exhibit pro-inflammatory or pro-regenerative phenotype. Following SCI, the majority of resident astrocytes become pro-inflammatory; however, our understanding of the role of NPC-derived astrocytes after SCI is limited. Here, we show that engrafted and endogenous NPC-derived astrocytes exhibit pro-regenerative and anti-inflammatory phenotypes in rats with SCI compared to pro-inflammatory resident spinal astrocytes, which was verified by transcriptomics analysis *in vitro*. Our co-culture system indicates that reactive NPC-derived astrocytes enhance NPC neurogenesis and increases maturity and synapse formation of NPC-derived neurons in a paracrine fashion. Mechanistically, we demonstrate that NPC-derived astrocytes perform regenerative CSPGs remodeling by receptor mediated endocytosis, and degradation of CSPGs through production and release of ADAMTS1/9 enzymes, a capacity that spinal astrocytes lack. Taken together, our novel findings show that newly generated astrocytes from NPCs can promote the repair processes after SCI, at least in part, by regenerative remodeling of CSPGs.

POSTER #93

The Impact of Omega-3 fatty acids on macrophage cell metabolism and function

Floriane Houenagnon[1], Michael Byun[1], Tamaris Souza[2,3], Ayesha Saleem[2,3] and Samantha Pauls[1,3]

[1]College of Pharmacy, University of Manitoba, Winnipeg, Canada.

[2]Faculty of Kinesiology and Recreation Management, University of Manitoba, Winnipeg, Canada.

[3]Children's Hospital Research Institute of Manitoba, Winnipeg, Canada.

Introduction

Obesity has become an escalating global epidemic, and it poses significant social, economic and health burdens. Obesity is a complex disease that can be accompanied by inflammation and often by comorbidities, including cardiovascular diseases, type 2 diabetes, certain cancers, and many others. One of the hallmarks of obesity-associated inflammation is the infiltration of immune cells, such as monocytes and macrophages, into dysfunctional adipose tissue. Many studies highlight the pivotal role of cellular metabolism in regulating macrophage phenotype and function. These key metabolic pathways include glycolysis and mitochondrial respiration. One promising therapeutic approach against obesity-associated inflammation is the use of omega-3 (n-3) polyunsaturated fatty acids (PUFA), such as docosahexaenoic acid (DHA) and α-linolenic acid (ALA). n-3 PUFA have anti-inflammatory effects on macrophages. Preliminary work in our lab has shown that n-3 PUFA increases glycolytic rate and decreases mitochondrial respiration rate in monocytes; however, the impact of n-3 PUFA on metabolic pathways in macrophages remains unclear.

Methods

Macrophages, including murine RAW 264.7 cells and human THP-1-derived macrophages (TDM), were treated with the following: vehicle, DHA, ALA, and Oleic acid (OA, a monounsaturated fatty acid control) and incubated for 24 hours. Then, the ATP rate assay was conducted using a Seahorse XFe24 instrument. The lysates were collected to determine protein content by BCA assay to normalize the data. Cell viability was also assessed using the CYQUANT XTT assay.

Results

Preliminary data in TDM suggests that ALA increases the percent of ATP derived from glycolysis at the expense of mitochondrial respiration. In murine RAW 264.7 macrophages, ALA and DHA had no apparent effects on glycolysis and mitochondrial respiration rates. Cell viability was not impacted. Experiments in primary bone marrow-derived macrophages are ongoing.

Conclusion

Targeting metabolic pathways, including potentially through n-3 PU FA-rich diets or supplements, may be a new therapeutic strategy for chronic inflammation.

POSTER #10

Docosahexaenoic Acid shows Therapeutic Potential in Managing SARS coronaviruses Infection by Transcriptomics

Shiqi Huang[1,2], Carla G. Taylor[1,2,3], Peter Zahradka[1,2,3]

[1] Department of Physiology and Pathophysiology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB R3E 0T5, Canada.

[2] Canadian Centre for Agri-Food Research in Health and Medicine, St. Boniface Hospital Albrechtsen Research Centre, Winnipeg, MB R2H 2A6, Canada.

[3]Department of Food and Human Nutritional Sciences, Faculty of Agricultural and Food Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada.

Introduction

Our laboratory has previously demonstrated that docosahexaenoic acid (DHA) may not only upregulate immune response pathways in human monocytes, but also reduce angiotensin converting enzyme 2 (ACE2), the cellular receptor for SARS coronaviruses (SARS-CoV), in various rat tissues and human cultured cells. Additionally, DHA was found to inhibit cellular entry of SARS-CoV-2 pseudovirus. Thus, we hypothesized that DHA can help manage SARS-CoV infection.

Methods

RNA-sequencing was performed on DHA-treated (20 or 125 µM for 8 h) and control human EA.hy926 endothelial cells in both the growing and quiescent states. The data were processed by the RSEM-STAR-DESeq2 pipeline, and then subjected to gene set enrichment analysis (GSEA) and over-representative analysis with cluster Profiler.

Results

Only in quiescent cells, 20 µM DHA was found to downregulate pathways related to SARS-CoV-1/2-host interactions in the KEGG database, specifically the processes by which the virus disrupts host protein translation and global mRNA splicing to suppress host defences. The term “potential therapeutics for SARS” in the Reactome database was positively enriched by both 20 and 125 µM DHA in quiescent cells only, including genes related to nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, interleukin-6 pathway, heat shock proteins, and *TBK1*. However, in growing cells, terms related to SARS-CoV-1/2-host interactions were upregulated by DHA. Beyond derailing SARS-CoV via translation machinery and ACE2, DHA was also previously found to concomitantly reduce ACE1 protein levels, thus preserving the ACE1/ACE2 balance. This is significant because the balance of ACE1/ACE2 is important for maintaining renin-angiotensin system homeostasis, a factor critical in the pathogenesis of long COVID.

Conclusion

Our findings proposed a novel perspective on DHA’s therapeutic potential for managing SARS-CoV infection, by hindering virus-host interactions. Furthermore, the beneficial effects of DHA only occurred in quiescent (healthy) endothelial cells but not growing (dysfunctional) endothelial cells, implying that COVID-19 patients without CVD may be more responsive to DHA treatment compared to patients with underlying CVD. Further *in vitro*, *in vivo*, and even clinical studies are required to validate these effects of DHA on SARS-CoV.

POSTER #113

STIM-dependent Pannexin 2 channel activation via N-terminal protein-protein interaction in response to calcium store depletion.

Christian L. Humphreys[1,2], Natalie E. Lavine[1,2], Michael F. Jackson[1,2]

[1]Department of Pharmacology and Therapeutics, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0T6.

[2]PrairieNeuro Research Centre, Kleysen Institute for Advanced Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0Z3.

Introduction

Pannexins are non-selective ion channels known for ATP release. While Panx1 is known for its involvement in pathophysiology, including stroke induced neuronal injury, little is known regarding Panx2 function. Interestingly, both Panx2 and Panx1 knockdown is necessary to prevent stroke induced neuronal damage. Previously, our lab discovered a novel activation mechanism for Panx1 involving ER resident stromal interaction molecules (STIMs). Sequence conservation and my own preliminary data shows that this mechanism can activate Panx2 as well. *I hypothesize that STIM1/2 activates Panx2 through a physical interaction with its intracellular domains.*

Methods

To test this hypothesis, I perform electrophysiology on human embryonic kidney (HEK) cells expressing Panx2 and STIM, and apply thapsigargin (Tg) to induce establish STIM-dependent activation. To identify the minimal region of Panx2 required for STIM-dependent activation we generated N-terminal deletion mutants. Using mice hippocampal neurons, I test whether Tg or oxygen-glucose deprivation (OGD), an in vitro stroke model, can stimulate Panx2-like currents.

Results

My findings show that Tg treatment stimulates STIM-dependent activation of Panx2 in HEK cells and confirm STIM coimmunoprecipitation with Panx2. N-terminal deletions of Panx2 are capable of membrane expression except for deletions targeting amino acids 29-53, suggesting this region is important for membrane trafficking. Notably, membrane expressing mutants exhibit reduced co-immunoprecipitation with STIM1, compared to full length Panx2. Supporting that STIM-dependent activation occurs in neurons, treatment of mouse hippocampal neurons with Tg or OGD generated large currents with properties consistent with those of Panx2. When Panx1 is blocked or deleted, activation is maintained, suggesting a role of Panx2 in mediating these currents.

Conclusions

Our evidence supports that Panx2 activation by Tg requires STIM. We show that STIM-binding to Panx2 N-terminal deletion mutants is attenuated compared to full length Panx2, suggesting a role for this region in STIM-dependent activation. Work is underway to test whether STIM likewise interacts with the C-terminus of Panx2. These studies characterize a first activation mechanism for Panx2 and will provide the basis to prevent STIM-dependent function of Panx2, which is essential to elucidate contributions of Panx2 in neurological disorders.

POSTER #59

Screening viral host dependency factors and human loss of function polymorphisms identifies broad-acting host-directed antiviral candidates.

Rubendren Jamilchelvan[1,2], Riley H Tough[1,2], Michelle Perne-Lemire[1,2], Xia Liu[3], Eric Enns[3], Paul J McLaren[1,2]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba,
[2]National HIV and Retrovirology Laboratory at the JC Wilt Infectious Diseases Research Centre, National Microbiology Labs, Public Health Agency of Canada,
[3]Bioinformatics Group, National Microbiology Labs, Public Health Agency of Canada

Introduction

Multiple genome-wide knockout/knockdown studies of viral infection have identified sets of host dependency factors (HDFs) that are essential for viral replication. Although these factors may be candidates for developing novel antivirals, defining candidates that do not lead to drug toxicity is challenging.

Methods

One opportunity to identify promising targets is to leverage available human genome resources to determine which HDFs harbour homozygous loss of function polymorphisms in healthy people. To that end, we sought to combine data from 27 genome-wide host dependency factor screens that covered HIV, Hepatitis C, Hepatitis D, SARS-CoV-2, SARS-CoV, Ebola, Influenza A, Zika, Dengue and West Nile virus, with the genome aggregation database (gnomAD) including >125,000 human exome and >15,000 whole-genome sequences.

Results

We identified 2,907 unique HDFs combined across all viruses, including 353 which were essential for $\geq$2 viruses and 2 which were essential for 5 viruses. Of the combined list, 137 targets were deemed non-essential by the observed/expected constraint score and the presence of homozygous loss-of-function variants found within the gnomAD control population. When functionally annotated, the 2,907 unique HDFs were enriched for several biological processes, notably protein transport, macromolecule catabolism, autophagy, and vacuole organisation. We also found that HDFs implicated in more than one virus were highly intolerant to a loss of function variant, suggesting they are likely to be involved in host-essential processes.

Conclusion

In silico and *in vitro* screening of HDFs harbouring homozygous loss of function variants in healthy people may aid in developing novel broad-acting antivirals capable of targeting more than one virus.

POSTER #144

Characterizing the neuronal role of histone acetyltransferases KAT6A and KAT6B using Drosophila melanogaster

Delaram Jebreili Rizi[1,2,3], Harsimran Kaur[1,2], Zoe Goldstine-Joubert[1,2], Catherine Bland[1,2], Paul Marcogliese[1,2,3]

[1]Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Canada. [2]Children's Hospital Research Institute of Manitoba, Winnipeg, Canada
[3]Excellence in Neurodevelopment and Rehabilitation Research in Child Health (ENRRICH) Theme, Winnipeg, Manitoba

Introduction

Dominant variants in either histone acetylation genes, *KAT6A* or *KAT6B*, cause neurodevelopmental conditions (Arboleda-Tham syndrome and SBBYSS syndrome) with overlapping features. Children show developmental delay, motor and speech impairment, and some develop seizures. We propose to study these disorders by examining the role of the single *Drosophila* ortholog, *enok*, in neurons. Moreover, we will overexpressing human KAT6A or KAT6B as well as patient variants to assess their function in flies to discern genotype-phenotype correlation for pathogenic variants associated with a range of symptoms in humans.

Methods

We will study the effects of developmental and adult-specific neuronal knockdown of *enok* using the UAS-GAL4 system in flies. Moreover, we have generated transgenic flies that express *KAT6A* and *KAT6B* and disease variants and assessed functional outcomes in vivo. We drove enok-RNAi using ubiquitous (Act-GAL4) and neuronal GAL4s (elav-GAL4, nSyb-GAL4) and examined lethality, lifespan, climbing, and seizure behaviour. The KAT6A/B variants were made using site-directed mutagenesis and commercial transgenesis.

Results

The neuronal knockdown of *enok* showed significant phenotypes like seizures and climbing defects with elav-GAL4. With nSyb-GAL4, the lifespan of the flies was diminished, and some RNAi lines caused lethality. We successfully generated 19 *KAT6A/B* variants and preliminary data has found a range of function when expressed ubiquitously in the fly.

Conclusion

Together, the function of enok in neurons is essential for the proper development of flies and knockdown can cause detrimental deficits. Moreover, flies can be used as a simple system to test the function of potential KAT6A or KAT6B variants in disease.

POSTER #128

Automated and Highly Accurate FDG-PET Reading for Differential Diagnosis of Dementia Disorders

Neil Junankar[1,2], Jarrad R. Perron[2,3], Ji Hyun Ko[1,2,3]

[1]Department of Human Anatomy and Cell Science, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB R3E 0W2, Canada.
[2]PrairieNeuro Brain Research Centre, Kleysen Institute for Advanced Medicine, Health Sciences Centre, Winnipeg, MB R3E 3J7, Canada.
[3]Graduate Program in Biomedical Engineering, Price Faculty of Engineering, University of Manitoba, Winnipeg, MB R3T 2N2, Canada.

Introduction

Neurodegenerative dementia disorders may be characterized by a progressive decline in cognitive functions which impact daily life, however these contain a broad spectrum of diseases each with unique pathology and diagnostics. The standard practice for their differential diagnosis is brain fluorodeoxyglucose positron emission tomography (FDG-PET) because these may be differentiated by unique spatial patterns of resting cerebral glucose metabolism. It remains common practice that these studies are interpreted by subjective visual impressions of tracer uptake throughout the brain by a physician. In this study, we present an automated system for differential diagnosis of neurodegenerative dementia spectrum disorders with FDG-PET.

Methods

Brain FDG-PET studies were performed on 212 people who were referred to our PET centre between 2011 and 2019 through local dementia clinics. These were a mix of patients with Alzheimer's disease (AD), behavioral-variant frontotemporal dementia (bvFTD), dementia with Lewy body (DLB) and primary progressive aphasia (PPA). Fifty-four of these received clinical follow-up with diagnosis of dementia (AD = 25, DLB = 17, bvFTD = 6, PPA = 6). Twenty-six patients did not have dementia but stayed below normal threshold for >6 months follow-up period (mild cognitive impairment; MCI). Nine patients' cognitive performance was normal, and twelve patients had non-cognitive conditions (e.g., psychiatric disorders). Other patients (n=111) were unincluded due to the lack of clear clinical diagnosis and/or no follow-up data. Static FDG-PET images were affinely registered to the O^{15} perfusion template provided in SPM12 and smoothed with an isotropic 8mm Gaussian filter. Using a novel combined approach of scaled sub profile modeling (SSM) and k-nearest neighbors (KNN), we trained two 7-group classifiers with and without data augmentation with mixing method.

Results

Without data augmentation, the classifier accuracy was 48% which was dramatically improved after the data augmentation upto 97%. When leave-one-out cross-validation was applied, the accuracy was reduced without data augmentation (25%), yet it was preserved with data augmentation (96%).

Conclusion

Results show that data augmentation combined with SSM feature selection and a KNN is an effective means of automating reading of FDG-PET images for differential diagnosis of neurodegenerative dementia disorders.

POSTER #108

Investigating the impact of MeCP2 mutation on the epigenetic landscape of brain cells

Ashraf Kadar Shahib[1], Ghanan Bin Akhtar[1], Khatereh Saei Arezoumand[1], James R. Davie[1], and Mojgan Rastegar[1]

[1]Department of Biochemistry and Medical Genetics, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada

Introduction

Rett Syndrome (RTT) is a neurological condition primarily affecting females and typically identified in infants by 1-2 years of age. **Methyl-CpG-binding protein 2 (*MECP2*)** gene mutations are the major cause of RTT. MeCP2 is the main methyl-binding protein in the brain. MeCP2 is a chromatin-binding nuclear protein, that binds to methylated DNA. The mechanistic basis of the MeCP2 interaction with the chromatin structure is significant for understanding the role of **MeCP2** in gene regulation. The chromatin structure consists of an array of nucleosomes, each being made up of an octamer of **core histones**, with 147 bp of DNA wrapped around each **nucleosome**. The linker **histone-H1** binds to the linker-DNA sequences helping in linear compaction of the chromatin. **MeCP2** competes with **histone-H1** binding to the linker DNA, with significant importance in neurons.

Methods

In this project, I employ experiments including **histone extraction** and **total cell protein extraction** from different brain regions of RTT-associated MeCP2 mutations using male and female transgenic mice with knock in mutations (**T158M and R255X**) and their **wild-type controls**. Extracted proteins are further separated by the use of **SDS-PAGE** and specific proteins and histones are studied. Finally, the results of wild-type mice are compared with those of the **sex- and age-matched RTT transgenic mice**, to study the impact of MeCP2 mutations on histone proteins, DNMTs (DNA methyltransferases), TETs (Ten-eleven translocation family proteins), and chromatin accessibility.

Results

We have evidence suggesting that the levels of **histone H1** is significantly increased between **wild-type** and **R255X mutant** in a specific **brain region**. We also have a trend suggesting that the levels of histone H1 decrease in the mutants compared to the wild-types in the case of another **brain region**, but increase in the case of a specific **brain region**.

Conclusion

The aim of this project is to study the effects of **MeCP2 loss-of-function** on the global epigenome landscape. Understanding the interplay between **MeCP2** and **histone H1**, and their relation to **core histones**, is critical in the **brain development** and **neurological** complications associated with Rett Syndrome. Moving forward, I aim to investigate the differences in the chromatin architecture between the **wild-types** and **MeCP2 mutants**.

POSTER #90

Characterization of the effect of sex steroids on CD8+ T-cell differentiation *in vitro*

Kamali Kannan[1,2], and Janilyn Arsenio[1,2,3]

[1] Department of Immunology, Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada.
[2] Manitoba Centre for Proteomics and Systems Biology, Winnipeg, Manitoba, Canada.
[3] Department of Internal Medicine, Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada.

Introduction

CD8+ T-cells diversity is pivotal in host response to infections and chronic inflammation. Upon acute infection, naïve CD8+ T-cells are activated, then differentiate into short-lived effector and long-lived memory cells. However, in chronic infection, CD8+ T-cells may become exhausted, characterized by weakened effector functions and elevated expression of inhibitory receptors compared to effector and memory T-cells. Biological sex differences in the immune system can be due to sex hormones modulating immune responses. In general, females mount stronger immune responses than males; however, they are more susceptible to developing autoimmune and inflammatory diseases. Understanding how sex hormones influence CD8+ T-cell differentiation in chronic viral infection remains a critical knowledge gap.

Methods

To investigate the impact of sex hormones on CD8+ T-cell differentiation *in vitro*, transgenic P14 CD8+ T-cells, expressing T-cell receptors that specifically recognize the gp-33 peptide, were activated by gp-33 peptide and cultured with sex steroids such as estradiol, dihydrotestosterone, and progesterone, along with IL-2 or IL-15 cytokines, to induce effector and memory CD8+ T-cell differentiation, respectively. Effector and memory T-cells were analyzed at 72 hours post-activation based on the activation markers (CD44, CD25, & CD62L), proliferation marker (Ki-67), and cytokine production (Interferon- γ, TNF- α). To generate exhausted T-cells, P14 CD8+ T-cells were cultured with continuous peptide for an additional 48 hours and analyzed based on exhaustion markers (PD-1, Tim-3, TCF-1, & Ly108) and cytokine production.

Result:

Preliminary findings indicate no difference in the activation of T-cells based on the expression of an activation marker, CD25, in the presence of sex steroids. However, an increased frequency of cytokine production such as, IFN-γ and TNF-α was observed on CD8+ T-cells cultured with estradiol and IL-15.

Conclusion

The preliminary result shows that CD8+ T-cells cultured with estradiol and IL-15 exhibit a higher frequency of cytokine production compared to dihydrotestosterone and progesterone-treated groups. This study has the potential to reveal how sex steroids affect CD8+ T-cell differentiation and function. This knowledge can enhance our understanding of sex-specific immune responses and to potentially inform personalized therapeutic strategies for infectious and autoimmune diseases.

POSTER #130

Role of astrocyte N-methyl-D-aspartate (NMDA) receptors and purinergic signalling in regulating cortical neuronal function in mice

Meher Kantroo[1], Noushin Ahmadpour[1], Michael J. Stobart[1], Jessica Meza-Resillas[1], Shahin Shabanipour[1], Jesus Parra-Nuñez[1], Tetiana Salamovska[1], Anna Muzaleva[1], Finnegan O'Hara[1], Dustin Erickson[1], Bruno Di Gaetano[1], Sofia Carrion-Falgarona[1], Bruno Weber[2], Alana Lamont[3,4], Natalie E. Lavine[3,4], Tiina M. Kauppinen[3,4], Michael F. Jackson[3,4], Jillian L. Stobart[1,5]

[1]College of Pharmacy, University of Manitoba, Winnipeg, MB, Canada.
[2]Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland.
[3]Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, MB, Canada., [4]PrairieNeuro Research Center, Health Sciences Center, Winnipeg, MB, Canada., [5]Centre on Aging, University of Manitoba, Winnipeg, MB, Canada.

Introduction

Astrocytes, a type of glial cells in the brain, communicate with nearby astrocytes and neurons via calcium signalling. These cells express N-methyl-D-aspartate receptors (NMDARs) which can cause a rise in astrocytic calcium levels. Furthermore, increases in astrocyte calcium is associated with release of purines like Adenosine triphosphate (ATP), which can modulate neuronal activity. The mechanisms by which astrocytic NMDARs can modulate neuronal activity via ATP has yet to be investigated.

Methods

In this study, NMDARs in astrocytes have been selectively targeted and knocked down in mice whisker barrel cortex. Immunohistochemistry and immunocytochemistry were performed to characterize the astrocytic NMDAR knockdown (KD). Awake *in-vivo* 2-photon calcium imaging was performed to study resultant astrocytic and neuronal calcium imaging. Texture discrimination behavior tasks were performed to investigate sensory acuity, i.e., the ability of mice to discriminate between two textures. Finally, acute pharmacology was done by applying ATP to the barrel cortex and studying possible rescue of astrocytic NMDAR KD related neuronal and behavior impairments.

Results

We observed a KD of astrocytic NMDARs in culture cells as well as brain tissue. Awake 2-photon imaging showed KD dependent calcium impairments in astrocytes and neurons. There were texture discrimination deficits present. Interestingly, upon ATP application, there was a rescue of neuronal and behavioral impairments.

Conclusion

This study highlights the functional relevance of astrocytic NMDARs in modulating cortical circuits via purinergic signalling. We show that astrocytic NMDARs are important for neuronal-astrocyte communication and sensory information processing in the whisker barrel cortex. This is relevant for diseases such as schizophrenia where NMDA receptors are poorly activated and thus, can help us shed light on disease mechanisms.

POSTER #143

Mutational analysis to Classify Thyroid Nodules

Amandeep Kaur, Kumar A. Pathak and Anuraag Shrivastav

Introduction

Indeterminant cytology is a challenge for pathologists for differentiated thyroid cancers (TC). Both adenomas and carcinomas have similar cytomorphology, making it difficult to differentiate without invasive procedures. The current diagnostic protocols include screenings, fine needle aspiration and surgeries all of which are invasive and inefficient in differentiating between thyroid tumors. The indeterminate diagnosis leads to cancer risks up to 30%. A major wave with molecular markers may provide potential support to differential diagnosis.

Research Objectives and Methodology

I aim to investigate the gene expression of N-myristoyltransferase 1 (NMT1), N-myristoyltransferase 2 (NMT2), Insulin growth factor receptor 1 (IGF1R) and Claudin 1 (CLD1) by qPCR in Follicular carcinoma (FC), Follicular adenoma (FA) and Papillary carcinoma (PC) samples. Furthermore, I investigated the specific point mutations and DNA methylation patterns by targeted next generation sequencing tNGS). The regulation patterns of these genes will determine its validity as a prognostic marker for Differentiated TC and whether it would be able to identify between the different thyroid cancers.

Results

The expression of four genes were compared between FA, FC, and PC. We did not observe any significant difference in *NMT1, NMT2* and *IGF1R* expression between FA, FC, and PC. *CLD1* gene showed similar expression for FA and FC, whereas in PC, the expression of *CLD1* was remarkably low. Similar pattern of *CLD1* gene was observed in the paired samples (adjacent normal tissue and tumor tissue). The tNGS data showed hyper and hypo methylation patterns in both NMT1 and NMT2, in the intronic and exonic regions. The DNA methylation patterns observed were different between the normal adjacent tissue and the tumor samples in numerous regions of NMT1 and NMT2. Further analysis is in progress with more samples to determine the point mutations and methylation status which could serve as differentiating and prognostic markers for TC.

Conclusion

Other than operative approaches for the diagnosis of nodules concerning papillary and follicular cytology do not exist, leading to false positive cases that receive unnecessary surgeries that could cause further implications. My research will allow us to distinguish between different TC without operative procedures thus reducing the risks of implications.

POSTER #48

A Descriptive Examination of Antenatal Care and Sub-regional Inequalities in Pakistan

Khan, Eefa[1]; Wehrmeister, Fernando[1,2]; Boerma, Ties[1]; Blanchard, James[1]

[1]Univeristy of Manitoba, Winnipeg, Canada.
[2] Federal University of Pelotas, Brazil.

Introduction

Pakistan has seen notable improvements in maternal and child health in the past few decades. However, there are concerns that the gains are modest as well as inequitable. Furthermore, there is a dearth of literature on the trends of inequalities, which rank among the widest regionally. It is crucial to address specific disparities and reach the most marginalized populations. This is the first study that explores utilizations and inequalities within Pakistan with district representativeness. Focusing on district-level variations allows for a deeper and more granular understanding of the unique challenges faced by specific regions, especially given that Pakistan's health decision-making has devolved to provincial authorities since 2010.

Methods

Data were analyzed from the Pakistan Multiple Indicator Cluster Survey (MICS) 2017-2020. Inequity measures to quantify the magnitude of disparities in antenatal care across wealth tiers between provinces and within provinces through summary measures of inequality including both simple and complex measures were utilized.

Results

We categorized districts into three tiers within each province based on the Multidimensional Poverty Index (MPI) – the poorest, middle and the richest. Our findings reveal variations in both coverage and quality between provinces, between wealth quintiles within provinces and between provinces. For example, wealth quintiles played a crucial role within province as well as between province in ANCq (quality in antenatal care indicator) - the poorest tiers in the wealthier province - Punjab performed better than the richest quintile in the poorest province - Balochistan. The analysis of antenatal care initiation times showed that wealthier quintiles commenced care sooner, and late initiation was particularly prominent in the lower quintiles of the wealthier provinces. A consistent pattern of rural-urban disparities was not observed, but rural areas often outperformed urban ones.

Conclusion

Wide wealth-based inequalities exist in the various components of antenatal care in Pakistan. The findings from this research can support the development of targeted strategies to reduce the impact of these wealth-based inequalities.

POSTER #15

Association between taste genetics and the dental plaque microbiome in early childhood caries

Mohd Wasif Khan[1,2], Vivianne Cruz de Jesus[2,3,4], Betty-Anne Mittermuller[2,3,4], Robert J. Schroth[2,3,4,5,] Pingzhao Hu[1,2,6*,] Prashen Chelikani[1,2,3,7*]

[1]Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MB, Canada.
[2]Children's Hospital Research Institute of Manitoba, Winnipeg, MB, Canada.
[3]Manitoba Chemosensory Biology Research Group, Oral Biology, University of Manitoba, Winnipeg, MB, Canada.
[4]Department of Preventive Dental Science, University of Manitoba, Winnipeg, MB, Canada.
[5]Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB, Canada.
[6]Department of Biochemistry, Western University, London, ON, Canada.
[7]Department of Physiology and Pathophysiology, University of Manitoba, Winnipeg, MB, Canada.

Introduction:

Early childhood caries (ECC), which is tooth decay in preschool children under six years, is influenced by a complex interplay of environmental, microbial, and genetic factors. Twin studies suggest that genetics may account for over fifty percent of the variation in dental caries. Taste genes further influence caries through preferences and immune responses mediated by taste-related proteins. This study aims to investigate the interplay between taste genetics and the dental plaque microbiome in ECC.

Methods

We explored the multifactorial aspects of ECC by analyzing genetics, microbiome, and host characteristics data from a cohort of 554 children. Dental plaque samples underwent 16S rRNA and ITS1 sequencing to profile bacterial and fungal abundance, respectively. The resulting amplicon sequencing data were processed with Qiime2, and MaAsLin2 in R was used to identify differentially abundant species. Variations in human taste-associated genes were identified using the GATK pipeline. We then examined the associations between these genetic variations and microbial features using PLINK, mbQTL, and mediation analysis methods. In our analysis, age, sex, and place of residence were used as covariates, and p-values were adjusted for false discovery rates.

Results

Significant associations were identified between ECC status and missense variations in taste genes TAS2R4, TAS2R40, and TAS2R43. Specifically, the missense variation in TAS2R43 was found to be associated with ECC-associated bacterial species *Veillonella_dispar* and *Gemella_morbillorum*. This genetic variation changes the amino acid at position 12 from leucine to valine, located near the protein's N-terminal region.

Conclusion

Our research underscores the significant role of variants in taste-related genes in modulating the oral microbiome, which may influence the development of dental caries. This study highlights the critical roles of genetic and microbial factors in the susceptibility to dental caries. It also advocates for the development of multidisciplinary approaches that enhance diagnostic, treatment, and preventive strategies in pediatric dentistry, leading to more personalized care for young children.

POSTER #77

Lactating Parents Attending Winnipeg Breastfeeding Centre: A Descriptive Study

Narges Khodabandehloo[1,3,4] Larisa Lotoski[2,3,4] Katherine Kearns[3,5,6,10] Christina Raimondi[3,5,6] Alexander Singer[6,7] Nathan C. Nickel[1,3,4,8,9] Meghan B. Azad[1,2,3,4]

[1]Department of Community Health Sciences, University of Manitoba, Canada.
[2]Department of Pediatrics and Child Health, University of Manitoba, Canada.
[3]Manitoba Interdisciplinary Lactation Centre (MILC).
[4]Children's Hospital Research Institute of Manitoba (CHRIM).
[5]Winnipeg Breastfeeding Centre, Manitoba, Canada.
[6] Department of Family Medicine, University of Manitoba, Canada.
[7]Manitoba Primary Care Research Network (MaPCReN).
[8]Manitoba Centre for Health Policy (MCHP).
[9]Faculty of Kinesiology and Recreation Management, University of Manitoba, Canada.
[10] Department of Obstetrics, Gynecology and Reproductive Sciences, University of Manitoba, Canada.

Introduction

The World Health Organization recommends exclusive breastfeeding for the first 6 months of life and continuation of breastfeeding for at least 2 years, with complementary food introduction at 6 months. Despite these recommendations, in Canada, almost 91% of mothers initiate breastfeeding, yet only 34% continue exclusive breastfeeding for the first 6 months of their child's life. This low rate of exclusive breastfeeding is also reflected in Manitoba (42%). Breastfeeding difficulties coupled with low specialized breastfeeding support, especially among first-time parents, lead to early cessation and non-exclusive breastfeeding. Winnipeg Breastfeeding Centre (WBC) is the only clinic in Manitoba exclusively dedicated to delivering diagnostic care at the physician level for breastfeeding dyads. Our project aims to describe the WBC patient population characteristics, breastfeeding difficulties, diagnoses, and treatments.

Methods

This is a retrospective longitudinal study describing the WBC client population (2017-2023). Data resources (~4000 records, ~1600 dyads) include clinic electronic medical records, free text format visit notes documented by the center physicians and an intake questionnaire gathering data on study population demographic characteristics, breastfeeding difficulties, risk factors and comorbidities. Data analysis includes manual variable extraction from the visit notes and descriptive statistics on a representative sample of the data (frequency, mean, standard deviation, and incidence).

Results

Data access and analysis is pending submitted Human Research Ethics Board and Provincial Health Research Privacy Committee approval. Expected results from this study:

1) Enhancing our understanding of who has access to the clinic, empowering stakeholders to adopt strategies to grow healthcare provision accessibility.
2) Informing the practices within the clinic and enhancing the quality of clinic care provision.
3) Offering additional avenues for research given the novelty of our study on this clinic data.

Conclusion

Identifying the population characteristics referred to WBC will shed light on who is and who is not accessing WBC, which is critical knowledge for equity and inclusivity in healthcare provision. This will pave the way for future explorations like WBC service delivery evaluation.

POSTER #69

Development and immortalization of *Peromyscus maniculatus* endothelial cell lines for analysis of infection with Sin Nombre virus

Levi Klassen[1,2], David Safronetz[1,2]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Manitoba, Canada.

[2]Special Pathogens Program, National Microbiology Laboratory Branch, Public Health Agency of Canada, Winnipeg, Manitoba, Canada.

Introduction

Sin Nombre virus (SNV), the etiological agent of Hantavirus Cardiopulmonary Syndrome (HCPS), is a pathogen of significant concern in the North American context, causing approximately 4-5 causes annually in Canada with a case fatality rate of 25%. The host reservoir of SNV are *Peromyscus maniculatus* rodents, and in all hosts, endothelial cells are considered the primary target of SNV. However, previous work has shown that, when *P. maniculatus* rodents are challenged with SNV obtained from human clinical samples or Vero E6 adapted SNV, they do not develop an infection. Similarly, Rhesus and Cynomolgus Macaques only develop severe disease when challenged with *P. maniculatus* passaged SNV, but not from Vero E6 adapted SNV. Because the pathology of SNV is hypothesized to be associated with increased expression of certain cytokines, the differences in pathology between different hosts may be visible at the in vitro level in endothelial cells from different host species. The overarching purpose of this project is to investigate the differences in cytokine expression from human and *P. maniculatus* endothelial cells when interacting with SNV sourced from human and *P. maniculatus* samples, and Vero E6 cells. Thus, the first stage of this project required the development of *P. maniculatus* endothelial cell lines.

Methods

Lung, adipose, and heart tissues were collected from male and female *P. maniculatus* rodents and endothelial cells were isolated using the respective Miltenyi dissociation and CD31+ isolation kits. These cells were immediately immortalized via SV40 large T antigen, and subsequently underwent G418 antibiotic selection.

Results

Cell types that successfully underwent the procedure resulted in cell lines developed originated from male heart, male adipose, female lung, and female adipose tissues. Flow cytometry will be conducted to confirm CD31 expression.

Conclusion

Further work will involve confirming CD31 expression of these cell types using flow cytometry, infecting these cell types with SNV from human and *P. maniculatus* samples, and Vero E6 cells, and comparing these results to infections conducted using HUVEC and HMVEC-L cells. Results from this project will help elucidate mechanisms of SNV in human hosts.

POSTER #150

Examining mental disorders among persons with and without intellectual and developmental disabilities: A retrospective population-based study

Kayla Kostal[1], Yona Lunsky[2], Alyson Mahar[1,3], Shay-Lee Bolton[1,4], Deepa Singal[1], and Shahin Shooshtari[1,5]

[1]Department of Community Health Sciences, University of Manitoba, Winnipeg, Canada.
[2]Department of Psychiatry, University of Toronto, Toronto, Canada.
[3]School of Nursing, Queen's University, Kingston, Canada.
[4]Department of Psychiatry, University of Manitoba, Winnipeg, Canada.
[5]St.Amant Research Centre, Winnipeg, Canada.

Introduction

33% of Canadians have a lifetime prevalence of mental disorders. Previous research examining the prevalence of mental disorders among persons with intellectual and developmental disabilities (IDD) found a significantly higher prevalence compared to persons without IDD or the general population. There has been no research examining the prevalence of mental disorders among persons with IDD in Manitoba. The purpose of this study was the calculate and compare the prevalence of any mental disorders, psychotic disorders, substance use disorders, and mood and anxiety disorders among persons with IDD and their matched comparison group.

Methods

A matched, retrospective cohort study was designed. Data were analyzed from the Manitoba Population Data Repository housed and maintained by the Manitoba Centre for Health Policy. To examine mental disorders through a period prevalence, 5 years of data (April 1, 2015 to March 31, 2020) were linked from various data sources. To identify persons with IDD, all data available from selected data sources and codes before April 1, 2015 were used. We matched adults with and without IDD at a ratio of 1:3 based on age, sex, and region of residence. Descriptive analyses, the generalized estimating equation technique, odds ratios, and 95% confidence intervals were conducted.

Results

Compared to the matched comparison group, persons with IDD had a significantly higher prevalence of any mental disorders (OR = 2.13; 95%CI: 2.06, 2.21), psychotic disorders (OR = 7.43; 95%CI: 6.82, 8.09), substance use disorders (OR = 1.9; 95%CI: 1.78, 2.03), and mood and anxiety disorders (OR = 1.87; 95% CI: 1.80, 1.94). We examined differences in the prevalence by age, sex, and region of residence.

Conclusion

Findings inform the mental disorder prevalence of Manitobans with IDD. Given the higher prevalence, more attention needs to be given to persons with IDD. These findings advocate for improvements in the mental health of persons with IDD. It is important to conduct more comprehensive health checks and screening among persons with IDD. Findings help inform policy, programs, and training of staff. Future research should be conducted to understand the underlying causes of the higher prevalence of mental disorders among persons with IDD.

POSTER #74

Multi-omic Characterization of the Temporal Metabolic Adaptations Underpinning Therapy Resistance and Tumor Recurrence in Glioblastoma

Helgi Kuzmychova[1], Emma Martell[1,2], Harshal Senthil[1], Esha Kaul[3], Ujala Chawla[1], Chitra Venugopal[4], Donald Miller[5,6], Tamra E. Werbowetski-Ogilvie[7,8], Sheila K. Singh[4,9], Tanveer Sharif[1,2*]

[1]Pathology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada. [2]Human Anatomy & Cell Science, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada. [3]Faculty of Science, University of Manitoba; Winnipeg, MB, Canada. [4]Surgery, McMaster University, Hamilton, Ontario, Canada [5]Pharmacology and Therapeutics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada. [6]PrairieNeuro Research Center, Kleysen Institute for Advanced Medicine, Health Sciences Centre, Winnipeg, Manitoba, Canada. [7]Pediatrics, Section of Hematology-Oncology, Baylor College of Medicine, Houston, Texas, USA. [8]Biochemistry and Medical Genetics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada [9] Biochemistry, McMaster University, Hamilton.

Introduction

Glioblastoma (GBM), the most prevalent malignant brain tumor in adults, boasts a bleak 5year survival rate below 10%, with prognosis stagnating over the past two decades. Despite the standard of care (SOC) involving surgical resection, temozolomide (TMZ) chemotherapy, and radiation (RT), 80-90% of patients experience relapse within 6-12 months, with recurrent GBM often proving refractory to subsequent treatments, rendering it ultimately fatal. Thus, an urgent need exists for novel therapeutic strategies to target recurrent GBM and enhance patient outcomes.

Recently, metabolic alterations have emerged as a hallmark of cancer that fuels uncontrolled cell proliferation and offers protection from therapy-induced stress. Metabolic pathways not only provide energy and biosynthesis but also essential co-factors for epigenetic modifications thus affecting transcriptional cell profile. Yet, the role of metabolism in GBM therapy resistance remains poorly understood. We hypothesize that specific metabolic adaptations underpin therapy resistance in GBM, presenting a viable therapeutic target to mitigate GBM relapse.

Methods

We developed a therapeutically relevant *in vitro* model that utilized samples derived from multiple patients and mimics SOC treatment regimen and *in vivo* patient-derived orthotopic xenograft mouse model. Using these models therapy resistant GBM cells were generated. We perform single-cell and bulk multiomics profiling at multiple treatment time points throughout TMZ-RT therapy to discern metabolic and transcriptomic alterations that follow TMZ-RT treatment.

Results

Temporal single-cell profiling unveiled a notable shift in heterogeneous populations of TMZ-RTresistant cells towards a more stem-like state. This transition was concurrent with alterations in multiple metabolic processes evolving throughout therapy, validated by metabolomics analysis and metabolic profiling of samples from multiple patients. Furthermore, consistent trends were observed in vivo. This metabolic rewiring encompassed various biosynthetic and energy-producing pathways, unfolding in a temporal and highly organized manner.

Conclusion

Our data provides a comprehensive and robustly validated picture of the transcriptional and metabolic adaptations that follow TMZ-RT treatment in patient-derived GBM cells both *in vitro* and *in vivo*. These findings emphasize the metabolic flexibility of the GBM cells and provide a solid foundation for the development of metabolism-based therapeutic strategies to mitigate cancer relapse in GBM.

POSTER #105

Gut Microbial Metabolites Inhibit Interferon-Lambda Signalling in Epithelial Cells Which May Be Involved in Regulation of NFkb:TLR2:NLRP3 Pathway that is Activated By Dietary B-Fructan Fibres in IBD

Samuel A. Lawal[1-3], Hana Olof,[4] Testimony Olumade[4], Terri Jeanson[2,5], Edgar Delbert[2,6], Wael El-Matary[2,6], Charles N. Bernstein[2,5,] Deanna M. Santer[4], Heather K. Armstrong[1-5]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Canada.
[2]IBD Clinical and Research Centre, University of Manitoba, Winnipeg, Canada.
[3]Manitoba Centre for Proteomics and Systems Biology, University of Manitoba, Winnipeg, Canada.
[4]Department of Immunology, University of Manitoba, Winnipeg, Canada.
[5]Department of Internal Medicine, University of Manitoba, Winnipeg, Canada., [6]Department of Pediatrics, University of Manitoba, Winnipeg, Canada.

Introduction

Inflammatory bowel diseases (IBD) involve altered immunity, gut microbiome and diet. High fibre diets are typically healthy. However, IBD patients express intolerance of high-fibre foods. We showed this is due to altered gut microbiome resulting in reduced fermentation of fibres by gut microbes. We found β-fructan fibres can induce inflammation via TLR2 and NLRP3 if not fermented by gut microbiota in IBD. However, this pro-inflammatory response occurred only in select IBD patients, suggesting altered gut immunity may predispose response to β-fructans. TLR2 may drive NLRP3-induced secretion of pro-cytokines via NFkB. A study suggested anti-inflammatory IFN-λ may inhibit NF-Kb, suggesting IFN-λ may impact TLR2:NLRP3 activity. We previously showed IFN-λ receptor is decreased in IBD patient gut tissues, suggesting the anti-inflammatory effect of IFN-λs may be dampened in IBD, perhaps indicating loss of IFN-λ drives sensitivity to B-fructans. As infectious microbes have been reported to impact IFN-λs signalling, and changes in the gut microbiota are a hallmark of IBD, I hypothesized that changes in microbiota in IBD inhibit IFN-λ signalling, allowing the B-fructan:TLR-2:NLRP3 inflammatory cascade to continue unimpeded.

Methods

Mucosal gut microbiota were collected from pediatric IBD (n=3) and non-IBD (n=3) patients during colonoscopy. Microbiota were cultured in modified brain heart infusion (BHI) media (15 hours, 37°C) under anaerobic conditions and supernatants were collected (microbial secretions). Caco2 cells were cultured with β-fructans (2mg/ml), +/-IFN-λ3 (50ng/ml), +/-microbiota secretions, or controls (LPS [1ug/ml], TNFa [10ng/ml]); cytotoxicity was examined by prestoblue, qPCR performed to determine IFN-λ activity (IFN-stimulated genes; ISGs), and ELISA to examine cytokine secretion.

Results

Treatments were not cytotoxic at indicated concentrations. IFN-λ3 upregulated ISGs. The addition of 4% ($P \leq 0.001$) or 10% ($P \leq 0.0001$) microbiota secretions inhibited IFN-λ3-induction of ISGs. I expect B-fructan induces IL-8 secretion in caco2 supernatant; I expect combining IFN-λ3/B-fructan reduces IL-8 secretion compared to B-fructan only; I expect adding IBD microbiota secretions with IFN-λ3/B-fructan inhibits IFN-λ, thus restoring B-fructan induction of IL-8 secretion.

Discussion

Our findings suggest gut microbial secretions can inhibit IFN-λ3 activity. I hope to uncover changes in IFN-λ in IBD impact B-fructans sensitivity and the molecular mechanisms underlying these changes in cell signalling pathways in IBD.

POSTER #20

Precise fine-tuning of CD19 CAR expression to mitigate T-cell exhaustion in CAR T cell therapy.

Paul Jerard M. Layug[1,2], Huda Alfarekh[2] and Yale Michaels[1,2]

[1]Department of Biochemistry and Medical Genetics, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada.

[2]Paul Albrechtsen Research Institute, CancerCare Manitoba, Winnipeg, Canada.

Introduction

Chimeric Antigen Receptor (CAR) T therapy has revolutionized the immunotherapy landscape, especially for leukemia and lymphoma. CARs are engineered fusion proteins comprised of an extracellular ligand binding domain that recognize cancer antigens, linked to an intracellular activation domain that licenses the T cell for cancer-killing. The majority of patients treated with CAR T achieved complete remission and have been living disease-free for years. To date, six CAR T products have been FDA-approved. Despite its clinical success, suboptimal durability and safety continue to delimit the advancement of CAR T as first-line treatment for cancers. Such limitations lie in the overly high levels of CAR expression on the transduced T cells, resulting in two major challenges: T cell exhaustion rendering them dysfunctional, and excessive inflammation resulting in immunopathology such as cytokine release syndrome.

Methods

Utilizing the microRNA silencing-mediated fine-tuners (miSFIT) technology, we created a library of lentiviruses that enables us to have precise, stepwise control of CD19 CAR transgene expression in human T cells. miSFITs are engineered target sites that recruit endogenous microRNA, thereby controlling the expression of a gene of interest. We tested the functionality of these CAR T cells *in vitro* against NALM-6 cells, a CD19-positive B-cell acute lymphocytic leukemia cell line. After 48-72 hours of co-culture, we quantified tumour cell killing, and performed immunophenotyping for T cell activation, cytokine production, memory subtypes, and exhaustion markers by flow cytometry.

Results

Our *in vitro* co-culture assays show that reducing the expression of CD19 CAR does not hamper tumour-killing capacity. However, after successive rounds of re-challenging– by replenishing the NALM-6 target cells– we observe notable differential dynamics of T cell activation, inflammatory cytokine production, and exhaustion phenotypes when we modulated expression levels of the CD19 CAR in T cells.

Conclusion

We demonstrate that it is feasible to precisely control CD19 CAR expression levels in human T cells using miSFIT technology. More importantly, these CD19 CAR-miSFIT T cells display effective anti-tumour properties but differing T-cell fate specifications. Ultimately, our goal is to define a “goldilocks” level of CAR expression where T cell exhaustion is mitigated, whilst maintaining effective and durable tumour-killing capacity.

POSTER #56

Development of a Flow Cytometric Approach to Study Immune Markers of HIV Risk in T Helper Subsets

Toby Le[1], Catherine M. Card[1,3], Julie Lajoie[1,4] and Keith R. Fowke[1,2,4,5].

[1]Medical Microbiology and Infectious Diseases
[2]Community Health Science University of Manitoba,
[3] Division of sexually transmitted and blood-borne infections, National Microbiology Laboratory Branch, Public Health Agency of Canada
[4]Medical Microbiology University of Nairobi
[5]Partner for Health and Development in Africa.

Background

HIV remains a major global health issue, with over 39 million people living with the virus and 1.3 million new infections in 2022 alone. During infection, HIV specifically targets the CD4 molecule found on T helper cells (Th cells) to mediate its viral entry. There are five well-characterized subsets of CD4 T cells: Th1, Th2, Th17, regulatory T cells, and follicular helper T cells. The Th subsets are particularly important to the immune system due to their specialized cytokine productions, enabling them to target specific classes of pathogens. Moreover, different Th subsets can exhibit varying levels of susceptibility to HIV infection. Toinvestigate these HIV target cells, we are developing a flow cytometric assay to detect Th subsets and measure their expression of biomarkers associated with increased HIV risk.

Methods

The development of this assay involved utilizing peripheral blood mononuclear cells (PBMCs) from healthy Winnipeg donors (n=4). Following PBMC isolation, cells were pre-stained for chemokine receptors to identify specific Th subsets: Th1 ($CCR6^{-}CXCR3^{+}$); Th2 ($CCR6^{-}CXCR3^{-}CCR4^{+}$); Th17($CXCR3^{-}CCR6^{+}CD161^{+}$); Th1/17 ($CCR6^{+}CXCR3^{+}$). Following pre-staining, the cells were subjected to *ex vivo* staining for T cell markers (CD3, CD4, and CD8 T cells) and activation markers (CD38, HLA-DR, and CD69).

Results

This assay has been optimized for clone interactions, fluorescence compensation, voltage titration, and antibody titration. Upon implementation, this assay can measure the % frequency of the following Th subsets in PBMCs: Th1 (% mean ± SD: 12.10± 1.54), Th2 (12.60 ± 8.37), Th17 (6.38 ± 3.36), Th1/Th17 (6.53 ± 2.52). Moreover, the assay can also measure activation markers associated with increased HIV risk (CD38, HLA-DR, CD69, CCR5) in each Th subset, resulting in 24 potential readouts.

Conclusion

Moving forward, this assay will be employed to characterize HIV susceptibility in PBMCs collected from sex workers in Nairobi, Kenya.

POSTER #127

Establishing iPSCs from primary fibroblast cell lines derived from Ursus maritimus to model neural development in vitro.

Nicolas Leclerc[1], Evan Richardson[2], Colin Garroway[3], David Yurkowski[4], Stephen Petersen[3,5], Meaghan Jones[1], Mohammed Mostajo-Radji[6], Sabine Mai[1], Robert Beattie[1]

[1] University of Manitoba, Rady Faculty of Health Sciences, 745 Bannatyne Ave, Winnipeg, Canada; Department of Biochemistry and Medical Genetics.

[2] Environment Canada, Environment and Climate Change Canada, 344 Edmonton Street Winnipeg, Canada; Science and Technology Branch.

[3] University of Manitoba, Faculty of Sciences, 66 Chancellors Cir, Winnipeg, Canada; Department of Biological Sciences.

[4] Fisheries and Oceans Canada, 200 Kent St, Ottawa, Canada; Division of Arctic Research.

[5] Assiniboine Park Conservatory, 55 Pavilion Crescent, Winnipeg, Canada; Conservation and Research.

[6] University of California, Santa Cruz, 2300 Delaware Ave, Santa Cruz, United States; Genomics Institute.

Introduction

Polar bears (PB) are highly intelligent, having one of the largest brain-to-body ratios of any land mammal. Understanding the fundamental processes that make the PB brain unique at a cellular and molecular level is important to the future preservation of this species.

Objectives

In this study, we aimed to culture primary fibroblast cells withdrawn from PB skin biopsies and to investigate their neuronal development by reprogramming these cells into induced pluripotent stem cells (iPSCs). iPSCs have emerged as a recent advancement in stem cell biology and provide novel approaches to studying and capturing the genetic diversities of animal populations. Adjacent to this, we also aimed to differentiate early fibroblast cells directly into neuronal cells through chemical induction. With these cells we will have a platform to test the effects of environmental toxins on PB brain development.

Methods & Preliminary Results

We have generated primary fibroblast cell lines using skin biopsies derived from PB in Churchill, Manitoba. PB fibroblasts have been subcultured for over 20 passages, and karyotypic analysis confirms expected chromosome numbers (74 chromosomes diploid) and cell morphology. We also directly reprogrammed PB fibroblasts into neurons through chemical induction, as confirmed by Map2 and Sox2 immunostaining. Finally, we have begun differentiating PB fibroblasts into iPSCs using the CytoTune 2.0 commercial transduction kit. Colonies have formed and have been confirmed as stem cells by TRA-1-60 live cell staining.

Conclusion

This research program represents important impacts on conservation, and evolutionary brain research, potentially creating a paradigm shift in how we study and preserve endangered species.

POSTER #167

Monkeypox surveillance in GBMSM, Sex Workers and PLWH in Kenya

Candice Lemaille[1], Souradet Shaw[2], Lyle McKinnon[1], Jason Kindrachuk[1]

[1]Department of Medical Microbiology & Infectious Diseases, University of Manitoba, Winnipeg, Canada.,
[2]Department of Community Health Sciences, University of Manitoba, Winnipeg, Canada.

Introduction

Emerging diseases are one of the greatest threats to global health. For this reason, there is an urgent need to increase preparedness and response measures to prevent outbreaks. Monkeypox virus (MPXV), a member of family *Orthopoxiridae*, is the causative agent of mpox and is clinically similar to smallpox in humans. Historically, the absence of rapid diagnostic, vaccination deployment, and surveillance infrastructure have had significant effects on transmission containment. Further, mpox incidence is increasing in endemic regions. The main goal of this project is to conduct MPXV surveillance in high-risk population in Kenya.

Methods

Because the 2022 outbreak was mainly in those who identify as gay, bisexual, and other men who have sex with men (GBMSM) and through sexual contact, the participant cohort will include this community as well as sex workers, which are included in current mpox cases identified in Democratic Republic of the Congo. Sera and lesion samples will be collected from an ongoing anorectal health screening program and assessed for active or past MPXV infection. An enzyme-linked immunosorbent assay (ELISA) will be performed to assess prior exposure to MPXV. Active lesions will be analyzed by polymerase chain reaction (PCR) and sequencing to do phylogenetic linkage.

Results

Preliminary data from the International Mpox Response Consortium (IMReC) have shown seropositivity against human orthopoxviruses among sex workers in Nairobi. Because participants were not considered vaccinated against smallpox and there are no mpox-reported cases in Kenya, there is a need to investigate the circulation of MPXV in this country adjacent to endemic regions.

Conclusion

The significance of this project is to conduct early identification of new introductions of MPXV in Kenya. This project will help identify additional hotspots for cryptic virus transmission and provide additional justification for requesting rapid access to vaccines and therapeutics for mpox in sub-Saharan Africa.

POSTER #141

Viral Immunogenicity as a Potential Determinant of Virulence: Impacts of Antigenic Variations in the SARS-CoV-2 Omicron BA.1 Variant of Concern

Katherine Li[1,2], Paul Sandstrom [1,2], Hezhao Ji [1,2]

[1]National Microbiology Laboratory at JC Wilt Infectious Diseases Research Centre, Public Health Agency of Canada, Winnipeg, MB, Canada.

[2]Department of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Winnipeg, MB, Canada.

Introduction

With the advent of the SARS-CoV-2 Omicron Variant of Concern, the COVID-19 pandemic quickly shifted towards an endemic phase, driven by Omicron's increased transmission rate yet reduced disease severity. Despite this, continued viral evolution within Omicron's descendant lineages has raised concerns regarding immune evasion, as both vaccinated and previously infected individuals remain susceptible to new infection.

Methods

Comprehensive computational analyses compared the SARS-CoV-2 Omicron BA.1 variant to other SARS-CoV-2 Variants of Concern (VOCs), as well as the ancestral Wuhan lineage. T cell (class II) epitopes were predicted for the spike protein of each lineage with the IEDB-AR TepiTool. The resulting putative epitopes were sorted by sequence similarity with our self-developed tool, CAVES. Mutated epitopes from the VOC lineages were assessed by the quantity of human leukocyte antigen (HLA) alleles predicted as binders, as well as their relative binding affinities to determine how immune recognition differed between viral lineages.

Results

Regular and promiscuous epitope predictions suggested that the Omicron BA.1 variant possessed a larger number of epitopes recognizable by the host immune system compared to other VOC lineages. Mutated epitopes from the Omicron variant were also predicted to be recognized by a broader range of HLA alleles per epitope than those from the ancestral Wuhan strain. Furthermore, analyses of the HLA binding affinities indicated that mutated Omicron epitopes often exhibited enhanced binding to HLA alleles compared to those from the Wuhan strain, which may potentially influence the host immune response.

Conclusion

These results demonstrate the impact of mutations on immune recognition in the Omicron BA.1 VOC. As the reported patterns were not seen in other VOC lineages, this further supports the distinction of Omicron from the original Wuhan strain, as well as from other pre-Omicron VOCs. This work highlights the importance of ongoing research to understand the evolutionary progression of SARS-CoV-2 variants and the downstream effects of antigenic variation in the context of viral immunogenicity and virulence.

POSTER #8

Functional Characterization of *Slc23a3*: a novel ascorbic transporter in mouse

Ruotong Liu[1], Peter K. Eck[1]

[1]Department of Food and Human Nutritional Science, University of Manitoba, Manitoba, Canada.

Background

The murine solute carriers *Slc23a1* and *Slc23a2* are well characterised sodium-dependent ascorbic acid transporters which mediate its uptake into cells. *Slc23a1* is expressed in epithelial tissues and therefore determines ascorbate uptake and retentio. *Slc23a2* distributes ascorbate into somatic cells. A third member in the family, *Slc23a3*, remains uncharacterised. The mouse *Slc23a3* shares 50.26% and 53.32% protein sequence similarity with *Slc23a1* and *Slc23a2*, respectively. *Slc23a3* co-expresses with *Slc23a1* in epithelial tissues.

We therefore hypothesize that *Slc23a3* encodes an ascorbic acid carrier, specifically the protein mediating cellular release in epithelial tissues.

Objective

This project aimed to identify if *Slc23a3* mediates ascorbic acid transmembrane transport and its mode.

Method

The mouse *Slc23a3* coding sequence was synthesised and subcloned into the pGEM5Zf(+)-Xenopus expression vector. From this template, capped complementary RNA was transcribed using the HiScribe® T7 ARCA mRNA Kit, and 18.4 ng was injected into individual *Xenopus Laevis* oocytes. Three days after injections the uptake and release of radiolabelled C^{14}-ascorbic acid was determined through scintillation spectroscopy.

Result

Oocytes expressing mouse Slc23a3 had higher internal radioactivity compared to sham injected oocytes when incubated with 300 µM and 3 mM C^{14}-ascorbic acid as external substrate. This uptake was not sodium dependent. Moreover, oocytes expressing mouse Slc23a3 released more radioactivity compared to sham injected oocytes after prior loading.

Conclusion

The internal accumulation of C^{14}-ascorbic acid radioactivity and its release from loaded oocytes under reductive experimental conditions identifies mouse *Slc23a3* as a transmembrane channel mediating ascorbic acid transport.

Relevance

Existing expression evidence combined with the presented experimental data strongly indicate that the mouse *Slc23a3* gene encodes the protein mediating ascorbate release form absorptive epithelial cells and selected somatic cells.

POSTER #5

Thioredoxin-interacting protein mediates chronic corticosterone treatment-induced depressive-like behavior and cognitive decline in mice.

M. Alejandra Llanes-Cuesta[1,2], Hua Tan[1,2], Azar Aghazadeh Khasraghi1[,2] and Jun-Feng Wang[1,2]

[1]Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, MB, Canada.

[2]Kleysen Institute for Advanced Medicine, Winnipeg, MB, Canada.

Introduction

The stress response in organisms is mediated mainly by the hypothalamic-pituitary-adrenal axis (HPA). During stress, glucocorticoids [cortisol in humans; corticosterone (CORT) in rodents] are released by the HPA axis targeting different organs including the brain. Although acute stress allows for adaptation to environmental stimuli, chronic stress impairs brain function and is a major risk factor for the development of depression. Thioredoxin-interacting protein (Txnip) is an inhibitor of thioredoxin (Trx), a protein that plays an essential role in regulating redox balance. Recently, our lab found that Trx may regulate cAMP response element binding protein (CREB) neurotrophic signaling by preserving the cellular redox balance, thus promoting neuronal differentiation. In addition, Trx inhibits apoptosis-signaling kinase 1 (ASK1) and prevents apoptosis. This evidence suggests that inhibition of Trx activity by Txnip could impair neuronal differentiation and lead to neuronal death, thus contributing to the development of depression.

Aim

This work aims to understand the role of Txnip in chronic CORT treatment-impaired neuronal differentiation and CORT-induced depressive-like behaviors in mice.

Methods and Results

Primary cultured mouse cerebrocortical neurons were treated with 0.01, 0.1, 1.0, and 10 µM CORT at 2 DIV for 5 days. Immunoblotting analysis showed that CORT treatment dose-dependently increased Txnip but not Trx protein levels. We also found that treatment with 0.1 µM CORT for 5 days increased phosphorylated ASK1 and decreased phosphorylated CREB protein levels in cultured neurons. Furthermore, we found that chronic treatment with CORT decreased dendritic outgrowth and branching, and that knocking down Txnip using CRISPR/Cas9 technology prevented CORT-impaired dendritic outgrowth. Finally, after female and male mice were exposed to 20mg/Kg CORT subcutaneous injections for 21 days, we found that chronic CORT treatment induced anxiety, anhedonia and behavioral despair depressive-like behaviors, and cognitive impairments. On the other hand, knocking down Txnip in the medial frontal cortex reversed CORT-induced depressive phenotype and cognitive decline in both female and male mice.

Conclusion

Altogether this data suggests that chronic CORT treatment upregulates Txnip, inhibits Trx activity, impairs neuronal differentiation and promotes neurodegeneration, which might contribute to the development of depressive-like behaviors in mice.

POSTER #41

Retrospective serosurveillance of historic monkeypoxvirus exposure among Kenyan sex workers from 2013-2018

Kristi Loeb[1*], Kieran Milner[1*], Candice Lemaille[1], Brielle Martens[1], Derek Stein[1,2], Julie Lajoie[1], Souradet Shaw[3], Anne W. Rimoin[4,5], Placide Mbala-Kingebeni[6,7], Keith Fowke[1], Joshua Kimani[1,8], Lyle McKinnon[1], Jason Kindrachuk[1,9]

[1]Department of Medical Microbiology & Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada., [2]Cadham Provincial Laboratory, Winnipeg, MB, Canada., [3]Department of Community Health Sciences, Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada., [4]Department of Epidemiology, UCLA Fielding School of Public Health, Los Angeles, California, United States.,[5]UCLA-DRC Health Research and Training Program, UCLA Fielding School of Public Health, Los Angeles, California, United States., [6]Pathogen Genomics Laboratory, Epidemiology and Global Health Department, Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo., [7]Service de Microbiologie, Cliniques Universitaires, Faculté de Médecine, Université de Kinshasa, Kinshasa, Democratic Republic of the Congo ., [8]Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya., [9]Department of Internal Medicine, Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada.

Introduction

Mpox is a zoonotic infectious disease caused by monkeypox virus, an orthopoxvirus considered endemic in West and Central Africa. Though monkeypox virus was first identified in humans in 1970, it has largely remained a neglected pathogen. The 2022 global outbreak brought a renewed interest, including the need to study not only endemic areas but nearby regions as well to fully delineate the impact of the virus.

Methods

656 cryopreserved serum samples taken from previous studies of female and male sex worker populations collected in Nairobi, Kenya between 2013-2018. This cohort included people aged 19 to 74 as of 2024, with most individuals falling between 20-39 and 40-55, and largely included people living with HIV (563 of 656). All samples were initially screened by a UV-inactivated vaccinia virus ELISA. 121 positive samples were then tested by meso scale discovery (MSD) containing five monkeypox virus antigens (A29L, A35R, B6R, E8L, and M1R) and five vaccinia virus antigens (A27L, A33R, B5R, D8L, and L1R) to confirm the ELISA results.

Results

99 of the 656 samples were found to be positive by VACV ELISA, with the highest positivity among the 20–39-year-olds (37.4%, 37/99 positives) and 40-55 year olds (36/99 positives) and in female sex workers (63/99 positives) compared to male sex workers or community samples. Seropositivity was also associated with HIV status, with 88 of 99 positives coming from individuals living with HIV. Of the 121 samples tested by MSD confirmed the VACV ELISA results, two samples in the 20–39-year age group had mpox antibody concentrations >1000 AU/mL indicating prior orthopoxvirus exposure.

Conclusion

Given the elimination of variola virus in 1970 and conclusion of the Kenyan smallpox vaccination campaign in 1972, the presence of orthopoxvirus antibodies in individuals younger than 52 years indicates previous exposure to an orthopoxvirus aside from variola virus or vaccinia virus. While camelpox virus has been detected in camels in northern Kenya, human cases have not been described in the region. Therefore, our results suggest that mpox has been circulating among sex workers in Kenya predating the current global outbreak.

POSTER #107

Examining colistin-resistant *Escherichia coli basS* R93P mutant outer membrane vesicles for their proteomic signatures and antimicrobial properties

Genevieve Loiselle[1], Shelby Reimer[1], George Zhanel[1], Jorg Stetefeld[2], Neillier Júnior[2], Dan Beniac[3], Timothy Booth[3], Patrick Chong[3], Garrett Westmacott[3], and Denice Bay[1]

[1]Department of Medical Microbiology and Infectious Diseases, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada.

[2]Department of Chemistry, Faculty of Science, University of Manitoba, Winnipeg, MB, Canada.
[3]National Microbiology Lab, Public Health Agency of Canada, Winnipeg, MB, Canada.

Introduction

Colistin (COL) antibiotics are last-resort treatments for multidrug-resistant pathogens such as *E. coli*. Recent studies show that prolonged exposure to COL by *E. coli* K-12 can increase mutations to a two-component sensor histidine kinase *basS*/*pmr*B (R93P) responsible for extreme COL resistance (512 µg/ml COL MIC). These mutants also demonstrate higher rates of outer membrane vesicles (OMVs) formation, which may be related to the resistance mechanism.

Methods

Here, we characterize the properties of these COL-resistant (COLR) OMVs to determine their composition and antimicrobial properties when purified and supplemented back to COL susceptible *E. coli* in a modified antimicrobial susceptibility (AST) assay. To compare COLR OMV properties, a hypervesiculating *E. coli* mutant Δ*tolA* and wildtype (WT) *E. coli* OMVs were included as controls, and all OMVs were isolated from 1 L culture ultradiafiltration purified supernatants.

Results

A comparison of OMV formation rates using dynamic light scattering and nanoparticle tracking analysis revealed that COLR-OMVs had a broad range of sizes as compared to controls and transmission electron microscopy images of COLR-OMVs were large and tubular in shape. Proteomic analyses of COLR-OMVs show a shift in uniquely identified membrane and periplasmic protein compositions, particularly in the outer membrane, suggesting COLR-OMVs made have distinct biomarkers as compared to WT cells and OMVs. Modified AST of isolated COL-OMVs revealed that their supplementation to WT cells with and without added COL had an antagonistic inhibitory effect on COL MIC values when compared to WT-OMV supplementation. To determine if COL drug carryover in OMVs was a factor, COLR-OMVs isolations were repeated without COL selection, and these vesicles lacking COL exposure did not show the same inhibitory affect as the COL OMVs where the cultures were grown with selection.

Conclusions

In conclusion, COLR-OMVs appear to have unique properties as compared to WT OMVs in overall shape, abundance, and proteome. COLR-OMVs also carry over and magnify COL drug in vesicle isolations; this factor can give COL-OMVs additional antimicrobial properties and the presence/absence of COL exposure by *basS* R93P mutants and their impact on OMV formation should be further explored.

POSTER #76

Do EpOMEs and DiHOMEs cause Airway Smooth Muscle Contraction?

McKay J.C. Lowry[1,2], Dina Mostafa[1,2], Luke Ziemanski[1,2], Christopher D. Pascoe[1,2]

[1]Department of Physiology and Pathophysiology, University of Manitoba.
[2]Biology of Breathing, Children's Hospital Research Institute of Manitoba.

Introduction

Oxylipins are a diverse group of lipid mediators found within the lung. Oxylipin profiles can differ between individuals with asthma and following exposure to environmental irritants. These oxylipins can regulate key aspects of airway physiology. Classical oxylipins, including the prostaglandins and leukotrienes, can mediate Airway Smooth Muscle (ASM) **contraction** or **relaxation** and play an important role in asthma pathophysiology. However, the function of most oxylipins in the context of ASM physiology remains unexplored. For example, epoxy-12-octadecenoic acids (EpOMEs) and dihydroxy-12-octadecenoic acid (DiHOMEs) are elevated in asthmatic lungs following exposure to pollen, but how they regulate ASM contractility remains completely unexplored. Recent data suggests that EpOMEs and DiHOMEs activate TRPA1 and TRPV1 channels in neuronal tissue. TRPA1 and TRPV1 are calcium permeant channels that are abundant in ASM and can regulate ASM contraction. **In this study, o*ur objective is to determine whether EpOMEs and DiHOMEs cause ASM contraction via TRPA1 and TRPV1 channels.***

Methods

hTERT immortalized human ASM were loaded with calcium indicator dye (Fura-2) and changes in intracellular calcium concentration was measured after addition of 9,10-EpOME, 12,13-EpOME, 9,10-DiHOME, and 12,13-DiHOME (10µM). Allyl-isothiocyanate (TRP activator, 1 µM) and histamine (10 µM) were used as a positive control. Phosphorylated Myosin Light Chain (p-MLC) was measured as a surrogate for contraction via Western Blotting. Data was analyzed via One-Way ANOVA and GraphPad.

Results

Intracellular calcium concentration was increased in ASM (Figure 1.) by exposure to **9,10 EpOME (319ηM±157), 12,13 EpOME (437ηM±116), 9,10 DiHOME (60ηM±49), 12,13 DiHOME (472ηM±293).** Contextually, vehicle control elicited a small increase (15ηM±13), whereas positive controls allyl-isothiocyanate and histamine caused a 542ηM and 359ηM±116 increase, respectively. Peak calcium occurs within 40 seconds of oxylipin addition excluding 9,10 EpOME which elicited a later response. P-MLC abundance compared to control was increased following treatment with **9,10 EpOME (58%), 12,13 EpOME (113%), 9,10 DiHOME (106%), 12,13 DiHOME (86%)**. For context, histamine and allyl-isothiocyanate had a 45% and 54% increase, respectively.

Conclusion

EpOMEs and DiHOMEs all caused calcium release and increased p-MLC, but with varying efficacy. This data gives compelling evidence to suggest that EpOMEs and DiHOMEs are possible contractile agonists for ASM. More work is required to understand the variability in their activity and whether these effects are mediated via TRP channels. Understanding the contractile effects of non-classical oxylipins will help to discover whether they favor airway narrowing. This will indicate whether these oxylipins are detrimental or benign in lung diseases like Asthma.

POSTER #85

Milk-derived extracellular vesicles attenuate NF-κB activation and NLRP3 inflammasome formation in polarized human microglia.

Lu Jueqin[1] and Wijenayake Sanoji[1]

[1]Department of Biology, The University of Winnipeg, Winnipeg, Manitoba, Canada.

Introduction

Exposure to maternal stress is associated with the activation of pro-inflammatory responses in offspring cell and systems, including Nuclear Factor Kappa-B (NF-κB) pathway [1] and NLR family pyrin domain containing 3 (NLRP3) inflammasome formation[2]. The pathways lead to the production of pro-inflammatory cytokines and chemokine precursors [3]. Milk-derived extracellular vesicles (MEVs) are bioactive nanovesicles that transport biological cargo, such as DNA, RNA, protein and microRNA to offspring. MEV supplementation has been shown to attenuate pro-inflammatory pathways in neonatal intestines, lungs, and kidneys[6], but it's ability to control neuroinflammation in the central nervous system is unknown[7]. My objective is to investigate if MEVs may induce pro-survival benefits by attenuating NF-κB pathway activation in microglia, the central immune cell of the brain parenchyma.

Method

Immortalized human microglia were primed with 10 ng/mL of interferon-γ to induce a pro-inflammatory phenotype. Microglia polarization was confirmed by measuring the transcript abundance of pro-inflammatory markers. 10 ng/mL MEVs were supplemented 24 hours post- IFN-γ priming. The cells were divided into 4 groups: Group 1: Baseline control, Group 2: MEV, Group 3: IFN-γ and Group 4: IFN-γ + MEV. Post-MEV supplementation, cells were harvested at 6/12/24 hours for downstream analysis (n=6/treatment). MEVs were isolated from unpasteurized human donor breast milk using serial ultracentrifugation and filteration[8]. MEVs characterizations were done via nanoparticle tracking transmission electron microscopy and western immunoblotting. Transcript and protein abundance of NF-κB and NLRP3 inflammasome markers were quantified via RT-qPCR and western immunoblotting, respectively.

Results

10ng/mL treatment of IFN-γ lead to HMC3 polarization into a pro-inflammatory phenotype as determined by increases in pro-inflammatory markers. Transcript abundance of NF-κB pathway genes (TLR4, IκBα, NF-κB p65) and NLRP3 inflammasome (caspase-1, IL-1β, and IL-18) remained unchanged. However, protein abundance of pro-inflammatory markers (TLR4, phosphorylated-IKKα/β and NF-κB p65) increased with IFN-γ priming and decreased with MEV supplementation at 6/12/24 hour timepoints. Level of anti-inflammatory markers such as phosphorylated-IκBα and pro-caspase-1, which is the inactive form of caspase-1 decreased with IFN-γ priming and increased after MEV supplementation.

Conclusion

MEV supplementation may attenuate the activation of NF-κB and NLRP3 inflammasome formation and promote pro-survival effects in stressed microglia.

POSTER #44

The Impact Of Sema3E/PlexinD1 Deficiency on IL-10 Expression in Macrophages

Chelsea Lukawy[1], Mojdeh Matloubi[1], Lianyu Shan[1], Abdelilah Soussi Gounni[1]

[1] Department of Immunology, Rady Faculty of Health Sciences, Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba R3E 0T5, Canada.

Introduction

Asthma is a chronic inflammatory disease of the lungs, affecting more than 300 million individuals worldwide. It has numerous phenotypes, including airway inflammation, remodelling, and hyperresponsiveness. Our lab has data demonstrating the regulatory role of semaphorin3E (Sema3E) and its receptor plexinD1 in critical features of allergic inflammatory response and airway remodelling. Recently, we found that a deficiency of the plexinD1 receptor in interstitial macrophages (IMs) impacts the IL-10 pathway, affecting the host immune response.

As IMs are replenished from the bone marrow, it is intriguing to investigate whether Sema3E/plexinD1 in bone marrow-derived macrophages (BMDMs) impact IL-10 expression. Further investigation is warranted to evaluate the potential molecular signalling mediated by the Sema3E/plexinD1 axis and how this affects macrophage function. These findings may lead to new approaches to understanding and managing asthma, a multifaceted disease with diverse phenotypes.

Hypothesis

Sema3E/plexinD1 deficiency in BMDMs up-regulates inflammatory phenotypes, influencing the IL-10 pathway.

Methodology

Bone marrow cells were extracted from the tibias and femurs of Sema3E KO and WT mice and cultured with M-CSF to differentiate bone marrow progenitors into macrophages. In addition, macrophage cell-specific plexinD1 KO ($CX3CR1cre^{ERT2}$-$Plxnd1^{fl/fl}$) bone marrow cells were collected, cultured and subjected to tamoxifen. Following, BMDMs were stimulated with LPS at various time points. A portion of BMDMs received Sema3E-Fc treatment before the LPS challenge. IL-10 levels in supernatant were measured using mesoscale and ELISA.

Results

Following LPS stimulation, IL-10 levels in Sema3E KO and plexinD1 KO BMDMs significantly increased. Similarly, TNF and IL-1B levels increased in Sema3E KO and plexinD1 KO BMDMs. When Sema3E KO BMDMs were pretreated with Sema3E-Fc, there was a significant decrease in IL-10 levels, similar to levels found in WT BMDMs exposed to LPS.

Conclusion

Our data suggests that the Sema3E/plexinD1 axis prevents excessive anti-inflammatory responses characterized by high IL-10 levels by limiting excessive pro-inflammatory responses.

POSTER #112

Unraveling Novel Role(s) of Semaphorin 3E in Regulating Natural Killer Cell Functions

Heqing Ma[1], Abdulaziz Alamri[1,3], Obinna Okeke[1], Manli Zhang[1], Abdelilah Soussi-Gounni[1] , Ruey-Chyi Su[2] and Sam K.P. Kung[1]

Department of Immunology[1], Medical Microbiology & Infectious Diseases[2], University of Manitoba, Max Rady College of Medicine, Rady Faculty of Health Sciences, Winnipeg, Canada. [3] King Saud University, Department of Biochemistry, Riyadh, Saudi Arabia.

Aim of Study

Natural killer (NK) cells are innate cells that are critical in tumor and viral immunity. Semaphorins (Sema) were first discovered as axon guidance molecules in the nervous system. Emerging data suggest that a member of the Semaphorins family, Sema3E, regulates diverse immune cell functions in disease models. We reported previously that Sema3E produced by immature DC regulated IL-2 activated NK-cell migration, and that expression levels of Sema3E and its cognate receptor PlexinD1 were tightly regulated in NK cells. My recent work showed that Sema-3E deficiency had resulted in a modest but significant decrease in splenic NK cell number (manuscript in preparation for re-submission). We hypothesized further that Sema3E/PlexinD1 signaling axis is a novel pathway that regulates NK cell-effector functions at steady-state.

Methods

Spleen were harvested from either BALB/c or Sema-3E -/- mice using a commercial NK cell isolation kit. IL-2 (1000 U/ml, 3 days)-containing complete culture medium was used to activate/culture NK cells in vitro. Functions of the NK cells were evaluated using CD107a degranulation assay alongside intracellular staining for IFNγ. RMA/RMA-s or Tap-1 knockdown/WT 4T1 cell lines were inoculated into the mice via i.p injection to assess the killing effect in vivo. Some IL-2 activated NK cells were preserved in Trizol reagent and processed for bulk RNA sequencing.

Results

The IL-2 activated Sema3E-/- NK were impaired in cytotoxicity when co-cultured with either PMA+Iono cocktail or target cells, while the IFNg level remains comparable between Sema3E-/- and the WT control. Consistently, preliminary in-vivo killing data suggests a higher target cell number remaining in Sema3E-/- mice. The bulk RNAseq analyses revealed multiple genes related with cell cycle and cytoskeleton reorganization that were differentially de-regulated Sema3E-/- NK cells.

Conclusion

Cytotoxicity, but not cytokine production, of the NK cells was impaired by the Sema3E deficiency when exposed to stimulants. My results further established the importance of Sema3E in the regulation of NK cell biology. A better understanding of this signaling axis will reveal novel therapeutic target(s) that allow one to manipulate NK cell cytotoxic function specifically.

POSTER #42

Discovery of A Novel and Selective Adenylyl Cyclase Isoform 6 Activator: Exploring A Potential Treatment for Respiratory Diseases

Saeid Maghsoudi[1,2], Vikram Bhatia[1,4], Martha Hinton[1,2], Alexander Pritchard [3], Prashen Chelikani[1,2,4], John L. Sorensen [3], Shyamala Dakshinamurti[1,2,5]

[1]Biology of Breathing Group, Children's Hospital Research Institute of Manitoba; Departments of [2]Physiology, [3]Chemistry, [4]Oral Biology and [5]Pediatrics, University of Manitoba, Winnipeg, Canada.

Introduction

cAMP is a crucial intracellular signalling mediator produced in mammals by nine membrane-bound adenylyl cyclases (ACs) (AC1-9). Although many drugs inhibit or stimulate AC activity through the upstream GPCRs, ACs themselves have not been major drug targets. ACs are allosterically activated by the parent compound forskolin (FSK). AC6 is dominant in neonatal pulmonary arteries (PA) and airway smooth muscle. AC6 is considered cardioprotective, and its content upregulated in hypoxia. However, we have reported AC enzyme inhibition due to S-nitrosylation in PA, and in PA myocytes exposed to hypoxia. By mutational analysis of S-nitrosylatable cysteines, we identified C1004, at the interface of AC6 with Gαs, as crucial to initiate AC6 activity. To rescue AC6 activity, we designed and synthesized novel forskolin derivatives targeting AC isoforms.

Methods

HEK cells stably overexpressing AC isoforms (AC 3, 5, 6, 7, 9) as well as cysteine-to-alanine mutants (AC6_C1004A, AC6_C1145A or AC6_C447A) were cultured in normoxia (21% O2) or hypoxia (10% O2) for 72 hours or challenged with nitroso donor S-nitrosocysteine (CysNO). Cells from all treatment groups were lysed for measurement of real-time AC catalytic activity, and detection of protein S-nitrosylation by Biotin Switch Method. Forskolin-dependent real-time cAMP generation was measured using a live-cell cAMP biosensor cADDis assay. A library of forskolin derivatives was synthesized, screened and tested for activation of AC isoforms 3, 5, 6, 7 and 9, and in PA by isometric myography.

Results

Among AC isoforms studied, only AC6 catalytic activity is inhibited by hypoxia and CysNO, impairing cAMP production; this correlates with increased cysteine nitrosylation of hypoxic AC6, but not of other AC isoforms. C1004 is conserved among AC isoforms. However, alanine substitution of C1004, but not of other exposed cysteines, prevents nitrosylation of hypoxic AC6; AC activity and cAMP accumulation are decreased in AC6_C1004A compared to AC6WT. Among forskolin derivatives synthesized, forskolin 1α,9α-carbonate offered relatively selective activation of AC6.

Conclusion

Although hypoxia inhibits AC6, forskolin can stimulate catalytic activity of AC6 under hypoxic conditions. The hypoxia inhibitory mechanism does not involve amino acids that interact with forskolin. Selective AC6 reactivation is a novel therapeutic target in pulmonary diseases.

POSTER #6

Cigarette Smoke Extract Induces Neutrophils to Release Citrullinated Proteins via NETosis

Jeba Atkia Maisha[1,2], Haziqa Kassim[4], Xinyun Liu[1], Maggie Jing Ouyang[1], Christopher Pascoe[4,5], Liam J O'Neil[1,2,3]

[1]Department of Immunology, University of Manitoba.
[2]Manitoba Centre for Proteomics and System Biology, Winnipeg, MB.
[3]Department of Internal Medicine, University of Manitoba.
[4]Department of Physiology and Pathophysiology, University of Manitoba; [5]The Children's Hospital Research Institute of Manitoba, Winnipeg, MB.

Introduction

Cigarette smoking is a major risk factor in autoimmune diseases. Although it promotes inflammation by releasing oxygen-free radicals, it is unclear if it has a direct effect on the immune response. Neutrophils are the first responders of host defense and two of their major immune responses are degranulation and neutrophil extracellular traps (NETs) formation. Granular proteins are released in both cases, which may serve as autoantigens in individuals with genetic risk. Here, we examined whether cigarette smoke extract (CSE) can induce neutrophils to release potential autoantigens such as citrullinated proteins.

Methods

Neutrophils from healthy donors stimulated with CSE were analyzed by flow cytometry, enzymatic profiling, extracellular DNA release, immunofluorescence, and citrullinated protein probing.

Results

There was no increase in degranulation and activation markers CD66b, CD11b, CD64, CD16, CD63 and CD32 in CSE-treated neutrophils (*ns*). No significant granular protein (NE, PR3) activity differences were seen in the cell culture supernatants (60 minutes, *ns*). To observe extracellular DNA release due to NETosis, cultures of neutrophils with CSE were measured by fluorescence (SYTOX green) over time (8 hours, $p < 0.01$). This was confirmed with immunofluorescence imaging (NE, DNA). Protein citrullination in NETs (isolated after 4 hours) was detected using a Phenylglyoxal probe. When PAD enzymes that are typically responsible for citrullination, are inhibited (Cl-Amidine, 500µM), NETosis significantly reduces in CSE-induced neutrophils ($p < 0.05$).

Conclusion

Our data suggest neutrophils induced with CSE release NETs but do not degranulate. This may indicate a mechanism by which CSE skews neutrophils to undergo NETosis and release citrullinated autoantigens. We are currently examining the specific PAD isoform involved in the cascade.

POSTER #2

Increased interleukin-1 receptor antagonist (IL-1Ra) in human islets protects beta cells from amyloid-mediated apoptosis – Implications in type 2 diabetes and islet transplantation.

Danish Malhotra, Rushie Tyagi, Lucy Marzban

[1]College of Pharmacy, Rady Faculty of health Sciences, University of Manitoba.

Introduction

Type 2 diabetes (T2D) is characterized by insulin resistance, impaired beta-cell function, and death. Islet amyloid, formed by aggregation of human islet amyloid polypeptide (hIAPP, amylin), contributes to islet inflammation and beta-cell death in T2D. Islet amyloid also forms in cultured and transplanted human islets. We previously showed that amyloid formation promotes interleukin-1 (IL-1) beta production in human islets, which leads to beta-cell upregulation of the Fas cell death receptor and apoptosis. Amyloid-induced IL-1beta production may lead to imbalance between IL-1beta and its natural inhibitor IL-1 receptor antagonist (IL-1Ra), thereby promoting islet inflammation. In this study, we tested if increasing the local production of IL-1Ra can reduce amyloid-mediated beta-cell death.

Methods

Isolated human islets (n=3 donors; purity: >90% assessed by dithizone) were transduced with adeno-IL-1Ra to increase the local expression of IL-1Ra in islets. Transduced islets were cultured in normal (5.5 mM) or elevated (11.1 mM) glucose (to form amyloid) for 5 days. Paraffin-embedded islet sections were used for quantitative immunolabelling for insulin, IL-1beta, thioflavin S (amyloid), TUNEL and caspase-3 (apoptosis). Islet culture medium was collected to assess islet IL-1beta release (by ELISA).

Results

Human islets cultured in normal glucose formed little or no amyloid and had low levels of IL-1beta. However, islets cultured in elevated glucose formed amyloid during culture which was associated with elevated islet IL-1beta levels, and the proportion of TUNEL and caspase-3 positive beta cells. Following culture, adeno-IL-1Ra transduced islets had lower number of TUNEL and caspase-3 positive beta cells than non-transduced islets.

Conclusion

These findings suggest that local elevation of IL-1Ra in human islets can reduce IL-1beta-mediated amyloid-induced beta-cell death. Modulation of islet IL-1beta/IL-1Ra balance may therefore provide a therapeutic strategy to protect human islets from amyloid-mediated beta-cell death in patients with T2D and islet grafts.

POSTER #137

Use of Oxylipins to Determine the Dietary Requirement for the essential fatty acid, α-Linolenic Acid (ALA)

Anne Manson[1,2], Karanbir K. Sidhu[1,2], Oleksandra Fedorova[1,2], Huy Hoang Khai La[1,2], Elizabeth Magaji[1,2], Le Kim Long Nguyen[1,2], Tanja Winter[1,2], Harold M. Aukema[1,2]

[1]Food and Human Nutritional Sciences, University of Manitoba, Winnipeg MB, Canada., [2]Canadian Centre for Agri-Food Research in Health and Medicine (CCARM), Winnipeg MB, Canada.

Introduct on

The dietary requirement for ALA remains unclear, as evidenced by the AI recommendation for this essential fatty acid. In previous studies we observed that the amount of dietary ALA required to maximize DHA oxylipin levels appears to be higher than the amount required to maximize tissue DHA levels. Further, we also observed that dietary ALA could reduce both arachidonic acid (ARA) and its oxylipins. However, only DHA levels have classically been used to estimate the ALA requirement. Since oxylipins mediate many fatty acid functions, this study was designed to examine whether both DHA and ARA oxylipins could be used to determine the dietary ALA requirement more accurately.

Methods

Rats and mice were provided a range of 0.1-2.5 g ALA and a consistent level of 2g of linoleic acid per 100g diet. Non-esterified oxylipins in serum, liver, kidney and brain homogenates underwent solid phase extraction and were analyzed by HPLC-MS/MS.

Results

In response to increasing levels of dietary ALA, DHA oxylipins initially increased rapidly and then plateaued whereas ARA oxylipins tended to decrease and plateau. Thus, the ratio of DHA/ARA oxylipins synthesized via the major common biosynthetic pathway (lipoxygenase) was calculated and the breakpoint of the transition from increase to plateau was estimated by piecewise regression. In serum, liver and kidney the estimated breakpoint indicated an average ALA requirement of ~0.7g/100g diet (1.7%energy), which would result in a recommended dietary allowance of ~1.0g/100g diet (2.4% energy). In contrast, in the brain this oxylipin ratio was constant across all levels of dietary ALA, indicating that the breakpoint occurred at less than 0.1g of ALA/100g of diet, and suggesting that the brain has priority for ALA metabolism.

Conclusions:

he combination of DHA and ARA oxylipins can be used to estimate the dietary ALA requirement and indicates that the requirement is higher than previously estimated using DHA alone. Further, this ratio could be used as a biomarker of ω-3 fatty acid status to address questions such as the bioequivalence of ω-3 fatty acids, or the optimal dietary ω-6/ω-3fatty acid ratio.

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Keywords: Nutrient assessment; Biomarkers; Omega-3 fatty acid; Fatty acid/lipid metabolism; Oxylipins.

POSTER #96

Differential modulation of airway inflammation by Innate Defence Regulator Peptide IDR-1002

Courtney Marshall[1], Mahadevappa Hemshehkar[2], Padmanie Ramotar[1], Dylan Lloyd[2], Neeloffer Mookherjee[1,2].

[1]Department of Immunology, [2]Manitoba Centre for Proteomics and Systems Biology, Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada.

Introduction

Sex-related differences in the prevalence, severity, and response to therapy in asthma are well established. Around 15% of asthmatics, primarily females, do not respond to available steroid therapies resulting in severe asthma. We have previously shown that an immunomodulatory small peptide, Innate Defence Regulator (IDR-1002), can alleviate airway inflammation, significantly suppress eosinophil and neutrophil accumulation in the lungs of allergen house dust mite (HDM)-challenged female mice, by suppressing IL-33 (cytokine that drives steroid-unresponsiveness). Here, we examine the mechanism and sex-related differences in the ability of IDR1002 to control IL-33 and airway inflammation in HDM-challenged mice.

Methods

HBEC-3KT were treated with IDR-1002 or IDR-1002.2 (10uM) before stimulation with IFNγ (30ng/ml). Kinetics of expression and abundance of IL-33 and Tristetraprolin (TTP) was examined over 24hs by RT-qPCR and ELISA. Male and female BALB/c mice (7-8 weeks) were challenged with intranasal administration of 35uL of whole HDM extract (0.7mg/mL) in saline for 2 weeks. IDR-1002 was administered at 6mg/kg subcutaneously 3 times a week for 2 weeks. Bronchoalveolar lavage fluid (BALF), lung tissue, and serum were collected 24h after final HDM challenge. Immune cell accumulation in the BALF was analyzed using flow cytometry. Abundance of TTP, IL-33, and ERα, in lung tissue was determined using ELISA. Abundance of cytokines were monitored by a Luminex assay by Eve Technologies™. Abundance of HDM-specific IgE in serum was determined using ELISA.

Results

IDR-1002 suppresses IL-33 and induces expression of TTP and MKP-1, negative regulators of IL-33, at 24h. In murine lung, suppression of IL-33 and known TTP targets TNFa, IL-6, and IP-10 by IDR-1002 was more robust in female mice. However, IDR-1002 mediated suppression of immune cells in BALF and Th2 cytokines IL-4, IL-5, and IL-13 did not show differences. IDR-1002 mediated suppression of HDM specific IgE was more robust in male mice. Further, HDM significantly enhanced abundance of estrogen receptor alpha (ERα) which was suppressed by IDR-1002 in female mice.

Conclusion

There are sex-related differences in the mechanism through which IDR-1002 suppresses cytokine targets of TTP, but not immune cell accumulation and Th2 axis and these differences may lie in differential modulation of ERα.

POSTER #121

A population-based study of SARS-CoV-2 IgG antibody responses to vaccination in Manitoba

Brielle Martens[1], Paul Van Caeseele[2,3], Jared Bullard[1,3], Joss Reimer[4], Lyle R. McKinnon[1,5,6], Souradet Y. Shaw[7], Jason Kindrachuk[1,8,9], Derek R. Stein[1,3]

[1]Department of Medical Microbiology & Infectious Diseases, University of Manitoba, Winnipeg, MB, Canada., [2]Department of Pediatrics and Child Health, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada., [3]Cadham Provincial Laboratory, Winnipeg, MB, Canada., [4]Winnipeg Regional Health Authority, Winnipeg, MB, Canada., [5] Centre for the AIDS Programme of Research in South Africa (CAPRISA), Durban, South Africa., [6] Department of Medical Microbiology and Immunology, University of Nairobi, Kenya., [7]Department of Community Health Sciences, University of Manitoba, Winnipeg, MB, Canada., [8]Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada., [9]Manitoba Centre for Proteomics and Systems Biology, Health Science Centre, Winnipeg, Manitoba R3E 3P4, Canada.

Introduction

Understanding variables that influence antibody responses to COVID-19 vaccination within a population can provide valuable information on future vaccination strategies. In this population-based study, we examined the antibody responses to COVID-19 vaccination in Manitoba using residual serum specimens collected between January 2021 and March 2022 (n = 20365).

Methods

Samples were tested for spike and nucleocapsid IgG against SARS-CoV-2 using clinically validated assays. We assessed the impacts of multiple factors on post-vaccination antibody titres including type of vaccine, age, sex, geographic location, number of doses received, and timing of vaccination.

Results

Our investigation demonstrated that vaccination with one dose of Moderna mRNA-1273 elicited higher anti-spike IgG titres overall compared to Pfizer BNT162b2 vaccination, while one dose of Pfizer BNT162b2 followed by a second dose of Moderna mRNA-1273 exhibited higher titres than two doses of Pfizer BNT162b2 or Moderna mRNA-1273, irrespective of age. Age and time post-vaccination had considerable effects on antibody responses, with older age groups exhibiting lower anti-spike IgG titres than younger ages, and titres of those vaccinated with Pfizer BNT162b2 waning faster than those vaccinated with Moderna mRNA-1273 or a combination of Pfizer BNT162b2 and Moderna mRNA-1273. Antibody titres did not appear to be affected by sex or geographic location.

Conclusions

Our results identify how factors such as age and type of vaccine can influence antibody responses to vaccination, and how antibody titres wane over time. This information highlights the importance of tailoring vaccine regimens to specific populations, especially those at increased risk of severe COVID-19 and can be used to inform future vaccination strategies, scheduling of booster doses and public health measures.

POSTER #53

COVID-19 Pandemic and Health and Quality of Life of Persons with Intellectual and Developmental Disabilities Living in the Community

Mahnoosh Matlabi[1], Kayla Kostal[1], Margherita Cameranesi[1], Maria Baranowski[1,2], Lindsay McCombe[2], Shahin Shooshtari [1,2]

[1]Department of Community Health Sciences, University of Manitoba, Winnipeg, MB, Canada., [2]St.Amant Research Centre, Winnipeg, MB, Canada.

Introduction

Persons with intellectual and developmental disabilities (IDD) residing in the community and receiving community-based health and social services experience more positive health and quality of life (QoL) outcomes compared to similar individuals residing in institutions. However, during the COVID-19 pandemic, persons with IDD residing in community homes and the direct support workers caring for them faced some challenges that might have impacted their health and QoL. This study aimed to explore the lived experiences of direct support professionals responsible for caring for adults with IDD residing in community homes in Manitoba about the impact that the COVID-19 pandemic had on them and their care recipients.

Method

This study investigated the unique lived experiences of direct support professionals supporting adults with IDD living in the community using a descriptive qualitative methodology. Twelve direct support professionals supporting adults with IDD living in community homes in Manitoba participated in semi-structured individual interviews. Respondents were all direct support professionals providing care to individuals who have been living in the community and receiving community-based services for at least three years. All interviews were recorded and transcribed verbatim. Narrative data was analyzed thematically to identify patterns of meaning in the data.

Results

What emerged from study participants' accounts will be presented and discussed, with a special focus on the changes in health status and QoL that persons with IDD experienced during the pandemic, including both challenges and opportunities. Some of the struggles reported by staff members include disruptions in support services, increased social isolation for persons with IDD, deteriorating communication, difficulty in adhering to safety protocols, and the emotional strain experienced by support staff.

Conclusion

The COVID-19 Pandemic negatively affected the health and quality of life of persons with IDD and created challenges for their direct support professionals**.** The study results will guide community service providers to implement interventions and more inclusive infection prevention policies to mitigate the negative impact of health crises such as COVID-19 on the health and QoL of persons with IDD and their caregivers.

POSTER #101

Mapping Gene Dysregulation in Cisplatin-Induced Ototoxicity at the Single-Cell Level

Deanne Nixie Miao[1], Emilia Luca[2], Janilyn Arsenio[3], John Pham[1] Alain Dabdoub[2], Britt Drögemöller[1,4-6]

[1]Department of Biochemistry and Medical Genetics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada., [2]Sunnybrook Research Institute, Toronto, Ontario., [3]Department of Immunology, Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada., [4]Children's Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada., [5]CancerCare Manitoba Research Institute, Winnipeg, Manitoba, Canada., [6]Centre on Aging, University of Manitoba, Winnipeg, Manitoba, Canada.

Background

Cisplatin, a major chemotherapeutic agent, causes hearing loss (ototoxicity) in up to 80% of patients, with children facing a three-fold higher risk of experiencing this adverse drug reaction. Genetics plays an important role in cisplatin-induced ototoxicity (CIO). Therefore, we hypothesize that genetic variants modulate cisplatin-induced changes in gene expression within the cochlea, leading to CIO. To provide insights into which genes, pathways, and specific cells are involved in CIO, we profiled single-cell gene expression changes in the cochlea.

Methods

Intraperitoneal injections of 3mg/kg of cisplatin or saline were administered to postnatal day 6 (P6) CBA/CaJ mice in the treatment (*n*=6) and control groups (*n*=6), respectively. 4-hours post-cisplatin administration, mice were euthanized, and whole cochlear ducts were dissected. Single nuclei were isolated using the 10XGenomics Chromium Nuclei Isolation Kit, followed by single-nuclei RNA-sequencing (snRNA-seq) using the Single Cell Gene Expression + RNA Profiling Kit. Sequencing was performed on the NovaSeq X Plus Sequencing System. snRNA-seq data were processed using CellRanger and analyzed with Seurat, to evaluate our capacity to capture relevant cell types, detect gene expression changes, and identify differences in cell proportions between control and treatment groups.

Results

We sequenced 15,510 and 12,194 nuclei for the control and treatment groups, respectively. 45 clusters were identified, including rare cell types such as inner (IHC) and outer hair cells (OHC). Following cisplatin treatment, significant reductions in cell proportions were observed across 19 clusters, including the hair cell clusters (P = 0.02), with an 84.6% decrease in OHC and an 88.9% decrease in IHC observed in the treatment group relative to the control.

Conclusions

Our pilot study revealed significant decreases in several inner ear cells, including IHC and OHC, 4-hours post-cisplatin administration. Based on these findings, we will conduct a follow-up pilot study focusing on the 1-hour timepoint post-cisplatin administration. This timepoint was chosen to investigate gene expression changes preceding cell death, coinciding with the timepoint when platinum levels first peak in most organs, including the cochlea. Insights from these preliminary investigations will inform future snRNA-seq and snATAC-seq experiments examining cisplatin's effect on gene expression and chromatin accessibility in cochlear cell types.

POSTER #87

D-Serine facilitates aggressive migration and stemness of recurrent glioblastoma cells by interacting with host endothelial cells.

Ryan Mota[1,2], Ping Lu[1,2], Emma Martell[4], Maria Alejandra Llanes Cuesta[1,2], Ujala Chawla[3], Tanveer Sharif[3,4], Chris Anderson[1,2]

[1]Department of Pharmacology and Therapeutics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada., [2]PrairieNeuro Research Centre, Kleysen Institute for Advanced Medicine, Health Sciences Centre, Winnipeg, MB, Canada., [3]Department of Pathology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada., [4]Department of Human Anatomy & Cell Science, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada.

Introduction

Glioblastomas (GBM) make up over 50% of all gliomas, representing the most aggressive primary brain tumor. GBM cells infiltrate deep brain structures via perivasculature/endothelial pathways, exploiting stemness and malignant traits. This allows access to nutrient-rich environments causing infiltration and expansion of recurrent tumors. GBM cells release large amounts of glutamate, which leads to enhanced tumor malignancy by poorly understood mechanisms. Work from our group indicates a robust role for NMDA receptors expressed by brain endothelial cells (eNMDARs) in transducing signals initiated by parenchymal cells in the brain. Thus, we hypothesized that GBM cells release NMDAR co-agonists, glutamate and D-serine, leading to activation of eNMDARs and enhancement of endothelium-dependent GBM migration and stemness.

Methods

Human cerebral microvascular endothelial cells (hCMECs/D3) and patient-derived recurrent GBM cells were co-cultured in a transwell system to assess whether endogenous glutamate and D-serine acting on eNMDARs mediate GBM cell migration and stemness. GBM migration was quantified by transwell cell counts after 24 hours of migrated cells, and stemness was evaluated through sphere formation assays after a 5 day incubation. Both parameters were assessed in the presence or absence of hCMECs, and with various experimental manipulations including pharmacological inhibitors and CRISPR/Cas-9 mediated silencing of GBM and/or endothelial genes.

Results

GBM migration and stemness were both significantly potentiated by the presence of endothelial cells in co-culture. D-Serine release was detected from GBM cells in a manner experimentally mitigated by extracellular catabolism with D-amino acid oxidase, inhibition of serine racemase (SR) with phenazine methosulfate (PMS) and CRISPR-mediated silencing of SR. These manipulations significantly inhibited GBM migration and reduced markers of stemness. Glutamate and D-serine site NMDAR antagonists, as well as CRISPR-mediated GluN1 silencing in endothelial cells, also mitigated stemness and migration outcomes. In vivo, PMS administration reduced tumor volume at 4 and 6-weeks after GBM injection and extended mouse survival following GBM injection.

Conclusion

Our experiments demonstrate that interactions with brain endothelial cells support recurrent GBM cell malignancy. Specifically, GBM migration and stemness are enhanced by endothelial cells, dependent on GBM D-serine and endothelial NMDARs. Targeting this signalling axis represents an encouraging pre-clinical pathway for therapeutic development.

POSTER #158

H4R3me2a, a mark of super-enhancers

Sadhana Rithi Narayanan Sudhakar[1], Li Wu[2], Shrinal Patel[1], Athanasios Zovoilis[2], James R. Davie[1,3]

[1]Department of Biochemistry and Medical Genetics, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, MB, Canada, [2]Southern Alberta Genome Centre, University of Lethbridge, Lethbridge, AB, Canada, [3]Paul Albrechtsen Research Institute, CancerCare Manitoba, Winnipeg, MB, R3E 0V9, Canada.

Introduction

Histone H4 asymmetrically dimethylated at arginine 3 (H4R3me2a) is a modification catalyzed by protein arginine methyltransferase 1 (PRMT1). H4R3me2a is an active histone modification and stimulates the activity of lysine acetyltransferase, CBP/p300 which catalyzes the formation of H3K27ac. The association between PRMT1 and CBP/p300 promotes the interaction between the enhancer and the promoter of the β- globin gene in erythroid cells. As a result, it transcriptionally activates gene expression and maintains an active chromatin state. Our previous study with avian erythroid cells described the genomic distribution of H4R3me2a including its association with H3K27ac. This study aims to analyze the genomic landscape of H4R3me2a in human breast cancer cells, MCF7 using the chromatin-immunoprecipitation sequencing (ChIP-Seq) data and find their correlation with the avian data. The study will address the features of H4R3me2a genomic distribution including broad domain, super-enhancers, and correlations with other histone PTMs including H3K27ac.

Methods

ChIP-seq and RNA-Seq analyses, Correlation analyses and Mononucleosome Immunoprecipitation experiments.

Results

The genic distribution of H4R3me2a in human MCF7 cells was like that of chicken where they were observed to be in intronic (23.2%), intergenic (23.5%), and transcription start site (TSS – 31.8%) regions. H4R3me2a was distributed as a broad domain (BD) along the body of a small subset of genes. We inferred that genes presenting with H3K4me3 also had H4R3me2a and H3K27ac peaks forming the chromatin feature of genes called the broad epigenetic domain. H4R3me2a-BD overlapped with the top-ranked super-enhancers determined based on the H3K27ac peak intensity. A very similar genic distribution of H4R3me2a and H3K27ac was observed around the TSS. Correlation analyses for H4R3me2a and H3K27ac peaks produced a p-value of 0.719 indicating a stronger correlation. Further it was confirmed *in-vitro* that H4R3me2a and H3K27ac exist together in the same nucleosome using mononucleosome immunoprecipitation experiments.

Conclusion

The study establishes H4R3me2a as a distinctive marker associated with broad domains and super-enhancers. The association between H4R3me2a and H3K27ac was confirmed using correlational and *invitro* mononucleosomal approaches. PRMT1/H4R3me2a associates with CBP/p300, and other lysine acetyl and methyltransferases and recruits these chromatin-modifying enzymes that produce other active histone modifications contributing to the transcriptional regulation of gene expression.

POSTER #94

Influence of post translational modifications in neutrophil proteases in Rheumatoid Arthritis

Ramiza N. Zaman[1], Mario N. Lagos[1], Liam O'Neil[1]

[1]Manitoba Centre for Proteomics and Systems Biology, Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada

Introduction

Neutrophils are equipped with diverse mechanisms that activate in response to invading pathogens. These include release of neutrophil proteases such as cathepsin-G, neutrophil elastase, and proteinase-3, through a process called degranulation. However, during uncontrolled inflammation in diseases such as rheumatoid arthritis (RA), dysregulated activation can accelerate bystander tissue damage. During such responses, neutrophil proteases can be modified through post-translational modification (PTM), with citrullination and carbamylation amongst key players, as these modifications serve as autoantigens in RA. However, it remains unclear why these PTMs occur in specific proteins and how they might influence their function. This study introduces a potential link between RA-relevant PTMs and neutrophil protease activity, which may enhance the inflammatory response.

Methods

To illustrate role of PTMs in protein function, commercially available purified proteases and neutrophil supernatant following degranulation were treated with 330 mU PAD2/PAD4 and 50-800 µM potassium thiocyanate (KOCN) to induce citrullination and carbamylation, respectively. Protease activity is quantified via fluorogenic plate-based assays. Activity-based protein profiling using TAMRA-FP allowed in-gel visualisation of active species. Citrullination was visualised in gels by labelling proteins with citrulline-specific fluorescent probe Rhodamine-PG, while carbamylation is detected by western blot. Fibroblast-like synoviocytes (FLS) cells were cultured with citrullinated/carbamylated neutrophil proteases and quantified for inflammatory cytokines by ELISA. FLS cell surface activation by flow cytometry will be assessed using markers CD90, cadherin 11 etc.

Results

Our preliminary data suggests citrullination by PAD2, but not PAD4, increases purified and degranulated neutrophil protease activity. Specifically, we observed 2-fold increase in PR3 and 4-fold increase in NE activity in PAD2-treated proteases compared to control. Carbamylation of recombinant proteases by different KOCN concentrations did not show increased activity with increasing time-points. However, KOCN-treated degranulated protease activity was 9-fold higher than the untreated control. We discovered substantial rise in cytokine release in citrullinated/carbamylated FLS cells from ELISA and expect increased FLS cell surface activation in modified proteases.

Conclusion

These results suggest induction of RA-relevant PTMs may play dual roles through enhancing neutrophil protease function and potentiate formation of autoantibodies. Future work seeks to understand the autoantigenicity of modified neutrophil proteases and the precise modified sites that impart these phenotypic changes.

POSTER #64

Maternal Resveratrol (RESV) Supplementation and the Effects on Cardiac Hypertrophy, Mitochondrial Metabolism, and Calcium Transport

Marcelo Ninalaya[1,2,3], Gabriel M. Brawerman[1,2,3], Mateusz M. Tomczyk[1,2,3], Bo Xiang[1,2], Stephanie M. Kereliuk[1,2,3], Adrian West[2,4], Vernon W. Dolinsky[1,2,3].

[1]Diabetes Researched Envisioned and Accomplished in Manitoba (DREAM) Theme of the Children's Hospital Research Institute of Manitoba, Winnipeg, Canada., [2]Children's Hospital Research Institute of Manitoba (CHRIM), Manitoba, Winnipeg, Canada., [3]Department of Pharmacology and Therapeutics, Rady Faculty of Health Sciences, College of Medicine, University of Manitoba, Winnipeg, Canada., [4]Department of Physiology and Pathophysiology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada.

Introduction

Gestational diabetes mellitus (GDM) is a condition that manifests in pregnancy and is characterized by insulin resistance, glucose intolerance, and hyperglycemia, and impact maternal and offspring health. Medications have shown effectiveness but have associated risk of adverse pregnancy outcomes and the long-term effects on the offspring are unknown. Our previous studies in rats observed that cardiomyocytes of GDM-offspring exhibit hypertrophy, mitochondrial dysfunction, and impaired calcium flux. In this study, we hypothesize that administration of Resveratrol (RESV) to maternal GDM diet will mitigate mitochondrial dysfunction, cardiac hypertrophy and improve calcium flux in GDM-exposed offspring.

Methods

Female Sprague-Dawley rats were fed a low-fat (Lean, 10% kcal fat) or high-fat and sucrose (GDM, 45% kcal fat) diet six weeks before mating to induce GDM. A subgroup of GDM dams were switched to a diet containing RESV (GDM+RESV, 45% kcal + 4g/kg RESV). At e18.5 fetal echocardiography was performed to assess cardiac structure. To determine the effects of RESV on GDM-offspring, e20 pups were sacrificed for fetal cardiomyocyte isolation. Measurements of mitochondrial respiration were performed using the Agilent-Seahorse XFe24. Measurements of calcium flux were performed using fluo-4 on the Cytation-5.

Results

Fetal echocardiography revealed maternal RESV attenuated GDM-induced cardiac hypertrophy. GDM-exposed offspring showed 1.4X larger intraventricular septal and left ventricular posterior wall thickness compared to lean and GDM+RESV offspring (Lean vs. GDM, $p<0.05$) (Lean vs. GDM+RESV, $p<0.05$). Cardiomyocytes isolated form GDM-offspring had lower levels of maximal respiratory capacity compared to lean and GDM+RESV offspring (Lean vs. GDM, $p<0.05$) (Lean vs. GDM+RESV, $p<0.05$). Furthermore, cardiomyocytes isolated from GDM-offspring exhibited delayed calcium flux cycles compared to lean and GDM+RESV offspring upon angiotensin II stimulation.

Conclusion

Our data replicates the previous findings that GDM-offspring exhibit cardiac hypertrophy and mitochondrial dysfunction. Maternal RESV supplementation improved mitochondrial respiration which contributed to impaired calcium flux upon angiotensin II stimulation. Importantly, maternal RESV supplementation attenuated GDM-induced cardiac hypertrophy in GDM-offspring.

POSTER #157

Intraperitoneal Delivery of IL2-IL2mAb (JES6-1) Conjugate Induces Regulatory T Cells in the Female Genital Tract of FoxP3-GFP BL/6 Mice

Faisal Nuhu[1], Christina Gavino[1], Marina Fujisuma[2], Aloysious Ssemaganda[1], Naima Jahan[1], Thomas Murooka[1,2], Lyle R. McKinnon[1,4]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Canada., [2]Department of Immunology, University of Manitoba, Winnipeg, Canada., [3]Center for Proteomics and System Biology, University of Manitoba, Winnipeg, Canada., [4]Centre for the AIDS Programme Research in South Africa (CAPRISA), Durban, South Africa.

Introduction

Female genital tract (FGT) inflammation increases HIV acquisition risk and reduces the efficacy of topical pre-exposure prophylaxis. Expanding FGT Tregs is a potential strategy to reduce genital inflammation and HIV acquisition risk. In support of this, we recently showed that endocervical Tregs were associated with decreased genital inflammation and a lower abundance of HIV target cells. IL2 conjugated to IL2mAb clone JES6-1 (IL2C) has been shown to preferentially expand Tregs. This study aimed to investigate the impact of IL2C on the number and phenotype of Tregs in the FGT.

Methods

The estrous phases of FoxP3-GFP C57Bl/6 mice were synchronized with progesterone injection and randomized into three groups: those that received eight vaginal (n=6) or intraperitoneal (n=7) doses of 6µg IL2C and controls that received vaginal PBS (n=6). Following dosing, mice were euthanized to obtain the lower FGT and spleen for immunohistochemistry and flow cytometry. Tregs were defined as T cells expressing CD4 and GFP.

Results

By flow cytometry, intraperitoneal injection of IL2C significantly increased Treg frequency in the FGT compared to either vaginally dosed (83.3% vs. 18.2% FoxP3+ CD4+ T-cells, $p<0.0001$) or control mice (16.3%, $p<0.0001$). Similar results were obtained by immunohistochemistry; significantly higher FGT Treg counts were observed in the intraperitoneally dosed (mean=35 cells/field) and the vaginally dosed groups (mean=17 cells/field) compared to controls (mean=2 cells/field, $p<0.0001$). IL2C also induced higher expression of GITR on FGT Tregs compared to those dosed vaginally (98.2% vs. 48.5%, $p<0.05$). IL2C dosing had a limited effect on the frequencies and abundance of Th17 cells, NK cells, and Th1 cells.

Conclusion

Our findings suggest that IL2C delivered intraperitoneally is a viable strategy to induce FGT Tregs with minimal off-target effects. Further studies will assess the efficacy of these Tregs in reducing genital inflammation, which could represent a novel strategy to improve women's health.

POSTER #45

The pro-apoptotic effect of chronic contractile activity-induced extracellular vesicles on Lewis Lung Carcinoma cells

Patience O. Obi[1,2,3,4,5], Tamiris F. G. Souza[1,2,4,5], Benjamin Bydak[1,2,3,4,5], Adrian R. West[2,4,6], Joseph W. Gordon[1,2,4,7], and Ayesha Saleem[1,2,3,4,5]

[1]Diabetes Research Envisioned and Accomplished in Manitoba (DREAM) Research Theme, CHRIM, Winnipeg, MB., [2]Biology of Breathing Research Theme, CHRIM, Winnipeg, MB., [3]Applied Health Sciences, University of Manitoba, Winnipeg, MB., [4]Children's Hospital Research Institute of Manitoba (CHRIM), Winnipeg, MB., [5]Faculty of Kinesiology and Recreation Management, University of Manitoba, Winnipeg, MB., [6]Department of Physiology and Pathophysiology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB., [7]College of Nursing, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB.

Introduction

Regular exercise reduces tumor growth *in vivo* and *in vitro*, but the exact mechanisms have yet to be fully elucidated. Extracellular vesicles (EVs) are small, lipid membrane-bound structures that enclose biological cargo, and constitute an essential method of cellular communication. We have previously shown that chronic contractile activity (CCA) increases the concentration of skeletal muscle derived EVs, and these in turn increased mitochondrial biogenesis in C2C12 myoblasts. Here, we hypothesized that CCA-derived skeletal muscle-EVs will mediate the anti-tumorigenic effects associated with chronic exercise.

Methods

C2C12 myoblasts were differentiated into myotubes, and electrically paced (3 hrs/day x 4 days @ 14V, C-PACE EM, IonOptix) to mimic chronic exercise. EVs were isolated from 10-12 ml conditioned media from control and CCA myotubes using differential ultracentrifugation. Lewis lung carcinoma cells (LLCs) were treated with the total number of control-EVs or CCA-EVs for 4 days (N=5-7). A subset of CCA-EVs were treated with 0.1% Triton X-100 (Triton)+100µg/mL Proteinase-K (ProK) before co-culture with LLCs. Cell count, viability, apoptosis, senescence and migration were measured post-treatment.

Results

CCA-EV treatment reduced cell count by ~20% (p=0.01), and cell viability by ~6% *vs.* control-EVs (p=0.03). CCA-EVs increased the expression of pro-apoptotic protein markers: Bax by 24% (p=0.05) and Bax/Bcl-2 ratio by 60% (p=0.03), and senescence marker HMGB1 by 48% (p=0.07) *vs.* control-EVs. CCA-EVs increased the incidence of apoptotic hallmarks: DNA fragmentation by ~13% (p=0.04), Annexin V+/PI+ cells by ~21% (p=0.03), and number of senescent cells by 29% (p=0.007) *vs.* control-EVs. Interestingly when CCA-EV protein cargo was lysed and degraded with Triton and ProK respectively, the increase in apoptosis and senescence was abrogated. Finally, CCA-EVs did not have any effect on cell migration *vs.* control-EVs.

Conclusion

Our data show that CCA-induced skeletal muscle-EVs reduced cell count and viability, increased markers of cell death and senescence, but did not affect migration in LLCs, and these effects were likely mediated by CCA-EV protein cargo. This study illustrates the potential of CCA-derived skeletal muscle-EVs in mediating the anti-tumorigenic effects of chronic exercise. Our next step will be to identify and validate the proteins involved in regulating the anti-tumorigenic effects of CCA-EVs.

POSTER #159

Milk extracellular vesicles improve peroxisome proliferator-activated receptors function in offspring of maternal obesity.

Mon Francis Obtial[1] and Sanoji Wijenayake[1]

[1]Department of Biology, The University of Winnipeg, Manitoba, Canada.

Introduction

Perinatal exposure to maternal obesity (PEMO) is known to induce epigenetic, biochemical, and physiological alterations offspring at the time of exposure and later in life. A key outcome of PEMO is systemic proinflammation chronic metabolic dysfunction (reduction in fat and carbohydrate breakdown). Activation of the peroxisome proliferator-activated receptors (PPARs) during critical windows of early development, may mitigate PEMO-induced metabolic dysregulation, as PPARs modulate glucose uptake and fatty acid oxidation (FAO). Milk extracellular vesicles (MEV) may be potential activators of PPARs, as they transport cargo (*e.g.,* microRNA) known to modulate cellular expression and downregulate pro-inflammatory responses in offspring. However, the interactions between PEMO and MEVs, and how they impact PPARs remains to be investigated.

Methods

Adult female rats (n=6/diet) were fed either a high saturated fat (60% kcal fat) or control diet (10% kcal fat) diet for four weeks prior to mating, during gestation (21 days), and throughout lactation until offspring sacrifice at postnatal day (PND) 11. MEVs were isolated from human donor milk using differential ultracentrifugation and serial filtration, and characterized with transmission electron microscopy, western immunoblotting, and nanoparticle tracking. Offspring were divided into 3 treatment groups (2 males, 2 females per group): 1) Handling control, 2) PBS oral gavage, 3) MEV (50 μL per gram of bodyweight) oral gavage. Starting PND4, offspring were treated twice daily until sacrificed. Tissues from the heart, liver, hypothalamus, prefrontal cortex, amygdala were collected for downstream analysis. The transcript abundance of PPARs (PPARα, PPARδ, PPARγ) PPARγ coactivator 1α (PGC1α) and downstream FAO targets (carnitine palmitoyltransferase (CPT) 1, CPT2) was measured using RT-qPCR.

Results

Offspring with MO exposure had decreased transcript abundance of PPARs, PGC1α, and FAO targets. MEV supplementation increased transcript abundance of aforementioned targets, and in offspring with MO exposure, comparable to control diet offspring.

Conclusion

Elevated PPAR expression and FAO is associated with anti-inflammatory processes and reduction in adiposity. As such, our findings illustrate the potential ability of MEVs to mitigate metabolic dysfunction induced by perinatal MO exposure by enhancing PPAR function and FAO.

POSTER #51

Gut-specific downregulation of interferon-lambda responses in inflammatory bowel diseases

Olamide Ogungbola[1,2], Ramsha Mahmood[1,2,3], Vi Vu[1,2], Jing Ouyang[1,2], Athalia Voisin[2,3,4], Nancy Nguyen[1,2], Xinyun Liu[1,2], Kaelie Bittorf[5], Suellen Lamb[5], Wael El-Matary[2,6], Charles N. Bernstein[3], Eytan Wine[7], D. Lorne Tyrrell[5], Heather K. Armstrong[1,2,3,7], Deanna M. Santer[1,2]

[1] Department of Immunology, University of Manitoba, Winnipeg, MB, [2] Children's Hospital Research Institute of Manitoba [3] Department of Internal Medicine, University of Manitoba, Winnipeg, MB, [4] Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, MB, [5] Li Ka Shing Institute of Virology and Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, [6] Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB, [7] Center of Excellence for Gastrointestinal Inflammation and Immunity Research and Department of Pediatrics, University of Alberta, Edmonton, AB .

Introduction

Inflammatory bowel diseases (IBD) affect ~1:120 Canadians, resulting in significant inflammation and damage to the gut tissue and its mucosal barrier. Unlike other interferon (IFN) families, IFN-lambdas (IFN-λs) signal through IFN-λ receptor-1 and interleukin 10 receptor-beta (IFN-λR1/IL-10RB) to down-regulate gut inflammation and promote gut healing in mouse models. As such, we hypothesized that intestinal IFN-λR1 levels and downstream activities are decreased in IBD, which could contribute to IBD pathology (gut inflammation and mucosal damage).

Methods

During routine colonoscopy, biopsy samples were obtained from the colon of IBD (active or remission) or non-IBD (n=12 each non-IBD, IBD) patients. IFN-λR1 levels were quantified in intestinal biopsies (immunohistochemistry) and blood (flow cytometry) using an antibody specific for IFN-λR1. Fresh intestinal biopsies were cultured *ex vivo* for 24 hr in media +/- IFN-λ3 and +/- IFN-λ signalling inhibitor (JAKi) and gene expression was quantified by Nanostring nCounter and RT-qPCR.

Results

We found a significant decrease in IFN-λR1 levels especially in IBD during active disease compared to non-IBD samples ($p<0.01$, 30-50% reduction). nCounter analyses uncovered significant increases in multiple IFN-stimulated genes (ISGs), with a significant decrease in specific pro-inflammatory cytokine genes even in non-IBD colon tissue. IFN-λ gene induction was significantly inhibited by a JAKi used as an IBD therapy ($p<0.001$, >87% inhibition). IFN-λ3 was up to 8-fold less potent at inducing ISG expression in IBD compared to non-IBD gut biopsies ($p<0.01$). Interestingly, IFN-λR1 levels were not decreased in peripheral blood immune cells of IBD, indicating the altered IBD gut microenvironment potentially decreased IFN-λR1 levels.

Conclusion

Our results demonstrate that patients with IBD have dysregulated IFN-λR1 and downstream responses. Lower IFN-λ responses in the IBD gut could lead to the lower induction of key anti-inflammatory and gut healing pathways. This work supports the further study of mechanisms regulating the IFN-λ system to develop strategies to restore optimal IFN-λ responses as a novel IBD therapy.

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POSTER #16

Exploring Splicing Regulation by REPAG Elements in Health and Diseases

Samuel Ogunsola[1], Jiuyong Xie[1]

[1]Department of Physiology & Pathophysiology, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, R3E 0J9, Canada.

Introduction

Advances in genome sequencing technology have transformed mutation detection in genetic diseases. Mutations, beyond altering protein function, can impact RNA; an example is alternative splicing (AS). In AS, a single gene can create multiple mRNA transcripts, each coding for different protein isoforms. Remarkably, in humans, over 95% of genes undergo AS, and disruptions, like mutations in cis-acting RNA regulatory elements, can lead to genetic disorders like cancers, endocrine, or neurological diseases. In our lab, we have identified a novel group of guanosine (G)-rich cis-acting RNA regulatory elements between the polypyrimidine tract (Py) and 3' AG dinucleotide (REPAG) of introns. The REPAGs are enriched in mammals, with 2281 of them found in annotated 3' splice sites in the human genome. However, the consequences of genetic mutations on splicing regulation by REPAG, especially in diseases development, remain unexplored.

Methods and Results

Using quantitative splice site strength measurements, calculated through the maximum entropy (MaxENT) and advanced machine-learning algorithms, such as SpliceAI, we have identified over 2000 pathogenic REPAG-like genetic variants in critical genes such as MAX, QSOX1, PRKACA, and DYSF, cataloged in databases including the Framingham Heart Study (FHS), GWAS, HGMD, and ClinVar. Interestingly, hundreds of these SNPs, predicted by SpliceAI from the FHS-sQTL (i.e., SNPs associated with RNA splicing) and GWAS analyses ($p < 5E-08$), are implicated in human genetic diseases/traits. For example, one SNP is linked to the eosinophil percentage of white blood cells in GWAS ($p = 6E-10$, rs762810-T) at the alternative splice site of MAX gene, which is crucial in cancer development. The SNP reversed the complete skipping of exon 5 by REPAG in the Max gene, as validated by splicing reporter assays and RNASeq Data from the Genome Tissue Expression Project (GTEX Database).

Conclusion

These observations underscore the impact that REPAG mutations can have on human genetic diseases. As we continue to explore these mutations in more databases like the UK Biobank and the Framingham Heart Study, we anticipate gaining deeper insights into how genetic mutations influence alternative splicing, shedding light on their broader implications for disease mechanisms.

Keywords: Mutations, Alternative Splicing, REPAG

POSTER #103

Elucidating Novel Function of Prolactin Inducible Protein in Regulating NK-cell Migration

Obinna I. Okeke[1]**, Chidalu A. Edechi[2,3], Heqing Ma[1], Manli Zhang[1], Yvonne Myal[3], Jude E. Uzonna[1] and Sam K.P. Kung[1]

[1]Department of Immunology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada., [2]Temerty Faculty of Medicine, University of Toronto, Canada., [3]Department of Pathology, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada.

Introduction

Prolactin inducible proteins (PIP) are frequently observed in breast cancers (BCs) and are recognized as a significant biomarker for identifying the source of BC. Its presence correlates with favorable prognostic outcomes in human BC.

In collaboration with the Myal laboratory, we reported earlier that ectopic expression of PIP in the mouse 4T1 BC model led to decreased growth of primary breast tumors but an increased incidence of lung metastasis in vivo. Additionally, a greater proportion of NK cells was observed within the PIP-expressing tumors located in breast tissue. We therefore hypothesize that PIP is a novel host regulatory factor of NK cell functionality.

Methods

We re-derived PIP-expressing 4T1 cells (4T1-PIP) and control 4T1 cells (4T1-EV) from primary breast tumor tissue (BR) and metastatic lung tissue (ML). The conditioned media from these cell lines and recombinant PIP (rPIP) were used in a transwell migration assay. NK cytotoxicity (CD107a) and cytokine production assay (IFNG) were assayed using activated BALB/c NK cells (in 1000 u/ml IL-2,m 4 days). Surface and intracellular analyses of molecules of interests were examined in flow cytometry. PIP expression was confirmed in western blotting.

Results

I showed that a higher number of NK-cell migration was observed in the conditioned media from PIP expressing 4T1. There was no difference whether the PIP was expressed in the 4T1 derived from primary breast tumor site (4T1-PIP-BR) or metastatic lung site (4T1-PIP-ML). I confirmed further that rPIP promoted NK migration in a dose dependent manner. Preliminary results for in-vitro cytotoxicity and cytokine assay (CD107a and IFNG) showed no difference for both PIP-expressing 4T1 cells (4T1-PIP) and control 4T1 cells (4T1-EV) from breast and lungs.

Conclusion

My preliminary findings show that PIP regulates NK migration but not anti-tumor effector functions of NK cells. The receptor and signaling pathways remain to be determined. It is the first study that examines how PIP can affect NK cell biology in both normal and disease state. Preliminary data that showed NK cell effector functions were comparable in primary breast and metastatic lung tumors supported the development of NK cell therapy in controlling metastatic breast cancer in lung.

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POSTER #34

Investigating the Impact of the Thioredoxin System on DNA Methylation of Neurons During Neurodegenerative Diseases

Tobi Olanipekun, Md Imamul Islam, Eftekhar Eftekharpour

Introduction

The neuron is susceptible to oxidative stress, which can contribute to the development and progression of neurodegenerative diseases in Alzheimer's disease, and Amyotrophic lateral sclerosis. The thioredoxin system, an antioxidant defense mechanism, has proven to be an important neuronal antioxidant, and its disruption by the knockout/down of thioredoxin in SH5YSY cells and Thioredoxin-1 neuronal knockout animal models is associated with breakdown of nuclear lamina (Neuronal nuclear laminopathy), increased markers of direct DNA damage, and synaptic dysfunction. These pathologies points to potential underlying disease-causing mechanisms in neurodegenerative diseases and can be possibly linked/explained by abberant epigenetic mechanism such as DNA methylation.

DNA methylation is a key epigenetic mechanism that can influence gene expression and contribute to the pathogenesis of neurodegenerative disorders. Understanding the relationship between the absence of the antioxidant thioredoxin and DNA methylation patterns in neurons may provide valuable insights into the underlying mechanisms of these diseases and potentially identify novel therapeutic targets.

Methods

This study will use a Cre-loxP neuronal Thioredoxin knock out mice to investigate the impact of the thioredoxin system on the DNA methylation profile of neurons during neurodegenerative diseases. Cortices of wild type and knockout mice will be obtained at 8 weeks, and a combination of mechanical and magnetic separation techniques will be used to isolate pure populations of neurons. This will reduce confounding effects from other non-neuronal cells later during data analysis. Following the isolation, the extracted genome will undergo bisulfite conversion in preparation for methylation studies, then epigenome-wide association studies (EWAS) will be used to check and identify if there are regions and specific probe points of the epigenome that are dysregulated in comparison to healthy control samples.

Conclusion

This study findings will enhance our understanding of the pathophysiology of neurodegenerative diseases by investigating the relationship between DNA methylation, oxidative stress and how they contribute to the pathophysiology of neurodegenerative diseases. These insights can inform the development of targeted therapeutic intervention strategies against neurodegenerative diseases.

POSTER #166

Short-Chain Fatty Acids Inhibit Type III Interferon Signaling in Intestinal Epithelial Cell Lines

Testimony J. Olumade,[1] Heather K. Armstrong,[1,2,3] and Deanna M. Santer[1]

[1] Department of Immunology, University of Manitoba, Winnipeg, MB, Canada; [2] Department of Internal Medicine, University of Manitoba, Winnipeg, MB; [3] Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Canada.

Introduction

Type III interferons (Interferon lambdas, IFN-λs) are known to dampen inflammation in mouse colitis models. We found reduced IFN-λ receptor (IFN-λR1) levels in inflammatory bowel disease (IBD) patient gut tissue but what causes this decrease remains unknown. Presence of infectious microbiota impacts IFN-λs signalling, and changes in the gut microbiota is a hallmark of IBD. We found secretions from a *Bacteroides fragilis* colony live-isolated from an IBD patient inhibited IFN-λ signaling in the Caco-2 intestinal cell line, leading us to speculate microbiota metabolites (e.g., amino acids, enzymes, tryptophan metabolites [e.g., kynurenine] and short-chain fatty acids [SCFA]) play a role in regulating IFN-λ in the gut. As SCFAs are decreased in IBD, and intermediate metabolites (e.g., succinate) are increased, I *hypothesized* that higher concentrations of SCFA (butyrate and propionate) would improve IFN-λR1 levels and IFN-λ signaling in human intestinal epithelial cells, while succinate would inhibit IFN-λ.

Methods

Caco-2 cells were seeded overnight and pre-treated with physiologically relevant concentrations of SCFAs (acetate [2.5-40mM], butyrate [0.156-2.5 mM], and propionate [2.5-10mM]), succinate (2.5-10mM), or kynurenine (25mM & 100mM) for two hours; metabolites were removed and media was added +/- IFN-λ3 (50ng/ml) for 22 hours. Caco-2 RNA was quantified by RT-qPCR examining IFN-stimulated genes (*MX1* and *IFIT1*). IFN-λR1 transcript (RT-qPCR) and protein (flow cytometry) levels were measured. Cell viability was examined by PrestoBlue assay.

Results

In contrast to my hypothesis, all SCFAs displayed a concentration-dependent inhibitory effect on IFN-λ3 induction of *MX1* and *IFIT1* ($p<0.05$) with no impact on cell death. Succinate and kynurenine did not affect ISG induction by IFN-λ3. Preliminary flow cytometry results showed that IFN-λR1 surface levels did not decrease after treatment with SCFAs.

Conclusion

Interestingly, the most abundant SCFAs found in the gut directly downregulated IFN-λ3 responses in the Caco-2 cell line, with IFN-λR1 surface levels unchanged. These results highlight that SCFAs may help maintain IFN-λ homeostasis but also suggest other microbiota factors may be involved in dampening IFN-λR1 levels in IBD which I aim to examine further in my PhD.

Funding

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POSTER #54

The Predictive Role of Pre-HIV Infection CD4+ Th17 Cells in HIV Disease Progression: A Multi-Cohort Study

Tosin Omole[1], Can Nguyen[1], Agata Marcinow[1], Naima Jahan[1], Giulia Severini[1], Katherine Thomas[2], Connie Celum[2], Nelly Mugo[2,3], Andrew Mujugira[2,4], James Kublin[5,6], Lawrence Corey[5,6], Aida Sivro[1,7,9,10], Jairam Lingappa[2], Glenda Gray[5,8], Lyle McKinnon[1,7]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Canada., [2]Department of Global Health, University of Washington, Seattle, USA., [3]Kenya Medical Research Institute, Nairobi, Kenya., [4]Infectious Diseases Institute, Makerere University, Kampala, Uganda., [5]HIV Vaccine Trials Network (HVTN), Seattle, USA., [6]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Center, Seattle, Washington, USA., [7]Centre for the AIDS Program of Research in South Africa (CAPRISA), Durban, South Africa., [8]South African Medical Research Council, Cape Town, South Africa., [9]JC Wilt Infectious Disease Research Centre, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada., [10]Department of Medical Microbiology, University of KwaZulu-Natal, Durban, South Africa.

Introduction

Interleukin-17 producing (Th17) $CD4^+$ T cells are highly permissive to HIV infection and depleted early in people living with HIV. Here, we explored whether the frequency of circulating Th17 cells pre-HIV infection was associated with HIV disease progression.

Methods

We used archived cryopreserved blood cells collected <1 year prior to HIV infection from participants enrolled in HIV Vaccine Trials Network (HVTN) 503 (n = 35) and the Partners Pre-Exposure Prophylaxis Study in combination with the Couples Observational Study (PP/COS) (n = 32). We applied flow cytometry to quantify Th17 cell frequency. Both cohorts comprise of high-risk heterosexual individuals from South and Eastern Africa, respectively, all initially HIV negative. Specifically, we focused on participants who subsequently contracted HIV within these cohorts, referred to as HIV cases, for the purpose of our analysis.

Results

In HVTN 503, participants had a median age of 23 years (IQR: 22-27), while in Partners PrEP, the median age was 30 years (IQR: 25-40). We included 17 female and 18 male participants in HVTN 503, and 19 female and 13 male participants in Partners PrEP. In HVTN 503, $IL17^+CD4^+$ T cell frequency correlated inversely with CD4/CD8 ratio measured <180 days (Spearman rank r = -0.42, p=0.012) and >180 days (r = -0.55, p=0.001) post-HIV infection and was associated with a faster decline in $CD4^+$ T cells below 500 copies/mm^3 (Cox regression HR = 2.9, 95% CI = 1.2 – 6.9, p=0.015), including after adjusting for viral load (aHR = 2.5, 95% CI = 1.0 – 6.1, p=0.038). However, in Partners PrEP, the correlation with CD4 decline was not significant (HR = 1.2 , 95% CI = 0.4 – 3.4, p=0.795). In an analysis that combined both cohorts, $IL17^+CD4^+$ T cells remained a significant predictor of $CD4^+$ T cell decline in the unadjusted (HR = 2.2, 95% CI = 1.1 – 4.3, p=0.023) and viral load adjusted models (aHR = 2.1, 95% CI = 1.0 – 4.1, p=0.038). $IL17^+$ T cell frequency was not associated with peak or set-point viral loads in either cohort.

Conclusion

Our study highlights the potential importance of pre-HIV infection Th17 cell levels in shaping HIV disease progression.

POSTER #39

IRE1 signaling increases PERK expression during chronic ER stress

Gideon Ong[1], Rosemund Ragetli[1], Katarzyna Mnich[2,3], Bradley W. Doble[4], Wafa Kammouni[1], Susan E. Logue[1,5,6]

[1]Department of Human Anatomy and Cell Science, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada., [2]Apoptosis Research Centre, University of Galway, Ireland., [3]School of Biological and Chemical Sciences, University of Galway, Ireland., [4]Department of Paediatrics, Department of Biochemistry and Medical Genetics, Max Rady College of Medicine, Rady Faculty of Health Sciences University of Manitoba, Winnipeg, MB, Canada., [5]CancerCare Manitoba Research Institute, Winnipeg, Canada. [6]Children's Hospital Research Institute Manitoba, Winnipeg, Canada.

Introduction

The Unfolded Protein Response (UPR) is an essential stress response pathway regulated by the Endoplasmic Reticulum (ER)-anchored sensors: IRE1, PERK and ATF6. While the primary role of the UPR is to maintain ER homeostasis, and thereby cell health, recent findings associated UPR dysregulation with the progression of multiple diseases. Notably, IRE1 signaling has been linked to the advancement of Triple Negative Breast Cancer (TNBC). For this reason, IRE1 has emerged as a potential therapeutic target for TNBC treatment. Indeed, an IRE1 inhibitor, MKC8866 (Orin1001) which demonstrated promising efficacy in preclinical models of TNBC, has entered Phase 2 clinical trials for advanced breast cancer. While clinical targeting of IRE1 offers great potential, it also highlights some key deficits in our fundamental knowledge of the UPR, namely how interconnect IRE1, PERK and ATF6 signaling pathways are. If we suppress IRE1 activity, do we impact PERK or ATF6 signaling pathways?

Methods

A panel of tumourigenic and non-tumourigenic breast cell lines were subjected to ER stress in presence/ absence of IRE1 inhibitors. Inhibitor based strategies were complemented with cell lines genetically engineered to lack IRE1 or its downstream signaling mediator XBP1. Chromatin immunoprecipitation assays were employed to assess XBP1s binding sites. The outcome of attenuated IRE1 signaling upon the wider UPR network in ER stressed cells was assessed by immunoblotting and qPCR.

Results

IRE1 inhibitor MKC8866, in addition to blocking IRE1 signaling, reduced PERK expression and downstream signaling during chronic ER stress. This was observed across multiple cells lines, IRE1 inhibitors, and both physiological and chemical inducers of ER stress. Initial findings with IRE1 inhibitors were verified using IRE1 knockout cells. Dissection of downstream pathways revealed a mechanism wherein IRE1-XBP1s signaling increases PERK through direct binding towards its promoter during chronic ER stress.

Conclusion

This study highlights a new, previously unreported, crosstalk between the IRE1 and PERK arms during long-term ER stress. Aside from providing new insights into fundamental UPR signaling, this finding could have important consequences on how we interpret results with IRE1 inhibitors. Our results suggest IRE1 inhibitors have the ability to impact not one but two arms of the UPR.

POSTER #28

Leishmania major Dihydrolipoyl dehydrogenase (DLD) is a critical virulence factor and modulates host immune response to the parasite.

Somtochukwu Stella Onwah[1], Zhirong Mou[1], Gaurav Gupta[1], Ping Jia[1], Obi, Patience[3], Saleem, Ayeesha[3], Saeid Ghavami[2] and Jude Uzonna[1]

[1]Department of Immunology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB., [2]Department of Human Anatomy and Cell Science, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB., [3]Faculty of Kinesiology and Recreation Management, University of Manitoba, Winnipeg, MB.

Introduction

Cutaneous leishmaniasis (CL) caused by several species of protozoan parasites that belong to the genus *Leishmania* is endemic to the Middle East, Asia, Latin and Central America and North Africa. The disease affects millions of people in these regions, and it is estimated that over 1 million new infections occur each year. Currently, there is no approved vaccine against human cutaneous leishmaniasis because of the poor understanding of the mechanisms that regulate disease pathogenesis and correlates of protective immunity. We previously identified *Leishmania* Dihydrolipoyl dehydrogenase (DLD) as a critical target of host immune response as a peptide derived from this protein (DLD_{63-79}) strongly activates $CD4^+$ T cell proliferation and cytokine response in infected mice. Although DLD has been shown to play a vital role in virulence of Fungi and bacteria, its role in *Leishmania* virulence and disease pathogenesis is unknown.

Method

We generated DLD deficient *L. major* using a CRISPR-Cas9-based approach and a complementary add-back control by episomal reintroduction of *DLD* gene into the DLD deficient strain. In the present report, we showed that DLD is a major *Leishmania* virulence factor and target for host immune response.

Result

Targeted loss of DLD results in impaired proliferation of *L. major* in axenic culture and bone marrow-derived macrophages, leading to a highly attenuated pathology *in vivo* in both the susceptible BALB/c and relatively resistant C57BL/6 mice. This impaired proliferation and attenuated pathology was associated with dramatic reduction in the frequency of cytokine (IFN-, IL-4 and IL-10)-producing $CD4^+$ T cells in spleens and lymph nodes draining the infection sites. Furthermore, the impaired proliferation of DLD deficient parasites was associated with compromised mitochondrial function and respiration manifested as increased extracellular acidification rate, increased proton leak and decreased ATP coupling efficiency and oxygen consumption rates. Vaccination with DLD deficient parasites induced strong protective responses upon virulent *L. major* rechallenge and this was associated with strong IFN-g response.

Conclusion

Collectively, these findings show that DLD is a critical metabolic enzyme for intracellular survival of *L. major* and targeting this molecule could be a viable option for controlling the disease.

POSTER #32

The Impact of a Revised Preclinical MSK Curriculum on Learning and Knowledge Retention Among Postgraduate Physicians

Taylor Orchard[1], Alexa Hryniuk[1], Jason Peeler[1]

[1]Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada

Introduction

Musculoskeletal (MSK) injuries/disorders are among the most common medical conditions treated by a physician. Despite this, previous literature suggests that physician training in MSK medicine has been historically inadequate resulting in a lack of knowledge, confidence and clinical skills among postgraduate physicians. The University of Manitoba (UM) implemented a revised preclinical MSK medicine curriculum in 2015 that included 109 hours of MSK instruction (32 hours of MSK anatomy; 68 hours of instruction on "core or must know" topics in MSK medicine; 11 hours of MSK clinical skills). The goal of this investigation was to examine MSK learning and knowledge retention among postgraduate physicians from the UM following curricular reform.

Methods

Five hundred and sixty-eight postgraduate physicians from the program's classes of 2018 – 2023 were contacted and asked to complete a standardized and recently validated MSK competency exam that consisted of 30 multiple-choice questions on topics in MSK medicine that all physicians should be familiar with. Each question included knowledge and concepts that could be directly mapped to both course and session specific learning objectives of the program's new preclinical MSK curriculum.

Results

A total of 90 responses were received from postgraduate physicians and demographically, participants represented a heterogeneous group of individuals similar to that reported in the most recent AFMC data report. The average test score among respondents was 75.0% (± 10.2%; range 57%-100%). No significant differences were noted in test scores when data was organized by gender, year of graduation, or previous academic performance in medical school. Physicians who specialized in fields related to MSK medicine (i.e. Orthopaedics, PM&R, Sport Medicine, Rheumatology), or who participated in MSK-related electives during clerkship training performed significantly better ($P \leq 0.01$) on the MSK exam.

Conclusion

Data suggests that the medical program's new preclinical curriculum supports high levels of MSK learning and knowledge retention among postgraduate physicians. The results represent an important step towards the establishment of minimum curricula standards in the area of MSK medicine among AFMC-accredited medical programs, and provide medical educators/program directors with a model of enquiry that could be used to address curricular inadequacies in other programs.

POSTER #36

Examination of cationic antimicrobial adapted *Klebsiella pneumoniae* shows differences in phenotypes and antimicrobial cross-resistance profiles that are dependent on drug classification.

Peter O'Reilly[1], Brandon Gregorchuck[1], David Alexander[2], George Zhanel[1], and Denice Bay[1]

[1]Department of Medical Microbiology and Infectious Diseases, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada., [2]Cadham Provincial Laboratory, Province of Manitoba, Winnipeg, MB, Canada.

Background

Cationic antimicrobials (CAs) are frequently used as antiseptics in healthcare, agricultural, and domestic settings; they include chlorhexidine (CHX), benzalkonium chloride (BZK), and last-resort antibiotics (colistin; COL). Due to their overusage, CAs increase selective pressure on bacteria to become more resistant to these compounds. CA resistance often coincides with antibiotic cross-resistance. *Klebsiella pneumoniae* is one of the top 5 frequently identified multidrug-resistant critical priority pathogens and is regularly exposed to CAs.

Methods

Klebsiella pneumoniae DSM 6135 was adapted to three of the most overused CAs (BZK, CHX, COL) to identify CA-resistance mechanisms using whole genome sequencing (Illumina) of CA-adapted isolates. We also elucidate their contributions to antimicrobial cross-resistance using broth microdilution antimicrobial susceptibility testing (AST) techniques.

Results

All CA-adapted *K. pneumoniae* isolates grew in CA concentrations ≥4-fold of the WT minimum inhibitory concentration (MIC). All CA-adapted isolates exhibited stable CA-resistant phenotypes after 10-days without CA selection. CHG- and BZK-adapted isolates showed decreased growth rates when compared to WT (15-50% reduced OD600 values after 24-hour growth). Preliminary AST results from each CA-adapted isolate towards a library of 9 drugs showed minimal cross-resistance (generally <4-fold increase in MIC) towards the clinically relevant antibiotics that were tested. Significant increases (≥4-fold) in MIC of CA-adapted isolates towards other compounds was primarily observed only for similar CAs as those used for adaptation, similar to past findings for *E. coli* CA- adapted isolates. Exceptions to this include COL-adapted isolates towards kanamycin (4-8-fold increase), and CHX-adapted isolates towards ciprofloxacin (4-8-fold increase) and COL (>15-fold increase). Preliminary findings from ongoing whole genome sequencing (WGS) showed single nucleotide variant changes in numerous genes in BZK-adapted isolates. Continued WGS analysis in future studies may reveal mutations in genes related to membrane drug efflux, lipid transport, and lipid synthesis as seen previously in *E. coli* CA adaptation studies.

Conclusion

K. pneumoniae can rapidly develop elevated, phenotypically stable CA-resistance that primarily enhances cross-resistance to similar CA classes. Gradual CA-adaptation has genotypic consequences that will be further verified using WGS and cloned gene complementation assays.

POSTER #116

Exercise-induced extracellular vesicles mediate apoptosis in human colon cancer cells in a exericse intensity-dependent manner

Berkay Özerkliğ[1, 4,5] , İbrahim Türkel[1], Merve Yılmaz[2], Dilara Vaizoğlu[3], Beril Erdem[3], Handan Sevim[3], Ayesha Saleem[4,5] , Z. Günnur Dikmen[2], and Şükran N. Koşar[1]

[1]Faculty of Sport Sciences, Hacettepe University, Ankara, Turkey., [2]Department of Medical Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey., [3]Department of Biology, Faculty of Science and Literature, Hacettepe University, Ankara, Turkey., [4]Faculty of Kinesiology and Recreation Management, University of Manitoba, Winnipeg, Canada., [5]The Children's Hospital Research Institute of Manitoba (CHRIM), Winnipeg, Canada.

Introduction

Regular exercise is known to reduce incidence rates and improve the prognosis of all cancers, but the underlying mechanisms remain elusive. Ample evidence suggests that exercise exerts therapeutic effects through extracellular vesicles (EVs), essential for cellular communication. Here, we hypothesized that exercise-induced EVs from serum of healthy young male participants will exert anti-tumorigenic effects on human colon cancer HT-29 cells in an exercise intensity-dependent manner.

Methods

10 healthy young active males (25.4±6.2yrs, with maximal oxygen consumption= 45±3.7mL·kg(-1)·min(-1)) participated in a randomized controlled crossover trial. Participants underwent two different workload-matched, acute bouts of exercise: (1) moderate-intensity continuous exercise (MICE) at 50-55% V02max, and (2) high-intensity interval exercise (HIIE) at 90% V02max on a cycle ergometer. A control session of rest (Pre) was included. EVs were isolated from serum samples collected immediately after each exercise session, and after the rest period, using ExoQuick™ and frozen at -80 ˚C. EVs were thawed once, then incubated with colon cancer HT-29 cells (100µg EVs/ml, for 48-72hrs). Cell viability, migration and apoptotis were measured post-treatment.

Results

EV treatment reduced cell viablity in all groups (Pre, MICE, HIIE) by 35%, 43% and 47% respectively, *vs*. PBS (negative control, $p<0.0001$). EVs from HIIE group showed significantly higher effect on cell viability compared to Pre ($p=0.04$), therefore further analysis used these groups only. Wound healing assay showed reduced migration in Pre (by 27%, $p=0.04$) and HIIE (by 39%, $p=0.005$) groups. EV treatment also increased expression of pro-apoptotic markers: Bax/Bcl-2 ratio by 56% ($p=0.03$) and Caspase-3 by 30% ($p=0.02$) in HIIE group *vs*. PBS, and no change was observed in the Pre group. Lastly, 16% of cells in Pre ($p=0.006$) and 28% of cells in HIIE ($p<0.0001$) were TUNEL-positive.

Conclusion

Our data show that exercise-induced EVs reduced cell viability, in an exercise intensity-dependent manner, with high-intensity exercise exerting the most anti-tumorigenic effects: decreased cell viability, reduced cell migration, increase in pro-apoptotic protein expression and elevated DNA fragmentation. To our knowledge, this the first human study that illustarates the therapeutic potential of exercise-induced EVs in cancer treatment. Further mechanistic studies are needed to unravel the precise mechanisms underlying these observations.

POSTER #84

Determining the Role of Zeb1 and Zeb2 in Cardiac Fibroblast Activation

Rohini Pallikkara Suresh[1,2], Jessica Mc Bride[1,2], Jeffrey T. Wigle[1,2]

Introduction

ZEB1 and ZEB2, members of the Zinc finger transcription factor family, have crucial roles in embryonic development, angiogenesis, and Epithelial-Mesenchymal transition (EMT). Previous studies in our lab have shown that Zeb2 is expressed in activated cardiac fibroblasts in vitro and in the scar following myocardial infarction in vivo. Expression of ZEB2 can be downregulated by Ski, an inhibitor of TGFβ/SMAD signaling. Using siRNA knockdown and adenoviral-mediated overexpression, we showed that ZEB2 was sufficient to induce cardiac fibroblast activation but was not required for this process. This finding could be a result of ZEB1 also being expressed in cardiac fibroblasts and thereby compensating for the loss of ZEB2 function during the process of cardiac fibroblast activation.

We hypothesize that:
1) ZEB1 and ZEB2 initiate and maintain the activated cardiac fibroblast phenotype
2) ZEB1 and ZEB2 may form a negative feedback loop to maintain an optimal level of expression in cardiac fibroblasts.

Methods

We compared the protein levels of ZEB1 and ZEB2 in primary adult male and female rat cardiac fibroblast cells during the process of fibroblast activation in vitro by using western blotting. We studied the effect of overexpression as well as knockdown of ZEB1 and ZEB2, both in mouse NIH 3T3 fibroblast cell line as well as in primary rat cardiac fibroblast cells.

Results

Our results show that both ZEB1 and ZEB2 expression is increased during cardiac fibroblast activation. We observed that ectopic expression of ZEB2 in both NIH 3T3 fibroblast cell line and rat primary cardiac fibroblasts resulted in a corresponding decrease in endogenous ZEB1 expression. Furthermore, siRNA-mediated knockdown of ZEB2 resulted in increased ZEB1 expression in cardiac fibroblasts. Similarly, ZEB1 knockdown resulted in increased ZEB2 expression.

Conclusion

ZEB1 is expressed during primary rat cardiac fibroblast activation and ZEB1 and ZEB2 function to negatively regulate their expression. Therefore, the loss of one ZEB family member may be compensated for by increased expression of the other family member which maintains the overall level of ZEB signalling.

POSTER #134

Pathogenic ELFN1 variants role in mGluR4 function

Afroza Parvin [1, 2] & Henry A. Dunn [1, 2]

[1]Division of Neurodegenerative Disorders, St. Boniface Hospital Albrechtsen Research Centre, Winnipeg, Manitoba, Canada., [2]Department of Pharmacology & Therapeutics, University of Manitoba, Winnipeg, Manitoba, Canada.

Introduction

In the brain, excitatory neurotransmitter glutamate not only activates ionotropic receptors but also G-protein coupled receptors (GPCRs) termed metabotropic glutamate receptors (mGluRs). These receptors are critical for modulating excitatory glutamatergic neurotransmission and are considered as highly viable avenues for drug design for neurodevelopmental disorders (NDDs). Based on sequence homology and signal transduction mechanisms, this family can be categorized into three groups (I, II, and III). Interestingly, group III mGluRs are negatively coupled to adenylate cyclase and act as autoreceptors providing negative feedback to inhibit glutamate release. Recently, we have discovered that group III mGluRs are trans-synaptically regulated by synaptic adhesion molecules (SAMs). Extracellular leucine rich fibronectin type III domain 1 (ELFN1) is a type of SAM which engages group III mGluRs trans-synaptically and alters the accepted pharmacological principles of these receptors. Recently, disease causing human ELFN1 gene variants have been identified developing diverse NDDs. In this study, we aim to evaluate the consequence of ELFN1 genetic variants on mGluR4 function in hope of guiding novel therapeutic strategies for ELFN1-mediated NDDs. Here, the hypothesis is “pathogenic variants of ELFN1 have both mGluR4-dependent and mGluR4-independent roles in the etiology of ELFN1 specific NDDs”.

Methods

To accurately assess the impact of ELFN1 mutations, mammalian expression plasmid vector pcDNA was used to insert various human ELFN1 pathogenic mutations. Wildtype and the mutated plasmids were transfected into the prototypical cell line of GPCR pharmacology: HEK293. After harvesting cells, we performed expression analysis via Western blotting and co-immunoprecipitation experiments to assess the expression levels of the ELFN1 variants and their trans-cellular interactions with mGluR4, respectively.

Results

Western Blotting showed the expression of the mutated variants of ELFN1. Subsequently, co-immunoprecipitation demonstrated that wildtype and intracellular mutated ELFN1 interact properly with the mGluR4 suggesting mGluR4-independent mechanism; however, no interaction was observed in extracellular ELFN1 mutation which ultimately suggests an mGluR4-dependent mechanism in the pathogenesis of these patients.

Conclusion

This is ongoing research and provided here some preliminary observations. A holistic approach would provide an early and unique description of molecular consequences of pathogenic ELFN1 variants and seminal characterizations of the intracellular roles and signalling pathways of SAMs.

POSTER #153

Determining the role of IRF2BPL in neurological disease.

Danielle M. Pascual[1], Yina Her[1], Paul Houston[1], Robert Beattie[1,2], Paul C. Marcogliese[1,2]

[1]Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MB, Canada., [2]Children's Hospital Research Institute of Manitoba, Winnipeg, MB, Canada.

Introduction

De novo truncating variants in the gene IRF2BPL cause severe childhood-onset ataxia termed NEDAMSS (Neurodevelopmental disorder with abnormal movements, loss of speech and seizures). Additionally, IRF2BPL missense variants are associated with autism spectrum disorder. IRF2BPL is important for nervous system development and maintenance, but its function remains unclear.

Objective

We have generated the first Irf2bpl knockout mice and aim to characterize the behavioral outcomes and brain pathology in these mice.

Methods

We generated the Irf2bpl null allele by removal of the majority of the single exon (Δ17-651). We performed heterozygous crosses to assess viability, mass, and motor function by vertical pole test and inverted grid test across littermates of both sexes.

Results

We observed that Irf2bpl KO mice are born at lower Mendelian ratios. Although WT and HET mice did not have a significant difference in mass, KO are significantly runted. Lastly, three-month-old Irf2bpl KO mice display motor defects on the vertical pole test and inverted grid test. KO mice brains are also smaller in size and mass than WT mice at 3 months of age.

Conclusion

We have generated preliminary data on Irf2bpl mice. Our data shows that the Irf2bpl KO mice display motor defects as early adults which may model NEDAMSS which may act as a preclinical model to develop therapeutics for this devastating disorder.

POSTER #154

Brain Glucose Imaging-based Model Predicts Cognitive Stability in Prodromal Alzheimer's Disease

Jarrad R. Perron[1,2], Ji Hyun Ko[1,2,3]

[1]Graduate Program in Biomedical Engineering, Price Faculty of Engineering, University of Manitoba, Winnipeg, MB R3T 2N2, Canada., [2]PrairieNeuro Research Centre, Kleysen Institute for Advanced Medicine, Health Sciences Centre, Winnipeg, MB R3E 3J7, Canada., [3]Department of Human Anatomy and Cell Science, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB R3E 0W2, Canada.

Introduction

Anti-amyloid medications for the treatment of Alzheimer's disease (AD) have recently become available, however, there exist concerns about the safety, cost-efficiency and efficacy of these drugs. Differential diagnosis is modestly accurate (sensitivity: 70.9 %- 87.3%, specificity: 44.3% - 70.3%) and predicting cognition from prodromal stages is prohibitively difficult. Patients with biologically defined AD often remain cognitively stable for years prior to the onset of dementia, with many developing mild cognitive impairment (MCI). These prodromal AD patients are at elevated risk of further progression from MCI to dementia (pMCI), however, an overwhelming majority of these patients (77.7%) remain stable at the MCI stage (sMCI) over a 3-year period. Prophylactic treatment of prodromal AD would be cost-inefficient and require sMCI patients to risk possibly severe side-effects.

Objective

Develop a machine intelligence capable of forecasting cognitive in patients with prodromal AD to select appropriate candidates for anti-amyloid pharmaceuticals.

Methods

Fluorodeoxyglucose positron emission tomography (FDG-PET) is sensitive to changes in resting cerebral glucose metabolism prior to the onset of dementia. Patients from the Alzheimer's Disease Neuroimaging Initiative with FDG-PET studies (n = 594) were stratified according to the AT(N) classification scheme. An ensemble of convolutional neural networks were trained (n_{train} = 311*)* to forecast cognition over a 3 year period in patients with prodromal AD. 20% of the prodromal subjects and all non-prodromal subjects were used as independent test sets. The data were cross-validated and balanced with the test set for clinical covariates.

Results

Final testing was performed on baseline images of prodromal and non-prodromal subjects (n_{test} = 283). The model discriminated between prodromal pMCI and sMCI subjects with 88.6% (39/44) accuracy, 73.7% (14/19) sensitivity, and 100% (19/19) specificity. Non-prodromal subjects were classified with 86.4% accuracy (206/239), 77.4% sensitivity (33/42) and 88.23% (165/197) specificity.

Conclusion

By identifying patients with prodromal AD who will not progress to dementia, our model may significantly reduce societal burden if implemented as a screening tool for anti-amyloid therapy. It could also give clinicians a quantitative means of decision support when prescribing anti-amyloid treatment.

POSTER #161

Retinoic Acid deficiency phenocopies developmental defects due to acute prenatal alcohol exposure in an FASD mouse model

Petrelli B[1], McKay L[1], Pind M[1], Oztürk A[1], Fainsod A[2], and Hicks GG[1]

[1] Department of Biochemistry & Medical Genetics; Regenerative Medicine Program, Faculty of Medicine, University of Manitoba, Winnipeg, Canada., [2] Department of Developmental Biology and Cancer Research, Institute for Medical Research Israel–Canada, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel.

Introduction

Fetal Alcohol Spectrum Disorder (FASD) arises from maternal consumption of alcohol during pregnancy affecting 2%–5% of North America. Our Xenopus laevis studies showed that alcohol exposure during early gastrulation reduces retinoic acid (RA) levels at this critical embryonic stage causing craniofacial malformations associated with FASD sentinel facial features. It is now understood that acute ethanol exposure overwhelms the aldehyde metabolic enzymes that would normally convert retinol (Vitamin A) to retinoic acid (RA). We hypothesize that PAE reduces RA levels during critical developmental stages in early gastrulation drives the later craniofacial malformations associated with FASD sentinel facial features. A genetic mouse model that induces transient RA deficiency in the node during gastrulation is described.

Methods

To biochemically mimic the alcohol-induced RA deficiency at gastrulation, we genetically engineered a mouse expressing Cyp26A1 from the endogenous Goosecoid (Gsc) promoter. The Gsc promoter dictates spatial-temporal expression to the node during gastrulation. Cyp26A1 degrades endogenous RA in these cells, mimicking the reduced RA levels induced by acute alcohol exposure and dysregulating neural crest cells induction.

Result

These mice recapitulate the phenotypes characteristic of prenatal alcohol exposure (PAE) suggesting a molecular etiology for the craniofacial malformations seen in children with FASD with sentinel facial features. Gsc+/Cyp26A1 mouse embryos have a reduced RA domain and expression in the developing frontonasal prominence region and delayed HoxA1 and HoxB1 expression at E8.5. These embryos also show aberrant neurofilament expression during cranial nerve formation at E10.5 and have significant FASD sentinel facial feature-like craniofacial phenotypes at E18.5. In adulthood, Gsc+/Cyp26A1 mice develop severe maxillary malocclusions. Furthermore, we show that Vitamin A supplementation during gestation rescues the craniofacial malformation phenotypes caused by PAE and associated with FASD sentinel facial features.

Conclusions

Taken together, our data provides mammalian evidence that strongly supports retinoic acid deficiency during gastrulation as a major molecular etiology of craniofacial malformations associated with FASD sentinel facial features in children. Moreover, Vitamin A supplementation may significantly reduce or prevent FASD outcomes in children with PAE.

POSTER #142

Surveillance of carbapenemase-producing organisms from retail pork and beef purchased from 2016 – 2018: a Canadian One-Health study

Paula E. Pidsadny[1,2], Richard J. Reid-Smith[3], Audrey Charlebois[4], Michael R. Mulvey[1,2], James S. Katipunan[2,5], Denice C. Bay[1], George G. Zhanel[1], and George R. Golding[1,2]

[1]University of Manitoba, Rady Faculty of Health Sciences, Department of Medical Microbiology and Infectious Diseases, Winnipeg, Manitoba., [2]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba.,[3]Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ontario., [4]National Microbiology Laboratory, Public Health Agency of Canada, Saint-Hyacinthe, Quebec., [5]University of Manitoba, Faculty of Science, Winnipeg, Manitoba.

Introduction

Antimicrobial resistance (AMR) is a concern across the One-Health Continuum where bacterial AMR pathogens can both colonize and cause infections in human and animal hosts. This is particularly a concern along the farm-to-fork continuum, where humans can acquire antimicrobial resistant-infections through the handling or consumption of agri-food products. This study examines the prevalence of carbapenemase-producing organisms (CPOs) in retail pork and beef purchased in Canada.

Methods

Fresh retail beef (n=60) and pork (n=187) were purchased in Canada from 2016-2018 through the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS), rinsed in PBS, and frozen. The PBS rinsates were sent to the National Microbiology Laboratory for further processing. Combinations of selective enrichment broth and selective chromogenic agar (bioMérieux) were used to isolate carbapenem-resistant organisms, which were identified with MALDI-TOF MS and tested phenotypically for carbapenemase production using the modified carbapenemase inactivation method (mCIM) or its variant CIMTris. Select CPOs were sequenced using Illumina sequencing and carbapenemase genes were identified with AMRFinderPlus.

Results

From the meat rinsates, *Pseudomonas* spp. (n=2397), *Aeromonas* spp. (n=426), *Acinetobacter* spp. (n=11), various Enterobacterales spp. (n=23), and isolates that could not be identified with MALDI-TOF MS (n=82) were isolated. Of the isolates tested to date, carbapenemase production was detected in *Aeromonas* spp. (327/425, 76.9%) and *Pseudomonas* spp. (27/2388, 1.1%), but not in Enterobacterales spp. (0/23). Sequencing of 91 select *Aeromonas* spp. showed they harboured *cphA* (83/91, 91.2%) or *imiH* (8/91, 8.8%). Overall, we report the prevalence of CPOs in retail beef and pork to be 51.7% and 29.9%, respectively.

Conclusions

This study is ongoing; however, current progress highlights the abundance of *cphA*-positive *Aeromonas* spp. in retail meat and future work will aim at comparing these agri-food isolates to human clinical and environmental isolates to examine the possible transmission of these CPOs across the farm-to-fork continuum in Canada.

POSTER #110

T cells drive beta cell senescence in type 1 diabetes

Jasmine Pipella[1,2], Peter J. Thompson[1,2]

[1]University of Manitoba, Physiology and Pathophysiology, Winnipeg, MB, Canada., [2]Children's Hospital Research Institute of Manitoba; DREAM theme.

Introduction

In 2022, Teplizumab, an anti-CD3 immunotherapy received FDA approval to delay symptomatic Type 1 Diabetes (T1D). While studies indicate its potential in improving beta (β) cell function, its mechanism on β cell stress remains unclear. Evidence suggests a subset of β cells can undergo senescence and accumulate in T1D. This study aims to investigate whether anti-CD3 immunotherapy mitigates β cell destruction by cytotoxic T cells and reduces accumulation of senescent β cells during T1D.

Methods

Female non-obese diabetic (NOD) mice received weekly doses of anti-CD3 monoclonal antibody or IgG control at the prediabetic stage. Intraperitoneal glucose tolerance tests were conducted to assess β cell function before isolating whole pancreas/pancreatic islets. Senescent β cell frequency and protein expression were evaluated using immunohistochemistry (IHC). The anti-CD3 treatment was validated and immunophenotyping was conducted using flow cytometry. Studies indicate that ABT-199, a senolytic compound that causes apoptosis in senescent cells, can halt T1D in NOD mice. Therefore, we proposed a combination therapy, where NOD mice were treated with anti-CD3/ABT-199, IgG/ABT-199, or control and similar assays were completed.

Results

Decreases of total $CD3^+$ T cells, antigen-specific insulin tetramer T cells, and total $CD4^+$ T cells were observed in the anti-CD3 treated mice compared to the controls. IHC revealed β cell preservation and slowed disease progression, and a decrease in senescent β cells in the anti-CD3 mice. Remarkably, no difference in markers of β cell identity, proliferation, or unfolded protein response pathways were observed. We observed a decrease in senescent gene expression in anti-CD3 treated mice versus control. Notably, the combination treatment showed a synergistic effect, with further improvement in glucose tolerance, a decrease in senescent β cells, and a reduction in insulitis in CD3/ABT-199 mice.

Conclusions

These findings suggest a novel mechanism for the action of anti-CD3 at the late pre-diabetic stage, where the modulation of T cells with anti-CD3 appears to impede disease progression by limiting senescent β cell accumulation. Future studies will explore the interplay between T cells and β cell senescence using co-culture assays to address whether T cells are driving healthy β cells towards a senescent state.

POSTER #136

Investigating in vivo effects of aberrant SKP1 expression on chromosomal instability in tubo-ovarian, high grade serous cancer

Kaitlyn N Quinlan[1], Megan C Rodriguez[1], Katharina Haigh[3,4], Zelda Lichtensztejn[1,3], Martin Köbel[5], Kirk J McManus[1,3], Mark W Nachtigal[1,2,3].

[1]Biochemistry & Medical Genetics, University of Manitoba, Winnipeg, MB, Canada; [2]Obstetrics, Gynecology & Reproductive Sciences, University of Manitoba, Winnipeg, MB, Canada; [3]Paul Albrectsen Research Institute, CancerCare Manitoba, Winnipeg, MB, Canada; [4]Pharmacology & Therapeutics, University of Manitoba, Winnipeg, MB, Canada; [5]Pathology, University of Calgary, Calgary, AB, Canada.

Introduction

We have found evidence strongly suggesting that chromosomal instability (CIN) may be a contributing event in tubo-ovarian, high grade serous carcinoma (HGSC) formation. CIN is a type of genomic instability defined as a change in the rate at which gains, losses, or rearrangements of fragments of or whole chromosomes occur. We determined that CIN was present in ~95% of HGSCs and have identified that loss of one copy of the CIN gene *SKP1* is associated with HGSC. SKP1 plays an important role the SKP1-CUL1-FBOX (SCF) complex, an E3 ubiquitin ligase complex that is critical for chromosome segregation and the DNA damage response. Importantly, we showed that in fallopian tube cell lines heterozygous loss of *SKP1* results in CIN. My project will translate these findings to novel *in vivo* models biologically relevant for genetic changes in HSGC patients.

Methods

To understand the contribution of CIN to HGSC etiology, I will measure the frequency of CIN in serous tubal intraepithelial carcinoma (STIC) lesions obtained from high-risk *BRCA1/2* patients. CIN will be assessed using centromere enumeration probes (CEPs) and fluorescent in situ hybridization. This technique detects centromeric deviations from diploid in the target cells that is indicative of CIN. We generated $Skp1^{+/flox}$ mice with an inducible cell-specific Cre driver (*Pax8*-Cre-ERT) to generate $Skp1^{+/-}$ fallopian tube cells, and a second model to inducibly generate $Skp1^{+/-}$:$Trp53^{-/-}$. Once gene recombination is induced, mice will be aged for 1, 3 and 6 months before assessment of pathobiology and CIN in the target cells.

Results

CEP analysis of STICs is ongoing. We have generated all experimental mice and have shown inducible gene recombination. Mice are aging after inducing gene recombination.

Conclusions

These findings will give us a more complete understanding of CIN in HGSC *in vivo*, and lead to therapeutic intervention taking advantage of the CIN environment to improve patient outcomes.

POSTER #151

Forecasting Canadian Age-Specific Mortality Rates: Application of Functional Time Series Analysis

Azizur Rahman[1,2], Depeng Jiang[1,3]

[1]Department of Community Health Sciences, University of Manitoba, Winnipeg., [2]Social Innovation Office, Department of Families, Government of Manitoba., [3] School of Sciences, Nanjing Forest University, Nanjing, China.

Background

In the insurance and pension industries, as well as in designing social security systems, forecasted mortality rates are of major interest. The current research provides statistical methods based on functional time series analysis to improve mortality rate prediction for the Canadian population.

Methods

The proposed functional time series-based model was applied to the three-mortality series: total, male and female age-specific mortality rate over the year 1991 to 2019 for the Canadian population. The functional principal component regression model (fPCA) is used to predict next ten years mortality rate for three series and examine the impact of age group differences over the years. In addition, functional autoregressive model (fAR(1)) is used to measure the impact of one year age differences on mortality series for Canadian population.

Results

For total series, the mortality rates for children have dropped over the whole data period, while the difference between young adults and those over 40 has only been falling since about 2003 and has leveled off in the last decade of the data. Furthermore, for male and female groups the difference between youth and those over 40 has only been falling since about 2008, and the difference between young adults and those over 40 has only been falling since about 1997 and has leveled off in the last decade of the data, respectively. Finally, a moderate to strong impact of age differences on Canadian age-specific mortality series is observed over the years.

Conclusions

For each series, the overall temporal patterns of mortality rates are relatively constant throughout time and after 40 years of age. On the mortality series across time, there were some age differences. Wider application of FTSA to provide more accurate estimates in public health, demography, and age-related policy studies should be considered.

Key Words: Functional time series analysis, Age-specific mortality, functional principal component, Functional autoregressive model.

POSTER #29

SEMAPHORIN 3E downregulates MESENCHYMAL markers gene expression in human primary Epithelial cells

Huda Fatima Rajani[1], Lianyu Shan[1], Nisha Singh [2], Prashen Chelikani[2], Abdelilah S. Gounni[1*]

[1]Department of Immunology, Max Rady College of Medicine, University of Manitoba, Apotex Center 750 McDermot Ave, Winnipeg, MB R3E 0T5, Canada., [2]Department of Oral Biology, RFHS, College of Dentistry, University of Manitoba, Apotex Center 750 McDermot Ave, Winnipeg, MB R3E 0T5, Canada.

Background and Aim

Standard asthma therapies include corticosteroids, long-acting β2 agonists, and leukotriene antagonists. While these therapies effectively reduce inflammation, they fail to prevent and reverse airway remodelling.

We previously showed that bronchial epithelial cells are significant producers of Semaphorin3E (Sema3E) in the airways, and its expression is downregulated following allergen challenge in mice and in bronchial biopsies of severe asthmatics compared to healthy donors. Epithelial-mesenchymal transition (EMT) is considered a major mechanism of remodelling in asthma, which is unresponsive to standard asthma treatment. In this study, we aim to investigate the role of Sema3E in reprogramming primary human epithelial cells associated with EMT.

Methods

Human primary bronchial epithelial cells (BEC) (N=3) obtained from healthy controls were grown under an air-liquid interface (ALI). A549 cells were subjected to submerged culture. ALI-cultured BEC and A549 were stimulated with house dust mite (HDM) extract, IL-13, TGF-β, Sema3E, time-dependent. RNA was obtained to evaluate the expressions of E-cadherin, TWIST, SNAI, vimentin, SMAD and ZEB1.

Results

TGF-β significantly upregulates the expression of Sema3E in immortalized lung epithelial cells, A549 and primary BEC. At different time points, we report an increase in the expression of mesenchymal markers (SNAII, TWIST, Vimentin, ZEB1 and SMADII) with TGF-β, IL-13 and HDM treatment, whereas Sema3E reduced the expression of these markers. Additionally, Sema3E with TGF-β and HDM upregulates expression of E-cadherin.

Conclusion

Our data indicate that Sema3E is a critical factor regulating EMT markers in the airways. TGF-β induced Sema3E expression mediates EMT BECs via a negative autocrine loop.

POSTER #1

Studying the relationship between prenatal exposure to maternal diabetes and sensitivity to cigarette smoke in adulthood in mice.

Sriyani Ranatunga[1,2], Dina H.D. Mostafa [1,2], Sujata Basu [1,2], Shana Kahnamoui[1,2], Chinonye D. Onuzulu[3], Jignesh Vaghasiya [1,2], Meaghan J. Jones [3], Christopher D. Pascoe [1,2]

[1]Department of Physiology and Pathophysiology, University of Manitoba, [2]Biology of Breathing Group, The Children's Hospital Research Institute of Manitoba, [3]Department of Biochemistry and Medical Genetics, University of Manitoba

Introduction

Chronic obstructive pulmonary disease (COPD) is **the fifth leading cause of death in Canada**. COPD is primarily caused by smoking, but early-life exposure to environmental factors may also increase COPD risk by impairing lung development and altering response to cigarette smoke (CS). Prenatal diabetes increases the risk for premature birth and childhood asthma, which are risk factors for COPD. However, whether prenatal diabetes directly impacts offspring susceptibility towards COPD remains unexplored. Using a mouse model, I hypothesize that exposure to prenatal diabetes will worsen CS induced lung dysfunction and inflammation in offspring, information that might suggest changes to COPD risk.

Methods

Six-week-old C57BL/6NJ female mice were fed with a high-fat diet (HFD-45% kcal) to induce diabetes or low-fat (LFD-10% kcal) control-diet for 6-weeks. Diets continued throughout pregnancy and weaning. Weaned offspring were fed with standard research chow-diet until 8-weeks of age, at which point they were exposed to CS/Room air for 50 mins, twice/day, for four days. Lung function and cell counts (flow-cytometry) were assessed on day five. Data analyzed in Prism GraphPad using two-way ANOVA.

Results

Offspring from HFD-exposed mothers were significantly heavier at 3-weeks (male:14.84±0.74 g; female:13.31±0.52 g) than controls (male:11.51±0.19 g; female:10.77±0.25 g). At 8 weeks of age the body weight difference maintained only in males (offspring from HFD 25.28±0.42; control 23.82±0.54), but not in females. CS exposure significantly increased total lung resistance (14%) and airway resistance (18%) in male offspring from HFD dams compared to control. HFD influenced CS induced immune cell infiltration in a sex-specific manner. Female HFD offspring exposed to CS had significantly decreased neutrophils (-61%) and $CD4^+$ T-cells (-81%) compared to control smokers. Interestingly male HFD offspring exposed to CS had significantly elevated B-cells (+190%) compared to control smokers.

Conclusion

Prenatal exposure to HFD alters offspring susceptibility towards CS-induced lung dysfunction and inflammation, in a sex-specific manner. HFD may modulate response to cigarettes in early adulthood, suggesting a propensity to future COPD. Future experiments will explore how prenatal exposure to HFD changes DNA methylation and expression of genes in the IGF1 and Polycyclic Aromatic Hydrocarbon metabolism pathways, which have been connected to COPD pathogenesis.

POSTER #91

A Quantitative Analysis of MR Image Quality Using a Receive-Only Volume-type Wireless Coil for Alzheimer's Research

Yujie Ren[1], Haoqin Zhu[2], Yuanyuan Chen[2], Gong Zhang[3], Dr. Melanie Martin[3].

[1]The University of Winnipeg, Department of Biology. 2. Sino-Canada Health Institute (SCHI). 3. The University of Winnipeg, Department of Physics.

Introduction

Magnetic Resonance Imaging (MRI) is playing an increasing role in Alzheimer's disease (AD) research, offering non-invasive and detailed visualization of brain structure and function to detect associated abnormalities. Specialized RF coils improve signal-to-noise ratio (SNR) in hippocampal imaging[1] and could help improve image quality for AD. Inductive RF coils offer a cost-effective, streamlined approach for wireless RF coils in MRI, enhancing patient comfort by eliminating bulky components[2-5]. Our study quantitatively analyzed MRI image quality (SNR) using the Litzcage[6] design inductive wireless volume coil compared to a commercial wired coil for 1.5 T head imaging.

Methods

A 5.3L Siemens phantom was imaged using a GRE sequence on a 1.5T Siemens Sempra system. Three scenarios were investigated: (1) using only the body coil; (2) using the body coil with the proposed Detunable wireless Litzcage coil (SCHI); and (3) using a 12-element commercial local receive coil array (Siemens). Tuning, matching, and field shimming were completed. Images were collected using the same parameters. Regions of interest (ROIs) were drawn in the center position of the phantom, and corresponding identical ROIs were drawn in the noise region of the images to calculate the SNR.

Results

For the commercial wired coil, the SNR at the distal end of the birdcage was only 44% of the value at the coil's center. With the proposed wireless coil, this value improved to 87%. The wireless coil enhances SNR by approximately 3.9 times across all phantom areas compared with using the body coil alone. Compared with the wired commercial coil, the wireless coil demonstrates an approximately 12% increase in SNR in the central area.

Conclusion

The wireless Litzcage coil offers better SNR compared to a commercial wired coil. Quantitative testing can be extended to more complex samples such as fruits or human brains. This coil technology can assist with brain research and clinical diagnosis requiring high image quality.

Acknowledgements

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POSTER #119

Oral Fluids for the Early Detection of Classical Swine Fever in Commercial Level Pig Pens

Erin Robert[1,2,†], Kalhari Goonewardene[1,†], Ian El Kanoa[1], Orie Hochman[1], Charles Nfon[1], and Aruna Ambagala[1,2,3,4,*]

[1]Canadian Food Inspection Agency, National Centre for Foreign Animal Disease, Winnipeg, MB, R3E 3M4, Canada

[2]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, MB, R3E 0W2, Canada

[3]Department of Animal Science, University of Manitoba, Winnipeg, MB R3E 2N2, Canada

[4]Department of Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB T2N 4Z6, Canada

*Author to whom correspondence should be addressed.

[†]These authors contributed equally to this work.

Introduction

The early detection of classical swine fever (CSF) remains a key challenge, especially when outbreaks are caused by moderate and low-virulent CSF virus (CSFV) strains. Oral fluid is a reliable and cost-effective sample type that is regularly surveilled for endemic diseases in commercial pig herds in North America. Here, we explored the possibility of utilizing oral fluids for the early detection of CSFV incursions in commercial-size pig pens using two independent experiments.

Methods

In the first experiment, a seeder pig infected with the moderately-virulent CSFV Pinillos strain was used, and in the second experiment, a seeder pig infected with the highly-virulent CSFV Koslov strain was used. Pen-based oral fluid samples were collected daily and individual samples (whole blood, swabs) every other day. All samples were tested by a CSFV-specific real-time RT-PCR assay.

Results

CSFV genomic material was detected in oral fluids on the seventh and fourth day post-introduction of the seeder pig into the pen, in the first and second experiments, respectively. In both experiments, oral fluids tested positive before the contact pigs developed viremia, and with no apparent sick pigs in the pen.

Conclusion

These results indicate that pen-based oral fluids are a reliable and convenient sample type for the early detection of CSF, and therefore, can be used to supplement the ongoing CSF surveillance activities in North America.

POSTER #19

Characterizing the Neuro-molecular and Neuro-anatomical Landscape of the Brain in Rett Syndrome Mouse Models

Chris-Tiann Roberts[1], Nicole Raabe[1], Carl O. Olson[1], Khatereh Saei Arezoumand[1], Ghanan Bin Akhtar[1], and Mojgan Rastegar[1]

[1]Department of Biochemistry and Medical Genetics, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba

Introduction

Mutations in the X-linked methyl-CpG-binding protein 2 (*MECP2*) gene are responsible for Rett Syndrome (RTT), an early-onset, debilitating neurodevelopmental disorder with no known cure and limited therapeutic strategies. Despite extensive use of RTT mouse models, a comprehensive neuro-molecular and neuro-anatomical characterization of these mouse models has yet to be established. Research has implied that variable neuronal morphology observed in mouse models of loss-, partial loss-, and gain-of-function mutations in the *Mecp2* gene depends on cell type, age, and specific *Mecp2*/MeCP2 mutation. A key objective is to determine whether similar findings are observed in the male and female RTT mouse models which recapitulate loss-of-function and nonsense mutations in *MECP2* gene.

Methods

Using two mutations of T158M (missense) and R255X (nonsense) transgenic RTT knock in mouse models, we assessed gross neuro-anatomical and neuro-molecular changes at onset of RTT-like symptoms. To investigate gross neuro-anatomical changes, post-dissection measurements of brain weight, length, and volume of male and female RTT mice were statistically compared to their age- and sex-matched, wild-type controls. To investigate the neuro-molecular landscape in the brain, semi-quantitative analyses were performed by Western blotting with total cell protein extracts from both male and female RTT mouse models. Protein extracts were probed for MeCP2, selected synaptic proteins and cell signaling pathways in dissected brain regions of the murine brain.

Results

Compared to age- and sex-matched controls, the mean brain weight of both T158M and R255X male and female mice was significantly reduced. Similarly, we observed reductions in the brain volume of both T158M and R255X male mice. However, the brain length of male and female mice was not similarly impacted in either mouse model. Western blot analyses in different brain regions of T158M mice suggested significant differences in the protein expression profile of RTT male and female mice compared to controls. Our future studies will focus on the impact of specific drugs on the neuro-anatomical and neuro-molecular characteristics of RTT mice through pre-clinical studies.

Conclusions

Taken together, our results demonstrate that statistically significant differences exist in the neuro-molecular and neuro-anatomical landscape RTT mouse models and provide a basis for development of therapeutic strategies.

POSTER #102

Is Empagliflozin Equivalent and/or Synergistic with Ace Inhibition in the Prevention of Chemotherapy Mediated Cardiotoxicity?

Tim Rozovsky,[1] Sara M. Telles-Langdon,[1] David Y. C. Cheung,[1] Allison Ledingham,[1] James A. Thliveris,[2] Danielle Desautels,[3] Joerg Herrmann,[4] and Davinder S. Jassal [1,2,3]

[1]Department of Physiology and Pathophysiology, University of Manitoba, Manitoba, Canada
[2]Department of Human Anatomy and Cell Sciences, University of Manitoba, Manitoba, Canada
[3]Department of Internal Medicine, University of Manitoba, Manitoba, Canada
[4]Department of Cardiovascular Medicine, Mayo Clinic, Rochester, Minnesota, USA

Introduction

Breast cancer is a major health problem in Canada. Doxorubicin and Trastuzumab (DOX+TRZ) are two of the most common anti-cancer drugs used in the treatment of breast cancer. While these two anti-cancer drugs improve overall survival in women with breast cancer, they increase the risk of developing heart failure. As a novel anti-diabetic medication, several randomized controlled trials have demonstrated that sodium-glucose co-transporter 2 (SGLT2) inhibitors, including Empagliflozin (EMPA), reduce the risk of heart failure associated hospitalization and mortality in patients with and without diabetes. Little is known, however, about whether SGLT2 inhibitors are cardioprotective in the setting of chemotherapy mediated cardiotoxicity.

Methods

A total of 75 C57Bl/6 female mice were used for the chronic *in vivo* murine model of chemotherapy mediated cardiotoxicity. Mice received prophylactic treatment with EMPA (10 mg/kg), Perindopril (PER) (3 mg/kg), or EMPA+PER orally for a total of 3 weeks as a run-in period prior to weekly administration of DOX+TRZ (8mg/kg and 3mg/kg, respectively) intraperitoneally for an additional 3 weeks (total of 6 weeks). Serial echocardiography was performed on a weekly basis and at the end of week 6, the mice were euthanized for histological and biochemical analyses.

Results

In mice treated with DOX+TRZ, the left ventricular ejection fraction (LVEF) decreased from 75±3% at baseline to 41±4% at week 6. Prophylactic treatment with either PER, EMPA, or EMPA+PER improved LVEF to 57±3%, 66±3%, and 68±4%, respectively ($P<0.05$). Histological analyses confirmed significant disruption of myofibrils, vacuolization, and loss of sarcomere integrity in the DOX+TRZ treated mice. Prophylactic administration with PER, EMPA, or EMPA+PER, however, improved myofibril integrity at week 6 in mice receiving DOX+TRZ.

Conclusion

In a chronic *in vivo* murine model of DOX+TRZ induced cardiotoxicity, the prophylactic administration of EMPA or EMPA+PER was superior to PER alone in preventing adverse cardiovascular remodeling.

POSTER #168

Aberrant Ubiquitin Regulation Drives Chromosome Instability and Cellular Transformation

Kailee A. Rutherford[1,2], Amy Johnson[1], Cindy Atayan[1], Tooba Razi[1], Mirka Sliwowski[1], Zelda Lichtensztejn[1] and Kirk J. McManus[1,2].

[1]Paul Albrechtsen Research Institute CancerCare Manitoba, Winnipeg, MB
[2]Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MB

Introduction

Despite improved screening efforts, colorectal cancer (CRC) remains the second-highest cause of cancer-related deaths in Canada. A deeper understanding of the etiological events driving CRC development will be critical to identify novel therapeutic targets to improve CRC patient outcomes. Chromosome instability (CIN) is defined as an increased rate at which whole chromosomes or fragments thereof are gained or lost, and is an enabling hallmark of CRC; however, the genetic determinants of CIN (*i.e.*, CIN genes) remain largely unknown. In this regard, preliminary data suggest ubiquitylation and deubiquitylation genes are essential for chromosome stability. Accordingly, this study evaluated reduced ubiquitination and deubiquitination gene expression for roles in CIN and early CRC development.

Methods

Our established quantitative imaging microscopy platform was coupled with siRNA-based silencing to assess ubiquitination (582) and deubiquitination (94) genes for impacts on CIN in colonic cells. Changes in nuclear areas and micronucleus formation were assessed, and the top 10 genes were prioritized for further evaluation, including *USP4*. Conceptually, changes in nuclear areas are associated with large-scale changes in DNA content (*i.e.*, ploidy), while micronuclei often arise from chromosome missegregation and DNA double-strand breaks. A CRISPR/Cas9 approach was additionally employed in A1309 (non-malignant, colonic) cells to generate *USP4*-knockout clones. *USP4*-knockout clones were subsequently assessed for changes in CIN phenotypes and evaluated for potential impacts on proliferation (Cell Titre-Glo) and anchorage-independent growth (soft agar colony formation assay).

Results

The siRNA-based screen identified 633 putative CIN genes (*i.e.*, 541 ubiquitylation and 92 deubiquitylation genes). Heterozygous ($USP4^{+/-}$-1 and -2) and homozygous ($USP4^{-/-}$-A and -B) knockout clones were generated and exhibit dynamic changes in nuclear areas, micronucleus formation and chromosome numbers over time (*i.e.*, CIN), as well as cellular transformation phenotypes.

Conclusion

These data provide novel insight into the role ubiquitin has in chromosome stability maintenance and identify *USP4* loss as a novel driver of CIN and cellular transformation in a colonic context. This work contributes to expanding our understanding of CIN and early CRC development, which will be critical for the development of more effective therapies to improve patient outcomes.

POSTER #17

Quasimetagenomic Approach to Subtyping Priority Pathogens in Foods for Outbreak Detection and Response.

Jillian Rumore[1,2], Matthew Walker[2], Franco Pagotto[3], Jessica D Forbes[4], Christy-Lynn Peterson[2], Andrea D Tyler[2], Morag Graham[2,1], Gary Van Domselaar[2,1], Celine Nadon[2,1], Aleisha Reimer[2], Natalie Knox[2,1]

[1]University of Manitoba, Department of Medical Microbiology and Infectious Diseases, Winnipeg, Manitoba, Canada; [2]Public Health Agency of Canada, National Microbiology Laboratory, Winnipeg, Manitoba, Canada; [3]Health Canada, Food Directorate, Bureau of Microbial Hazards, Ottawa, Ontario, Canada; [4]Eastern Ontario Regional Laboratory Association, Ottawa, Ontario, Canada.

Introduction

Current protocols for detection of priority pathogens in foods is not conducive to rapid pathogen detection. Direct sequencing of primary selective enrichment cultures (quasimetagenomics) may provide a more rapid solution to expedite pathogen detection. This study aimed to optimize protocols and analysis tools currently used for Canada's foodborne pathogen (FBP) surveillance to develop an end-to-end quasimetagenomics-based workflow for rapid food pathogen detection and characterization.

Methods

Pathogen-food combinations previously implicated in outbreaks, including *Shigella sonnei* (carrots), *Listeria monocytogenes* (ready-to-eat; RTE ham, turkey, and roast beef deli meat) and *Escherichia coli* O157:H7 (lettuce, ground beef) were artificially created with outbreak and non-outbreak strains. Foods were homogenized in pathogen-specific enrichment broths, confirmed to be pathogen-negative and then spiked at three levels categorized as low (10^1 CFU/ml), medium (10^2 CFU/ml) and high (10^3 CFU/ml). *Listeria* primary enrichments were incubated for 28 hours while *E. coli* and *Shigella* primary enrichments were incubated for 24 hours. DNA was extracted with and without a saponin-based eukaryotic DNA depletion step, and prepared libraries were sequenced on an Illumina NextSeq 550 instrument. Low-quality reads (fastp) and eukaryotic sequences (bowtie2) were removed, followed by taxonomic classification using KrakenUniq and a customized reference database. Pathogen-specific reads identified by KrakenUniq were extracted, assessed for quality and then investigated for outbreak detection using whole-genome multilocus sequence typing (wgMLST) and the single nucleotide variant phylogenomics (SNVPhyl) pipeline.

Results

Saponin-based eukaryotic DNA depletion did not significantly improve the pathogen signal ($p>0.05$); the FBP of interest was detected across all datasets and spike-in levels and demonstrated quality metrics comparable to the sequenced reference strains. All extracted pathogen-specific reads clustered by few genomic differences with outbreak-related strains (≤12 SNVs; ≤14 wgMLST alleles) and by many genomic differences from unrelated strains (>100 SNVs; >100 wgMLST alleles). The number of genomic differences identified for each outbreak was identical or within a single SNV / wgMLST allele to the corresponding previously sequenced single isolate outbreak strains.

Conclusion

Quasimetagenomics can be applied for detection and subtyping of priority pathogens in foods and can seemingly identify clusters of contaminated foods more rapidly than current methods, thereby reducing the number of foodborne illnesses and outbreaks.

POSTER #13

The role of FDC-SP in mucosal tissue B cell expansion and IgA/G production in Ulcerative colitis

Milad Sabzevary-Ghahfarokhi[1], Aaron Marshall[1], Jean-Eric Ghia[1], Sen Hou[1]

[1]Department of Immunology, Rady Faculty of Health Science, Max Rady College of Medicine, University of Manitoba

Introduction

The infiltration of B cells into the lamina propria and the elevated release of IgG antibodies in the colon can exacerbate the inflammatory response in ulcerative colitis (UC). Previous studies have demonstrated an up-regulation of Follicular Dendritic Cell-Secreted Protein (FDC-SP) in a specific subset of fibroblastic reticular cells in UC patient colon tissue. In a prior investigation, we observed that mice deficient in *fdc-sp* gene exhibited lower levels of serum IgG and higher levels of both serum and intestinal lavage IgA. However, the role of FDC-SP in B cell function and antibody production in UC remains unclear.

Methodology

Using FDC-SP transgenic and littermate control mice, we determined the impact of FDC-SP over-expression in a model of colon inflammation. Colon inflammation was induced by administration of 2.5% (w/v) dextran sulfate sodium (DSS) dissolved in water for 6 days followed by 9 days of recovery time. The Disease Activity Index (DAI) was monitored daily based on weight loss, stool consistency, and blood in the feces index. The localization and phenotype of B cells were evaluated by immunofluorescence (IF) and flow cytometry. We examined the levels of IgA and IgG antibodies in colon tissue, colon lumen and plasma by ELISA.

Results

Our preliminary data revealed an abnormal accumulation of B220+ IgD+ IgA- B cells in the lamina propria of mice with colitis. The accumulated B cells appear to impede the induction of epithelial stem cells marked by LRG5+ in the crypts. We further observed a marked B cell expansion and reduced IgA switching in mesenteric lymph nodes. FDC-SP transgenic mice exhibited an increased IgG1/IgA antibody ratio in serum compared to the control group; however, they did not show significant change in DAI or immune cell infiltration.

Conclusion

These findings suggest that the accumulated non-switched B cells may interfere with the healing process in colitis mice. Mesenteric lymph nodes may represent a significant site for the B cell activation and generation of pathogenic IgG-producing cells. Moreover, FDC-SP overexpression induces IgG production in the colitis mouse model.

POSTER #22

The Metformin-mediated Sex-specific and Brain region-dependent impact on MeCP2-BDNF homeostasis

Khatereh Saei Arezoumand[1], and Mojgan Rastegar1

[1]Department of Biochemistry and Medical Genetics, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, MB, Canada

Introduction

Rett Syndrome (RTT) is a severe, postnatal, and X-linked neurodevelopmental disorder without any cure. After a normal period of development, by 6-18 months of age, symptoms appear including developmental regression, loss of acquired skills, and breathing abnormalities, accompanied by neurological deficiencies, and mental disability. In addition, RTT is linked with metabolic abnormalities including impairment in glucose metabolism. An anti-diabetic drug (metformin) has been suggested as a potential therapy in the context of neurodevelopmental disorders such as RTT due to its low side effects, and permeability through the Blood-brain barrier. Earlier, we reported a metformin-mediated induction of *MECP2E1* transcripts (dominant isoform in RTT) in a human brain cell line. Here, we report the molecular effect of metformin in different brain regions of the brain in male and female mice.

Methods

Male and female mice received daily intraperitoneal (IP) injections of the vehicle or metformin for 3 weeks at 200mg/kg concentration per day. At the end of 21 days of treatment, mice tissues were collected for molecular analysis. Then, total protein extraction and quantification of the murine brain and liver tissues were completed, and extracts were subjected to Western blot using different primary and secondary antibodies.

Results

Analysis of the murine tissues included 4 specific brain regions that were examined by immunoblotting. The results demonstrated that metformin induced the MeCP2 protein levels in the hippocampus of male mice, with differential impact on the level of BDNF precursors (pre-proBDNF and pro-BDNF) in the hippocampus of female mice in a sex-dependent manner. Examination of thalamus, cerebellum, and frontal cortex confirmed a sex-specific and brain region-dependent regulatory role for metformin on MeCP2-BDNF homeostasis. Our results provide a solid foundation for metformin future pre-clinical studies for RTT and similar diseases.

Conclusion

The present study offers a new vision regarding the multiple effects of metformin at the protein level by providing insight into the sex- and brain region-dependent molecular impact of metformin in mice.

POSTER #111

Abstract Title: The association of blood neurofilament light chain protein with multiple sclerosis and comorbid depression

Mira Safa[1], Ruth Ann Marrie[1], Ronak Patel[1], Ronald Booth[2], John D. Fisk[3], Charles N. Bernstein[1], Kaarina Kowalec[1]

[1]University of Manitoba, Canada, [2]University of Ottawa, [3]Dalhousie University, Canada

Introduction

Multiple sclerosis (MS) is a chronic neurodegenerative disease. Elevated levels of serum neurofilament (sNfL) are a hallmark of axonal loss and are associated with prognosis and treatment response in MS. Neurofilament levels are also elevated in those with depression. Understanding whether sNfL levels are synergistically elevated in MS and comorbid depression compared with controls will provide insight into disease pathology.

Methods

Data will be taken from a prospective 3-year Manitoba longitudinal project estimating the burden of psychiatric comorbidities in MS. Collected data included sociodemographic characteristics, BMI, psychiatric disease measures, MS disease activity, and blood samples. All measures were assessed at the baseline study visit and then annually for 3-years. We will compare sNfL levels cross-sectionally using linear regression with consideration of BMI, age, depression scales, treatment regimen, and disease duration. For the longitudinal analyses, we will use linear regression where sNfL levels are the outcome, and disease group is included as a covariate, along with the other covariables mentioned.

Results

We included 529 participants (113 MS-depression, 142 MS no depression, 170 depression no MS and 104 healthy). There was a preponderance of females across all participant groups, with an average age of 46.8 years. Further analysis is underway.

Conclusion

People with MS are at higher risk for developing depression compared to the general population. By studying the association of sNfL in MS with comorbid psychiatric conditions, we will gain insights into the pathophysiology of comorbidities.

POSTER #132

Treatment and Prevention of Transplant Vasculopathy using immunoengineered Ti3C2Tx MXene Nanosystem

Leena Regi Saleth[1], Alireza Rafieerad[1], Weiang Yan[1], Keshav N. Alagarsamy[1], Abhay Srivastava[1], Niketa Sareen[1], Sanjiv Dhingra[1]

[1]Institute of Cardiovascular Sciences, Albrechtsen St. Boniface Research Centre, Department of Physiology & Pathophysiology, Rady Faculty of Health Sciences, University of Manitoba, MB R3E 0W2, Winnipeg, Canada.

Introduction

The long-term survival of heart transplant recipients is limited by cardiac allograft vasculopathy (CAV), an aggressive form of atherosclerosis. The pathogenesis of CAV occurs via non-immune and immune-mediated remodeling of the transplanted cardiac vasculature, with inflammation playing a crucial role in the development of CAV. Current treatment strategies for CAV are largely ineffective, and novel bioengineering approaches may hold the key to leverage this disease burden. Hence, this study reports the immunoengineering of titanium carbide ($Ti_3C_2T_x$) MXene-based nanosystem for treatment of transplant vasculopathy. We postulated that this biocompatible $Ti_3C_2T_x$ MXene nanosystem overcomes concerns relating to long-term safety and subsequent clinical translation.

Methods

Colloidal suspension of layered $Ti_3C_2T_x$ MXene nanosheets were prepared and subjected to in-depth microstructural characterization, and its intrinsic anti-inflammatory properties were implored. Upon testing the biocompatibility of the synthesized MXene, the *in vitro* immunomodulation of MXene nanosheets was assessed using flow cytometry, quantitative PCR and western blot. Following this, bulk RNA-Seq analysis and gene set enrichment analysis were performed on MXene treated co-cultured allogenic lymphocytes. Finally, the immunomodulatory effects of MXene nanosheets were tested in vivo using a rat model of allograft vasculopathy.

Results

The detailed physiochemical characterization of the MXene nanosheets showed that the nanosheets were 2- 5 µm in size and were surface-enriched with biologically active functional groups. This compositional flexibility of MXene aided in enhanced material-cell interactions. The MXene nanosheets were selectively and spontaneously uptaken into antigen-presenting endothelial cells, altering their gene expression and reducing the activation of allogeneic T-lymphocytes. Bulk RNA-Seq analysis of MXene treated lymphocytes showed decreased immunological signatures responsible for transplant-induced T-cell activation, cell-mediated rejection, and development of allograft vasculopathy. Further, gene set enrichment analysis revealed significant negative enrichment of genes involved in interferon-alpha/beta and interferon-gamma signaling. Finally, *in vivo* testing showed that the nanosystem displayed strong immunomodulatory functions to effectively ameliorate the cellular and structural changes of early allograft vasculopathy.

Conclusion

These findings emphasize the potential of $Ti_3C_2T_x$ MXenes in effective treatment of cardiac allograft vasculopathy. This supports the future development of MXene based novel platforms for prevention of allograft rejection in the heart and other organs.

POSTER #7

Radiology in the UGME Curriculum: 4th year Students' Perspectives

Eola Saltibus[1], Alexa Hryniuk[1]

[1]Department of Human Anatomy and Cell Science, Rady Faculty of Health Sciences, University of Manitoba

Introduction

Radiology is central to healthcare and intersects almost every medical specialty. Recently, there has been a general decrease in the teaching time devoted to radiology in the undergraduate medical education (UGME) curriculum which, consequently, underprepares students for residency and future practice. The aim of this study is to determine 4th year students' experiences and perspectives of radiology in the UGME curriculum with emphasis on any existing gaps in radiology curriculum delivery.

Methods

A 24-question survey related to radiology education was given to medical students (n = 110) in the final 4 months of their fourth year at Max Rady of College. The survey was hosted through survey monkey and disseminated among students through email and QR codes. Likert-type (1-4) and open-ended questions were utilized to gather student feedback regarding their confidence levels in diagnostic image interpretation and their perceptions of radiology integration in the curriculum. The resulting data was coded and analyzed using basic statistics (frequencies, mean, percentages and confidence intervals) while general themes were highlighted for associated free-response questions.

Results

Twenty-seven students have participated in the study thus far. Students felt slightly confident in their ability to order appropriate diagnostic imaging tests for patients (54%). Most participants (46%) were very confident in their ability to educate patients concerning the risks associated with imaging tests. Students were slightly confident in their ability to interpret diagnostic images, but confidence levels differed based on body regions. Participants indicated that there should be more opportunities for general interpretation of diagnostic images with a specific focus on ultrasound. Participants also recommended more exposure to radiology throughout the UGME curriculum.

Conclusion

Medical students recognize the significance of learning radiological interpretation skills in the medical education curriculum and its relationship to future clinical practice. Curricula improvements should focus on the integration of students' perspectives to address the gaps and deficiencies in content delivery. Adequate exposure to radiology ensures that students are equipped with the pertinent skills to be informed and confident in their ability to deliver optimal patient care.

POSTER #88

Longitudinal Preclinical Study with Novel Edaravone Analogue (NS-1-2) Delayed Disease Onset, Extended Life Span and Prevents ALS-Induced Cachexia in a SOD1 Linked Model of Amyotrophic Lateral Sclerosis

Nitesh Sanghai[1], Geoffrey K. Tranmer[1]

[1]College of Pharmacy, Rady Faculty of Health Science, University of Manitoba, Winnipeg, MB R3E 0T5, Canada.

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive motor neurodegenerative disease of the brain and spinal cord, resulting in severe weakness and death due to respiratory failure within 2-5 years of first diagnosis. One of the known causes of ALS is a mutation in the SOD1 gene, which acquires a toxic gain of function due to a change in redox disturbance caused by reactive oxygen species. The abnormal misfolding aggregates of SOD1 play a role in the pathogenesis of ALS. Currently, more than 200,000 people around the world have been living with ALS, yet there is no cure available. Since, the discovery of ALS in 1869, only two fully FDA-approved drugs, Edaravone an antioxidant, and Riluzole, an antiglutamatergic are available, with minimal effects on disease course. Further, preclinical studies of both these drugs in SOD1 ALS mice models have not shown any significant survival benefits. Therefore, our overall goal is to develop a novel Edaravone analogue (NS-1-2), that could slow down the progression of ALS.

Methodology

We have designed an anti-neurodegenerative agent and utilized a novel 'green' microwave synthetic approach to synthesize NS-1-2. Further, we evaluated the safety profile and neuroprotective potential of NS-1-2 in vitro, using spinal cord motor neuronal cells (NSC-34 cells and primary cortical neuronal cells). We performed randomized, longitudinal studies with aged-matched littermates to evaluate the (acute toxicity, chronic toxicity, and efficacy of the NS-1-2) *in vivo*. We used humanized mutant G37R mice line 42, an aggressive ALS mice model with early onset and fast progression of disease. All animal experiments for this study followed **(protocol Ref # 21-014** (AC11693) approved by the CACS, (UofM).

Results

We synthesized EDR analogue NS-1-2, utilizing a novel green synthetic procedure. Single and 120 doses daily of NS-1-2 (10mg/kg body weight) demonstrated no signs of treatment-associated toxicity in tissues of wild-type G37R mice. Further, presymptomatic treatment of NS-1-2 (10mg/kg body weight) in mutant G37R mice demonstrated significant efficacy in modifying clinical disease phenotypes in terms of delaying onset, extension of life span, and preventing ALS-induced weight loss a marker for ALS disease progression.

Conclusion

A novel anti-neurodegenerative agent (NS-1-2) demonstrated significant preclinical efficacy in the humanized SOD1 mice models of ALS, validating the therapeutic potential of a novel scaffold (NS-1-2) for slowing down the progression of incurable ALS. Further, with these intriguing results, we filed a patent with the support of the (UofM) Technology Transfer Office.

POSTER #4

Role of DOT1L in epigenetic dysregulation of the HOXA9 gene expression in mixed lineage leukemia

Hedieh Sattarifard[1], James R. Davie[1], Ted M. Lakowski[2]

[1] Department of Biochemistry and Medical Genetics, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, MB, Canada, [2] College of Pharmacy Pharmaceutical Analysis Laboratory, University of Manitoba, Winnipeg, MB R3E 0V9, Canada

Background

Mixed lineage leukemia (MLL) is characterized by the presence of KMT2A-MLLT3 fusion proteins resulting from chromosomal translocations affecting the *KMT2A* gene at 11q23 and *MLLT3* at chromosome 9. KMT2A-MLLT3 is a transcription factor that aberrantly recruits the DOT1L enzyme to the *HOXA9* gene, inducing ectopic expression of this gene. Dysregulation of *HOXA9* is a prominent feature in most aggressive mixed lineage leukemia cases. My research project aims to identify the factors responsible for the higher activity and targeting of DOT1L to *HOXA9*, which leads to robust transcription of *HOXA9* in MLL. Histone H2B monoubiquitination at Lys 120 (H2Bub1), a major histone post-translational modification involved in gene expression elongation, has a direct effect on DOT1L, increasing its activity. This study is designed to assess the level of H2Bub1 and subsequently target it using proteasome inhibitors. By reducing H2Bub1 levels, we aim to observe the effects on DOT1L activity both globally and specifically along the *HOXA9* gene in MLL cell lines. By understanding these factors, it is possible to synthesize inhibitors that can effectively target the *HOXA9* gene with minimal side effects.

Materials and Methods

Two cell lines, MOLM-13 (MLL) and K562 (chronic myeloid leukemia (CML)) will be used. In this study, we will employ methods and techniques including, ChIP sequence data and RNA-Seq data analysis, RT-qPCR, acid extraction for histones, SDS immunoblotting techniques, Bradford assay, and inhibitor treatments.

Results

RNA-Seq data analyses showed that *HOXA9* had the highest transcript level in the *HOXA* cluster in MOLM-13 cells (*HOXA9* is not expressed in the K562 cells). Furthermore, ChIP-Seq data analyses showed that the *HOXA9* gene was covered by H3K79me2 and DOT1L in MOLM-13 cells. The transcript levels of *HOXA9* and *DOT1L* were assessed in our cells. Results revealed that the *HOXA9* transcript was high in MOLM-13 but undetermined (Cq > 35) in K562. *DOT1L* transcript levels were quite similar in both cell lines. Protein levels of DOT1L were consistent in both cell lines. However, the H3K79me2 modification level, a product of DOT1L, showed significant differences favouring MOLM-13. The level of H2Bub1 was higher in MOLM-13 (has KMT2A-MLLT3 and higher activity of DOT1L) compared to K562. Treatment of MOLM-13 cells with 5nM and 50nM bortezomib effectively reduced H2Bub1 and H3K79me2 levels after 4 hours, suggesting its potential to modulate DOT1L activity.

Conclusion

Our study highlights the importance of *HOXA9* in MOLM-13 cells, showing its high expression in MLL. We found that the *HOXA9* gene is associated with H3K79me2 and DOT1L, indicating the role of DOT1L and *HOXA9* in the leukemogenesis of MLL. While DOT1L protein and transcript levels were consistent in both cell lines, MOLM-13 cells exhibited higher H3K79me2 levels, suggesting context-dependent DOT1L activity regulation. Additionally, we observed elevated H2Bub1 levels in MOLM-13 cells, potentially contributing to increased DOT1L activity. Treatment with bortezomib, a proteasome inhibitor, reduced H2Bub1 and H3K79me2 levels, indicating its potential as a therapeutic strategy for conditions involving dysregulated DOT1L activity, such as MLL. These findings suggest new avenues for exploring epigenetic-based treatments.

POSTER #149

Delineating and targeting a novel metabolism-based post-translational mechanism regulating the abundance of the 'undruggable' oncoprotein c-MYC in medulloblastoma

Harshal Senthil[1], Emma Martell[1,2], Helgi Kuzmychova[1], Esha Kaul[3], Subir Roy Chowhury[4], Ludivine Coudière Morrison[5], Agnes Fresnoza[6], Jamie Zagozewski[5], Chitra Venugopal[7,8], Chris M. Anderson[9,10], Sheila K. Singh[7,8,11], Versha Banerji[4,6,12,13], Tamra E. Werbowetski-Ogilvie[5], Tanveer Sharif[1,2,4*]

[1] Pathology, Rady Faculty of Health Sciences, University of Manitoba; Winnipeg, MB, Canada. [2] Human Anatomy and Cell Science, Rady Faculty of Health Sciences, University of Manitoba; Winnipeg, MB, Canada. [3]Faculty of Science, University of Manitoba; Winnipeg, MB, Canada.[4] CancerCare Manitoba; Winnipeg, MB, Canada. [5]Biochemistry and Medical Genetics, Rady Faculty of Health Sciences, University of Manitoba; Winnipeg, MB, Canada. [6]Central Animal Care Services, University of Manitoba; Winnipeg, MB, Canada. [7]McMaster Stem Cell and Cancer Research Institute, McMaster University; Hamilton, ON, Canada. [8] Surgery, Faculty of Health Sciences, McMaster University; Hamilton, ON, Canada. [9]Neuroscience Research Program, Kleysen Institute for Advanced Medicine, Health Sciences Centre; Winnipeg, MB, Canada. [10]Pharmacology and Therapeutics, Rady Faculty of Health Sciences, University of Manitoba; Winnipeg, MB, Canada. [11]Biochemistry and Biomedical Sciences, McMaster University; Hamilton, ON, Canada. [12]Internal Medicine, Rady Faculty of Health Sciences, University of Manitoba; Winnipeg, MB, Canada. [13]Medical Oncology and Hematology, CancerCare Manitoba; Winnipeg, MB, Canada.

Introduction

Brain tumors are the leading cause of cancer death in children, and medulloblastoma (MB) is one of the most common pediatric central nervous system malignancies. Amplification of the c-MYC oncogene is frequently observed in the most aggressive and lethal subgroup of this disease, group 3 (G3), but not in other subgroups. Patients that have G3 MB tumors with high c-MYC abundance are more likely to present as metastatic and are prone to develop fatal recurrent tumors. Unfortunately, the functional ubiquity and disordered structure of c-MYC makes it difficult to target for cancer treatment. Therefore, it is critical to identify novel, out-of-the-box strategies to suppress oncogenic c-MYC in highly aggressive G3 MB brain tumors. Recently, metabolism has emerged as a major regulator of overall cellular signaling processes through post-translational and epigenetic mechanisms. While c-MYC is known to regulate cellular metabolism, whether metabolism plays a role in reciprocally supporting enhanced c-MYC abundance in cancer is unknown. We hypothesize that an intrinsic feedback mechanism may exist where metabolic activity modulates c-MYC abundance that could be exploited as a therapeutic strategy to improve outcomes for G3 MB patients.

Methods

Using various well-characterized G3 MB cells, orthotopic intracerebellar xenograft models, patient tumor bioinformatics, detailed biochemical characterization, and point-mutation analyses, we have identified a novel metabolism-dependent post-translational modification that regulates c-MYC stability.

Results

In-depth molecular analyses unveiled that c-MYC is susceptible to oxidation and proteasomal degradation under conditions of metabolic stress. Targeting mitochondrial respiration via inhibition of complex-I promotes the accumulation of reactive oxygen species (ROS) and leads to oxidation and degradation of c-MYC in G3 MB cells. Point mutation analysis combined with biochemical oxidation assays identified the specific cysteine residues of c-MYC that are susceptible to oxidation and ultimately responsible for enhanced c-MYC degradation following complex-I inhibition. Importantly, oral administration of a blood-brain barrier permeable complex-I inhibitor impaired the growth of intracerebellar MB xenograft tumors in mice, significantly prolonging animal survival.

Conclusion

Altogether, these findings unveil a novel mechanism through which metabolism regulates the post-translational stability of c-MYC and provides insights for designing rationale therapeutic strategies for the treatment of MB patients.

POSTER #73

Unraveling the role of Unfolded Protein Response signaling in NLRP3 inflammasome formation

Suraj Shaji[1] & Susan E Logue[1, 2, 3]

[1]Department of Human Anatomy and Cell Science, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg.

[2]CancerCare Manitoba Research Institute, Winnipeg, Canada

[3]Children's Hospital Research Institute Manitoba, Winnipeg, Canada

Introduction

Interleukin-1β (IL-1β) is a potent cytokine involved in inflammatory responses. Excessive IL-1β secretion is linked to Rheumatoid Arthritis, Inflammatory Bowel Disease, and certain cancers. IL-1β is produced as an inactive precursor (pro- IL-1β), which is processed into a bioactive form by caspase-1. Similar to pro-IL-1β, caspase-1 is initially produced in an inactive form (pro-caspase-1), which requires recruitment to the NLRP3 inflammasome to enable the formation of active caspase-1. Recent studies suggest that a cell stress pathway called the Unfolded Protein Response (UPR) can influence IL-1β production by regulating formation of the NLRP3 inflammasome. How UPR signaling regulates NLRP3 inflammasome formation is not understood. To address this question, we profiled UPR signaling upon NLRP3 inflammasome formation.

Methods

To study IL-1β production, processing and release, the human monocytic cell line THP-1 was treated with LPS (1 μg/ml, 24 h) followed by nigericin (10 μM, 45 min). Harvested cells were subjected to immunoblotting and qPCR to analyze levels of IL-1β and UPR proteins (IRE1, ATF6 and PERK). Supernatants were subjected to immunoblotting and ELISA to analyze and quantify IL-1β release. To study the impact of UPR signaling, pharmacological inhibitors of IRE1 (MKC8866), PERK (AMG44) and ATF6 (Ceapin A7) were utilised.

Results

THP-1 cells treated with LPS and nigericin displayed robust production, processing, and release of IL-1β, thus validating the model system. IRE1 activation, as assessed by splicing of XBP-1 mRNA, was detected in LPS/nigericin-treated cells. Combination with the IRE1 inhibitor MKC8866, blocked LPS/ nigericin-triggered IRE1 activity as shown by reduced re XBP-1s and reduced levels of IL-1β present in the conditioned medium. Assessment of the ATF6 and PERK signaling pathways in LPS/nigericin-treated THP-1 cells is ongoing.

Conclusion

Our results indicate that stimulation of pro- IL-1β production and NLRP3 inflammasome activation in THP-1 cells is associated with activation of the key UPR mediator IRE1. Inhibition of IRE1 signaling reduced levels of IL-1β in conditioned medium, verifying a role for IRE1 signaling in innate immune responses. Further work establishing how IRE1 signaling influences NLRP3 inflammasome activation is ongoing, as is the assessment of additional UPR mediators, ATF6 and PERK.

POSTER #23

Laser-assisted Interstitial Thermal Treatment (LITT) Immune Responses in a Mouse Glioma Model

Santhosh S. Anandhan[1], Jeremy Spence[1], Sabine Hombach-Klonisch[1,2], Thomas Klonisch[1-4]

[1]Human Anatomy and Cell Science, University of Manitoba,
[2]Pathology, University of Manitoba.
[3]Medical Microbiology & Infectious Diseases, University of Manitoba.
[4]CancerCare Manitoba, Winnipeg, MB, Canada.

Introduction

Brain tumours, including primary brain tumours and metastatic brain tumours, represent a significant and increasing unmet clinical need. Minimally invasive LITT is a laser cytoreductive procedure that uses heat for brain tumor tissue ablation. This laser treatment generates a heat gradient in brain tissue that coincides with heat-stress and modulates the brain microenvironment (TME), causing the induction of heat shock proteins and the release of inflammatory factors, which may activate the innate and adaptive immune system. Furthermore, laser-induced damage generates neo-antigens that may promote local and systemic anti-tumor responses. Post-LITT dynamic cellular immune responses remain poorly understood but emerge as an attractive new therapeutic target for the treatment of inoperable brain tumors.

Methods

We have established a LITT mouse model to investigate LITT effects on brain tumors. Murine glioma CT2A cells were allografted into immunocompetent C57BL/J6 mice and tumor size was monitored by T2-weighted MRI prior to LITT treatment using a fiberoptic laser device and thermocouple for temperature control on brain tissue. LITT-treated mice and sham-treated (no laser activation) groups were sacrificed 1, 5, and 10 days to study early post-LITT induced cellular modulations. Serial FFPE sections were analyzed by multiplexed immunofluorescence (mIF) for spatiotemporal changes in tumor parameters (Ki67, caspase 3) and immune cell profiles.

Results

Progression of orthotopically xenografted glioma formation and consecutive glioma targeted laser-induced hyperthermia are closely monitored by MRI. Activation of resident astrocytes and microglia at the site of LITT is an early event. The mIF analysis revealed a time-dependent accumulation of diverse myeloid phagocytic cell and T cell subpopulations at LITT ablation sites and surrounding brain tissues in tumor and normal brain.

Conclusion

The activation of resident astrocytes and microglia occurs early during LITT brain injury and is followed by an influx of macrophage and T cell subpopulations to the injury site. Our future research will characterize these immune cell subpopulations and cytokine profiles in the LITT tumor brain.

POSTER #21

Characterization of sex-related differences in a steroid-unresponsive allergen recall response model

Tiffany Shen[1], Hemshekhar Mahadevappa[2], Courtney Marshall[1,2], Anthony Altieri[1,2], Neeloffer Mookherjee[1,2]

[1] Department of Immunology, University of Manitoba, Winnipeg, MB, Canada; [2] Manitoba Centre for Proteomics and Systems Biology, Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada.

Introduction

Biological sex can impact asthma incidence, severity, and response to therapy. The impact of biological sex in neutrophilic airway inflammation, a feature of severe asthma often associated with IL-17-driven responses, is not well understood. Here, we examine sex-related differences in an allergen-recall murine model of neutrophilic airway inflammation.

Methodology

Male and female BALB/c mice were sensitized with house dust mite (HDM) extract (25µg) with or without lipopolysaccharide (LPS; 1µg) for 3 days, rested for 4 days, followed by HDM challenge for 8 consecutive days. Bronchoalveolar lavage fluid (BALF), blood, and lung tissue samples were collected 24 hours after the last HDM challenge. We characterized leukocyte accumulation in the BALF by flow cytometry, the abundance of a panel of Th17-related cytokines in BALF and lung tissue lysates by Luminex multiplex platform, and the abundance of neutrophil elastase (NE) and myeloperoxidase (MPO) in lung tissue lysates by ELISA. Sex-disaggregated data analysis was performed for all outcomes.

Results

We demonstrated that the levels of IL-17 (IL-17A and IL-17A/F), NE and MPO, and the accumulations of neutrophils, CD4+ T cell and B cells are all significantly higher in the lungs after allergen recall challenge compared to allergen-naïve mice. Allergen-driven neutrophil accumulation in the BALF and IL-17A levels in the lung tissue lysates were significantly higher in females compared to males whereas CD4+ T cells accumulation was higher in male mice compared to females.

Conclusion

Levels of IL-17 and airway inflammation are higher in females compared to males, in response to allergen recall challenge.

POSTER #79

The impact of COVID-19 public health measures on the utilization of antipsychotics in schizophrenia in Manitoba – A population-based study

Mina Shirinbakhshmasoleh[1], Laila Aboulatta[1], Christine Leong[1,2], Hayley Riel[1], Kun Liu[3], Joseph C. Delaney[1,4], James M. Bolton[3], Jamison Falk[1], Silvia Alessi-Severini[1], Sherif Eltonsy[1,5], Kaarina Kowalec[1,6]

[1]College of Pharmacy, University of Manitoba, Winnipeg, Canada
[2]Department of Psychiatry, College of Medicine, University of Manitoba, Winnipeg, Canada
[3]Manitoba Center for Health Policy, Winnipeg, Canada
[4]Department of Epidemiology, University of Washington, Seattle, USA
[5]Children's Hospital Research Institute of Manitoba
[6]Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden

Introduction

During the COVID-19 pandemic, public health measures were implemented to reduce the spread of infection, yet it is unknown whether these measures affected medication access to those with schizophrenia (SCZ). This study aimed to assess whether the antipsychotic utilization rates in SCZ changed before and during the pandemic.

Methods

We used dispensed prescription drug data from the Canadian province of Manitoba in those with SCZ. The quarterly incident and prevalent dispensation of antipsychotics at two time periods (April-June 2020 and 2021) were compared with the expected trend from the previous five years using linear autoregression. We stratified the primary results by age and sex and examined multiple subgroups.

Results

There were 9,045 individuals with SCZ in the first fiscal quarter of 2020. The prevalent use of antipsychotics remained stable during the pandemic compared with the expected trend (2020 and 2021, both $p>0.05$). A significant decrease in the incident use of antipsychotics in April-June 2020 (estimate: -1.3, $p=0.01$) was noted compared with the expected trend. A significantly higher incidence of atypical antipsychotics (including risperidone) ($p=0.04$) and risperidone ($p=0.03$) was noted in the same period in 2021 compared with the expected trend.

Conclusion

This study found a decline in the receipt of antipsychotics for patients with SCZ during the initial implementation of COVID-19 public health measures, particularly in incidence overall. Future work on investigating the impact of these trends on SCZ outcomes is needed to help inform future pandemic-related policies.

POSTER #124

High level of short-chain fatty acids (SCFAs) impairs the barrier function of cervicovaginal epithelial cells, independent of the pro-inflammatory cytokines/chemokines.

Abu Bakar Siddik[1,4], Vanessa Poliquin[2,3], Jennifer Chan[3], Robert John Lotocki[2], Erica Labossiere[4], Suzanne Gibbons[4], Alberto Severini[1,4], T. Blake Ball[1,4], Paul Sandstrom[1,4], Ruey-Chyi Su[1,4]

[1]Dept. Medical Microbiology & Infectious Diseases, University of Manitoba.
[2]Dept. Obstetrics, Gynecology & Reproductive Sciences, University of Manitoba.
[3]Women's Outpatient, Women's Hospital.
[4]JC Wilt Infectious Diseases Research Centre, NMLB, PHAC, Winnipeg, Manitoba, Canada.

Introduction

Short-chain fatty acids (SCFAs), microbial-derived metabolites, play a dual role in human health. They are vital for gut health but their elevated levels in the vagina are linked to dysbiosis, which could affect susceptibility to HIV-1 and HPV infections. This study explored how SCFAs influence cervicovaginal epithelial integrity and immune responses.

Methods

Using air-liquid interface cultures of ectocervical (Ect1) and vaginal (Vk2) cell lines, we assessed the impact of SCFAs (butyrate and propionate) on epithelial barrier functions and immune activation. Cells were exposed to SCFAs, followed by stimulation using Poly(I:C), simulating viral infections. Measures included trans-epithelial electrical resistance (TEER) and dextran diffusion for barrier integrity, confocal microscopy for tight junction integrity and apoptosis, and multiplex assays for pro-inflammatory cytokine/chemokine levels.

Results

High butyrate levels compromised barrier integrity, evidenced by reduced TEER ($P<0.05$) and increased dextran permeability ($P<0.01$), effects that were pronounced even without viral simulation. This disruption was associated with decreased e-cadherin at tight junctions and increased apoptosis ($P<0.01$). Moreover, butyrate-exposed cells showed an elevated immune response to Poly(I:C), with high TNFα levels ($P<0.01$), indicating a heightened pro-inflammatory state.

The study also investigated cervicovaginal fluid (CVF) from individuals with HPV infection and squamous intraepithelial lesions (SIL). Contrary to gut findings, SCFA levels in CVF did not correlate with cytokine/chemokine levels. However, higher SCFA levels (+86%, $P<0.05$) and reduced lactic acid (-50%, $P<0.01$) were noted in participants with SIL, suggesting epithelial damage. Notably, HPV infection alone did not significantly alter SCFA or cytokine levels in CVFs.

Conclusion

In summary, cervicovaginal exposure to high butyrate levels deteriorates epithelial integrity and exaggerates immune activation, unlike in the gut where SCFAs generally promote mucosal health. This indicates that SCFAs' effects are context-dependent, potentially influencing the cervicovaginal environment's susceptibility to infections. This underscores the complexity of SCFAs' role in human health and disease, particularly in the context of vaginal dysbiosis and related infections.

POSTER #24

Synaptic Mechanism Underlying Substance Use Disorder

Mohd Sarim Siddiqui[1,2], Nirmala Padmanabhan[1,2], Tabrez J. Siddiqui[1,2,3,4]

[1]Prairie Neuroscience Research Centre, Kleysen Institute for Advanced Medicine, Health Sciences Centre.

[2]Department of Physiology and Pathophysiology, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba.

[3]Program in Biomedical Engineering, University in Manitoba

[4]Children's Hospital Research Institute of Manitoba

Introduction

Addiction poses a significant public health concern. Drugs of abuse exert acute and persistent impacts on neuronal circuit organization and addiction-related behaviors. We have identified two synapse organizing proteins, referred to as NX and CX, that are critically important in controlling synapse development and function. Genome-wide association studies have linked CX to human risk-taking and drug abuse, particularly cannabis. Intriguingly, both CX and NX are expressed in brain regions associated with addiction.

Methods and Results

We identified CX as one of the strongest interactors of NX in unbiased proteomics screens. Additionally, Co-Immunoprecipitation assays confirmed the in vivo binding of CX to NX, highlighting the importance of NX-CX complex in synaptic function and their potential implications for addiction. In our investigation, we aim to unravel the molecular mechanisms underlying the NX-CX complex and its role in addiction-related synaptic plasticity. By integrating molecular and behavioral methodologies, our study seeks to deepen our understanding of addiction mechanisms. Specifically, through behavioral studies on region-specific CX knockout mice and NX-associated synaptic changes, we aim to find changes in synaptic function with changes in addiction-related behaviors. Our behavioral studies will include assessments of drug-seeking behavior, reward sensitivity, cognitive function, and social interaction.

Conclusion

This comprehensive approach will not only shed light on the molecular pathways involved in addiction but also identify novel therapeutic targets for intervention.

POSTER #109

Healthcare Providers and Treatment Withdrawal: A scoping review of experienced distress in intensive care units

Lea Soliman[1], Dr. Louise Chartrand[2]

[1] College of Rehabilitation Sciences, University of Manitoba
[2]Department of Respiratory Therapy, College of Rehabilitation Sciences, University of Manitoba

Background

Intensive care units house the most critically acute patients with that require extensive treatments and highly specialized care. Highly trained healthcare providers work tirelessly to perform life-sustaining measures, but at times, all possible patient-centered options have been exhausted and treatment withdrawal is being acknowledged as the next step. This is an incomparable event that heavily impacts healthcare providers as they support patients, their families, and themselves through withdrawing or withholding experiences.

Objective(s)

The purpose of this scoping review is to analyze current literature and assess the reported experiences of moral, psychological, or emotional distress experienced by healthcare providers that participate in the withdrawal or withholding of ICU treatments. Interventions identified in the search results were voluntary, life-ending measures enacted on end-stage patients, particularly with the cessation of mechanical ventilation and the removal of the artificial airway.

Results

Multiple factors cause distress on providers while participating in this event, such as lack of role appreciation, lack of collaboration, and lack of communication skills. There was also evidence to suggest that cultural norms influence beliefs about terminal interventions, and that experiences of emotional distress were related to witnessing deaths, performing extubations, the need for mental compartmentalization, and the need for breaks post event. Moral distress was related to supporting the family, promoting false hope, the interpretation of euthanasia, and health care team conflicts. Evidence also acknowledged unnecessary patient suffering and decisions made against the patient's directives influencing the choice to withdraw care and also causing moral distress for providers.

Conclusion

Provider-driven protocols, end-of-life education, team debriefing, and enhanced communication tools are all measures that help to alleviate distress experienced by healthcare providers performing terminal extubation on mechanically ventilated patients in the ICU. Supports for these providers are important and would significantly alleviate the distress experienced in the intensive care unit. While there are recommendations available for these improvements, they are not regularly provided or standardized within health care organizations.

POSTER #104

Human milk-extracellular vesicles promote heat shock response during neuroinflammation.

Jasmyne A. Storm[1], Sanoji Wijenayake[1]

[1] Department of Biology, The University of Winnipeg, Winnipeg, MB, Canada

Introduction

Exposure to maternal stress (*e.g.*, diet, immune, psychosocial) during the perinatal (prenatal and postnatal) period shapes developmental trajectories in offspring. Microglia are the primary immune regulators of the brain parenchyma and environmental stress leads to their polarization. Neuroinflammation mainly stems from microglia polarization and attenuation of cytoprotective, pro-survival cellular cascades. Milk-derived extracellular vesicles (MEVs) are lipid-coated nanovesicles that carry bioactive cargo (*e.g.*, microRNAs, lipids, peptides). MEVs have been shown to attenuate pro-inflammation in peripheral cells and tissues, however, the cytoprotective potential of MEVs remains unknown in the brain. I investigated whether MEVs may attenuate neuroinflammation in microglia by activating the heat shock response (HSR). The HSR involves molecular chaperones (heat shock proteins; HSPs) that function to restore proteostasis under stress conditions by refolding or degrading misfolded aggregates. My findings suggest that MEV-supplementation enhances the HSR under pro-inflammatory conditions, specifically by increasing the abundance of candidate HSPs.

Methods

MEVs were isolated from unpasteurized human donor milk (NorthernStar Milk Bank; Calgary, AB) using serial ultracentrifugation. Human microglia cell lineage 3 (HMC3) were plated ($2x10^5$ cells/well; n = 5/treatment) and treated with 10 ng/mL IFN-γ to induce a pro-inflammatory phenotype (previously tested). HMC3 were supplemented with 200 µg of MEVs 24h post-IFN-γ polarization, then harvested at 6h, 12h and 24h. HSF1 and HSPs 40/70/90/27/60 abundance were analyzed via RT-qPCR and western immunoblotting. HSF1 cellular translocation was determined using cyto-nuclear extractions.

Results

MEV supplementation promoted HSR in polarized microglia, compared to homeostatic controls. HSP60 transcript increased with MEV supplementation, especially in primed microglia. Also, MEV-supplementation increased HSF1, and HSP27, HSP40 and HSP60 protein levels. HSP70 and HSP90 protein levels decreased with MEV-supplementation.

Conclusion

MEV-supplementation increases the abundance of HSF1 (transcription factor) and most cytoprotective HSPs and promoted a longer activation state of HSF1 by downregulating HSP70-HSP90 complex formation, which acts to negatively regulate HSF1 activity, as an HSR “off switch”. This suggests that MEVs help sustain the HSR in response to pro-inflammatory stress, exerting robust and continued pro-survival benefits. These results support the use of MEVs as a biotherapeutic to alleviate chronic neuroinflammation, and potentially improve later-life neurodevelopmental trajectories.

POSTER #139

PTX3 Deficiency Exacerbates Neutrophilic Airway Inflammation in House Dust Mite Chronic Model of Severe Asthma

Sina Taefehshokr[1], Lianyu Shan[1], Mojdeh Matloubi[1], Fatemeh Sedaghat[1], Sujata Basu[2], and Abdelilah S. Gounni[1]

[1] Department of Immunology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

[2] Department of Physiology and Pathophysiology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

Introduction

Neutrophilic asthma is refractory to available therapies e.g. inhaled corticosteroids and represent a major economic and social burden. However, the machanisms underylying this subype of asthma are unkown.

Pentraxin 3 (PTX3) is a member of the family of pattern recognition receptors (PRRs) known as pentraxins. We have previously shown that PTX3 deficiency exacerbates airway inflammation and airway hyperresponsiveness (AHR) in an ovalbumin mouse model of allergic inflammation. Therefore, we aimed to study the unknown role PTX3 in modulating neutrophilic inflammation, leading to enhanced AHR, and remodeling in a chronic model of severe asthma.

Methods

PTX3 knockout and wildtype (WT) mice were subjected to HDM+c-di-GMP to induce chronic model of severe asthma. Lung tissue and BALF immunophenotyping were studied with flow cytometry. In addition, cytokines and serum immunoglobulins were assessed by mesoscale and ELISA, respectively. AHR parameters were measured with FlexiVent ventilator. Collagen deposition and mucus production were visualized by Sirius-red, and Periodic acid-Schiff and associated genes were investigated using real-time PCR. Neutrophil extracellular traps (NETs) were studied using myeloperoxidase as the marker with immunofluorescence.

Results

PTX3 deficient mice, subjected to chronic model of severe challenge, exhibit heightened lung tissue, airway resistance, and elastance. IL17A was elevated in BALF of PTX3 deficient mice compared to WT. Additionally, PTX3-/- mice exposed to HDM+c-di-GMP demonstrated elevated total and HDM-specific IgA and IgE production, while displaying comparable levels of mucus and collagen production, respectively, when compared to their WT counterparts. Furthermore, PTX3 deficient mice exhibit distinct NETs-associated morphological features in contrast to WT mice.

Conclusion

PTX3 deficiency enhances neutrophil dominant inflammation along with AHR in chronic model of severe asthma.

POSTER #33

A global perspective: the gene expression profile of tuberculosis disease among people living with HIV

Erwin Taguiam[1], Mariana Herrera[1], Katherine Peña[2,3,4], Will Riaño[5], Lazaro Velez[5,6], Paul McLaren[1,7], Yoav Keynan[1,8,9], Zulma Vanessa Rueda[1,3,10*]

[1] Department of Medical Microbiology & Infectious Diseases, University of Manitoba, Winnipeg, Canada. [2] Escuela de Microbiología, Universidad de Antioquia, Medellín, Colombia. [3] Grupo de Investigación en Salud Pública, Facultad de Medicina, Universidad Pontificia Bolivariana, Medellín, Colombia. [4] Grupo Bacterias & Cáncer, School of Medicine, Universidad de Antioquia, Medellín, Colombia. [5] School of Medicine, Universidad de Antioquia, Medellin, Colombia. [6] Grupo investigador de Problemas en Enfermedades Infecciosas-GRIPE, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia. [7] JC Wilt Infectious Diseases Research Centre, Public Health Agency of Canada, Winnipeg, Canada. [8] Department of Internal Medicine, University of Manitoba, Winnipeg, Canada. [9] Department of Community Health Sciences, University of Manitoba, Winnipeg, Canada. [10] School of Medicine, Universidad Pontificia Bolivariana, Medellín, Colombia

Background

In 2022, tuberculosis (TB) was the leading cause of death among people living with HIV (PLWH) worldwide. Currently, screening methods are symptom-driven, which makes detecting TB challenging, with up to 75% of TB going undetected among PLWH. This research aims to investigate the transcriptomic profile of TB in PLWH with hopes of delineating host and TB interaction and improving early TB diagnosis among PLWH.

Methods

Participants were recruited from Medellin, Colombia and additional datasets were retrieved from publicly available database from the United Kingdom, Uganda and India (GSE107991, GSE107104 and GSE162164). Data were classified into 4 different groups confirmed by TB microbiology and HIV serology testing. Inclusion criteria included age ≥ 18 years old, and naive to HIV and TB treatment. RNA-sequencing analysis and pathway analysis were done.

Results

A total of 96 participants were included in the study as follows: people diagnosed with TB (n=31), HIV (n=23), TB among PLWH cohort (n=29), and non-HIV/non-TB cohort (n=13). 11,618 differentially expressed genes were found between all four cohorts with 7,897 upregulated and 3,721 downregulated genes (adjusted p-value=0.05 & fold change= ±1.5). Upregulated genes unique to TB among PLWH include DPP8, AK9, ATF2, KLH9, CREBRF, PAG1, TAB3, MR1, PDK3, and ABL2. Downregulated genes unique to TB among PLWH include NONO, LCK, ARF6, ILF3, NCL, SGK1, MADD, RCC2, BAP1, and CDK9. 1,415 genes were shared in all four cohorts.

Conclusion:

A total of 2071 upregulated genes and 1143 downregulated are unique for TB among PLWH which have the potential to serve as diagnostic biomarkers. Upregulated genes such as ATF2 and TAB3 have been correlated to the IL-1 signaling and NF-κB signal transduction pathway. Downregulated genes such as NONO, LCK, ARF6, ILF3, NCL, SGK1, MADD, RCC2, BAP1, CDK9 have been correlated to the positive regulation of biological processes.

POSTER #72

Exploring the Modulatory Effects of Aspirin on Vaginal Immunity

Morgan K. Taverner[1], Marina Costa Fujishima[2], Deesha Nayar[2], Keith R. Fowke[1] and Thomas T. Murooka[1,2]

[1]University of Manitoba, Department of Medical Microbiology and Infectious Diseases
[2]University of Manitoba, Department of Immunology

Human cohort studies associate bacterial vaginosis (BV) with adverse reproductive health outcomes such as genital inflammation and increased HIV acquisition risk. We developed a bacterial challenge model to recapitulate BV-associated barrier disruption, elevated cytokine production, and increased T cell activation and recruitment. Damping of genital inflammation needs to be part of a comprehensive HIV-prevention strategy along with pre-exposure prophylaxis (PrEP). Acetylsalicylic Acid (ASA), commonly known as aspirin, is an anti-inflammatory drug that is widely available, affordable, and accepted in Kenya. Human cohort studies in this region found that ASA modulates mucosal immunity by reducing HIV targets (CD4+CCR5+ T cells) in the female reproductive tract (FRT), but its broad impact on the vaginal mucosa is unknown. In this study, we addressed whether ASA could dampen BV-associated inflammation and epithelial barrier disruption *in vivo* and whether these effects can lower HIV acquisition risk.

Methods

C57BL/6 mice were intravaginally challenged with BV-associated anaerobes *Mobiluncus mulieris* or *Gardnerella vaginalis*, with daily dosing of 40mg/kg ASA. *In vivo* assessment of vaginal epithelial layer function using Lucifer yellow dye, immunophenotyping of vaginal T cell subsets, and RNAseq were performed to evaluate therapeutic effects of ASA.

Results

Initial results show little effect of ASA on epithelial barrier function during intravaginal challenge with either *Mobiluncus mulieris* or *Gardnerella vaginalis*. Intravaginal challenge also resulted in no change to CD4+CCR5+ T cell subsets or IFNy expression. A 1.5-fold elevation of activated CD4+CXCR6+ T cell subsets and increased IL-17a, IL-21, and IL-22 expression signatures were observed after *Mobiluncus mulieris* challenge, and daily ASA treatment significantly reduced Th17-associated cytokine expression. Relative to the aspirin-treated group, significantly elevated expression of genes associated with immune activation were observed in the FRT-draining lymph nodes of the vehicle control group following intravaginal challenge.

Conclusion

Short-term ASA treatment significantly lowered key readouts of vaginal mucosal inflammation induced by BV-associated bacteria, such as the recruitment of CXCR6+ T cell subsets and pro-inflammatory cytokine production. Further assessment of ASA to lower HIV acquisition risk is warranted and currently underway.

POSTER #115

Innate cGAS-STING Signaling in Doxorubicin Cardiomyopathy

Raneeta Thingnam[1], Rimpy Dhingra[1] and Lorrie Kirshenbaum[1]

[1]Department of Physiology and Pathophysiology and Institute of Cardiovascular Sciences, Max Rady Faculty of Health Sciences, University of Manitoba

Introduction

Doxorubicin (DOX) is widely used to treat a variety of human cancers. However, a well-known but poorly understood side effect of DOX treatment is its cardiotoxic properties which triggers cardiac cell death and heart failure. Autophagy is a cellular process responsible for the removal and degradation of damaged cellular components and proteins through a lysosomal regulated pathway. Previous work by our laboratory demonstrated that autophagy is impaired in cancer patients and mice treated with DOX. The cGAMP synthase cGAS stimulator of interferon genes STING, (cGAS-STING) is part of the innate immunity signaling pathway activated by cellular DAMPs such as nuclear DNA and chromatin associated HMBG1. Activation of cGAS-STING leads to cytokine production and cell death. However, the involvement of cGAS – STING pathway in DOX- cardiomyopathy in not well understood. Herein, we investigated the relationship between autophagy and cGAS-STING pathway in DOX cardiotoxicity.

Methods

Neonatal cardiac myocytes (NCMCs) were isolated from 1-2 days old Sprague-Dawley rats were treated with DOX in increasing doses (0.5μM, 1μM, 2.5μM, 5μM and 10μM). After eighteen hours of treatment, the cells were processed for western blot and protein expression of autophagy markers and proteins involved in innate immune pathway. Cell viability was assessed using vital dyes calcein AM and ethidium homodimer-1 in presence and absence of cGAS and STING inhibitors in DOX treated cardiomyocytes.

Results and conclusion

We show that in contrast to vehicle treated cells, nuclear HMGB1 is released and present in the cytosol of cardiac myocytes treated with DOX. This coincided with activation of cGAS-STING, increased production of TNFα and wide spread cell death. Notably, pharmacologic inhibition of cGAS or STING independently suppressed DOX-induced cardiac cell death. Our data reveal for the first time the involvement on cGAS-STING innate signaling pathway in the pathogenesis of TNFα mediated DOX cardiotoxicity. We suggest that therapeutic interventions that modulate autophagy flux may prove beneficial to preserving cardiac function and mitigating cardiotoxicity in cancer patients treated with Dox.

POSTER #126

Comparison of the tumor-infiltrating and tumor-adjacent cytotoxic T-cells reveals the uncoupling of exhaustion marker expression to their effector functions in breast cancer tumors

Diana Topolnitska[1,2], Alen Paiva[1,2], Edward W. Buchel[3], and Afshin K. Raouf[1,2]

[1] Department of Immunology, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba. [2] Paul Albrechtsen Research Institute, CancerCare Manitoba, Winnipeg, Manitoba. [3] Department of Surgery, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba

Introduction

The manipulation of patients' tumor-infiltrating T-lymphocytes (TIL) as a form of immunotherapy has shown promising results in the treatment of select cancers; however, limited benefit has been observed in treating breast cancers. A reason for this is the development of a dysfunctional T-cell phenotype. We posit that the study of T-cells from the autologous tumor-adjacent tissue (TAT) will reveal important information about the mechanisms of TIL exhaustion and how to overcome them.

Methods

$CD8^+$ T-cells were isolated from *in vitro* expanded patient TIL- and TAT-$CD3^+$ T-cell samples using fluorescence-activated cell sorting. They were then expanded for an additional two weeks prior to being characterized for their expression of exhaustion and activation markers using flow cytometry and qPCR. These expanded $CD8^+$ T-cells were placed in 3D co-cultures with autologous breast cancer cells (BCC). Following 24 and 72 hours of co-culture, BCC viability and T-cell exhaustion marker expression was assessed. Conditioned media was collected for cytokine analysis.

Results

Interestingly, the TIL-$CD8^+$ T-cells expanded more rapidly than the TAT-$CD8^+$ T-cells. However, the TAT-$CD8^+$ T-cells were more effective in eliminating breast cancer cells. The enhanced activity of the TAT-T-cells against autologous BCC was further corroborated with enhanced secretion of cytokines associated with activated cytotoxic T-cell functions. Furthermore, in co-cultures, up to a 9-fold increase in exhaustion marker LAG-3 expression was observed on both the TIL- and TAT-$CD8^+$ T-cells. The expression of markers PD-1 and CD160, however, remained low and unchanged. This observation suggests that exhaustion marker expression may not be coupled to T-cells' anti-tumor reactivity. Moreover, our data suggests that LAG-3, rather than PD-1 and CD160, might be the major reason for T-cell exhaustion in breast cancer tumors.

Conclusion

Our findings indicate that the TAT is a reservoir of tumor-reactive T-cells. We also found that their effector functions may be uncoupled from exhaustion marker expression; therefore, it is important to gain further insight into the mechanisms by which dysfunction occurs in breast cancer. Also, our observations indicate that blocking LAG-3 might be more effective than blocking PD-1 and/or PD-L1 as immunotherapy against breast cancers.

POSTER #9

Functional Variants Restricting HIV Set-Point Viral Load Correlate with Increased CHD1L Expression: Insights from GWAS Fine-mapping

Tough RH, McLaren PJ

Introduction

A recent genome-wide association study in individuals of African ancestries identified a region on chromosome 1 significantly associated with decreased HIV set-point viral load. Knockout of the closest gene, *CHD1L*, enhanced HIV replication in vitro in a cell-type specific manner and addition of exogenous CHD1L decreased p24 production *in vitro*. However, how genetic variation in the chromosome 1 region impacts CHD1L expression remains unclear.

Methods

To address this, we selected 121 variants in linkage disequilibrium ($r^2 \geq 0.2$) to the lead genome-wide association study SNP, rs59784663, and variants were annotated using HaploRegv4.2 to prioritize overlapping H3K4me1, H3K4Me3, H3K27ac, H3K9ac, DNase, eQTLs, and/or protein binding sites in blood tissues. The potential impact of functional variants on *CHD1L* expression was assessed using colocalization analyses in eQTLs in lymphoblastoid cell lines from the African Functional Genomics Resource cohort and monocytes from the Multi-Ethnic Study of Atherosclerosis (MESA) cohort. Statistical fine-mapping was performed using BIMBAM to determine if combinations of functional variants, or a single causal variant, was driving the association signal. We used PrediXcan trained on monocyte eQTLs from the MESA cohort to impute genetically regulated *CHD1L* expression to determine the impact of likely causal variants on *CHD1L* expression.

Results

We observed that in lymphoblastoid cells and monocytes originating from individuals of African ancestry, functional variants that increased CHD1L expression were significantly correlated with HIV set-point viral load. Using BIMBAM, we determined that control of HIV is more likely driven by a two-SNP (rs7525622 and rs73004025) or three-SNP (rs7525622, rs73004025, and rs72999655) model than by the lead GWAS SNP alone. When we tested the association between rs7525622, rs73004025, and/or rs72999635 dosage with imputed *CHD1L* expression, the alternate allele was strongly associated with increased *CHD1L* expression in a dose-dependent manner. In this model, increased *CHD1L* expression was also significantly correlated with decreased HIV set-point viral load.

Conclusion

Here we identify that the chromosome 1 region likely has multiple causal variants, with the majority of HIV set-point viral load variance explained by rs7525622, rs73004025, and rs72999655. Variants in the chromosome 1 region are strongly associated with decreased HIV set-point viral load and increased CHD1L expression.

POSTER #37

A Preclinical Mouse Model of Colitis Reveals Sex-Linked Profile Changes in Spleen Immune Cell Composi ion

Diane M. Tshikudi[1], and Jean-Eric Ghia[1, 2, 3]

[1]Department of Immunology Internal Medicine, University of Manitoba, Winnipeg, MB, Canada . [2]Department of Internal Medicine, Section of Gastroenterology, University of Manitoba, Winnipeg, MB, Canada. [3]Gastrointestinal Basic Biology Research, IBD Clinical & Research Centre, Winnipeg, MB, Canada.

Introduction

Ulcerative colitis (UC) is a chronic gut inflammation associated with extraintestinal manifestations (EIMs) such as arthritis that vary across patients in a sex-dependent manner. EIMs in colitis may result from a systemic dysregulation of the immune system where the spleen plays a crucial role in regulating peripheral immune tolerance. Yet, the impact of colitis and sex-mediated differences on immune cell subtypes within the spleen is unclear. Here, in a preclinical model of colitis, we immunophenotype spleen immune cell populations of male and female mice.

Methodology

Male and female C57BL/6 mice (n=4-8) were treated with 5% dextran sodium sulfate (DSS) to induce colitis or water (control) for five days. Cells isolated from spleen were labeled for innate immune cells ($CD11b^+$ myeloid, CD11b-$CD103^+$ dendritic cells, $Ly6g^+$ neutrophils, natural killer [NK] 1.1^+, $F4/80^+CD86^+$ M1 and $F4/80^+CD209^+$ M2 macrophages), adaptive immune cells (lymphocytes $CD4^+$, $CD8^+$, $CD19^+$B, T follicular helper $CD45^-CD4^+CXCR5^+$ [Tfh], exhausted $CD4^+PD1^+$ and regulatory [Treg]) and measured by flow cytometry.

Results

In colitic conditions, the profile of innate and adaptive immune cell populations differ significantly in the spleen between sexes. We found enriched myeloid cells, neutrophils, and M1 macrophages and reduced dendritic, NK, and M2 macrophage cells in colitis compared to control male mice. Colitic female mice had lower dendritic and NK cells and enriched neutrophils, whereas the M1 and M2 macrophages were unaltered compared to control female mice. $CD4^+$, $CD8^+$, $CD19^+$, and Treg cell abundance were similar in colitic and control conditions. Although Tfh and $CD4^+PD1^+$ were significantly reduced in male mice, they were not in female mice.

Conclusions

DSS-mediated colitis alters the frequency of the spleen's innate immune cells and subsets of T helper lymphocytes in a sex-dependent manner. This finding highlights the importance of considering the sex when using a preclinical colitic model of colitis and when assessing colitis and EIM occurrence.

POSTER #92

Human Milk Oligosaccharides and Cognitive, Language and Motor Development at One Year in the CHILD Cohort Study

Sarah E. Turner[1,2,3], Leslie Roos[2,4], Nathan C. Nickel[1,3,5], Piushkumar J. Mandhane[6], Theo J. Moraes[7], Stuart E. Turvey[8], Elinor Simons[2,3,11], Lars Bode[9], Bianca Robertson[9], Padmaja Subbarao[10], Meghan B. Azad[1,2,3,11]

[1]Manitoba Interdisciplinary Lactation Centre (MILC), MB, Canada. [2]Children's Hospital Research Institute of Manitoba, MB, Canada. [3]Department of Community Health Sciences, University of Manitoba, MB, Canada. [4]Department of Psychology, University of Manitoba, MB, Canada. [5]Department of Kinesiology and the Manitoba Centre for Health Policy, University of Manitoba, MB, Canada. [6]Department of Pediatrics, University of Alberta, AB, Canada. [7]Department of Pediatrics, The Hospital for Sick Children, University of Toronto, ON, Canada. [8]Department of Pediatrics, University of British Columbia, BC, Canada. [9]Department of Pediatrics, Larsson-Rosenquist Foundation Mother-Milk-Infant Center of Research Excellence (MOMI CORE), and the Human Milk Institute (HMI), University of California, San Diego, California, USA. [10]Department of Pediatrics, Physiology & Dalla Lana School of Public Health, The Hospital for Sick Children, University of Toronto, ON, Canada. [11]Department of Pediatrics and Child Health, University of Manitoba, MB, Canada

Introduction

Human milk oligosaccharides (HMOs) are complex sugar molecules found in human milk. Emerging research has linked some HMOs to improved neurodevelopment in animal and human studies, but these relationships remain unclear. The objective of this research is to examine the link between 19 HMOs and cognitive development at one year of age.

Methods

This study includes a subset of 247 parent-infant pairs from the Canadian CHILD cohort study. At 3-4 months post-partum, breastfeeding parents provided a milk sample which was analyzed by liquid chromatography to identify the 19 most prevalent HMOs. Trained research assistants administered the Bayley Scale of Infant and Toddler Development at one year of age which comprised three scales: cognitive, language and motor development (standardized to a mean of 100 and a standard deviation of 15; higher scores indicate better development). Adjusted linear regression was used to estimate the relationship between HMOs and neurodevelopment, controlling for maternal, infant and birth factors. Interactions were tested with infant sex as well as maternal secretor status, a genetic predictor of HMO production.

Results

Higher concentrations of the HMOs 3'-sialyllactose (3'SL) and lacto-N-hexaose (LNH) were related to better language and cognitive scores, respectively (β= 2.09; 95% CI: 0.61, 3.58; β= 2.10; 95% CI: 0.77, 3.43). Higher concentrations of disialyllacto-N-tetraose (DSLNT) were related to lower cognitive, language and motor scores (β= -1.50, -2.15 and -4.00, respectively, all $p \leq 0.05$). In interaction models, higher concentrations of 3'SL were related to better language and motor scores for infants of maternal secretors and lower scores for infants of maternal non-secretors. The opposite was true for difucosyllactose-N-hexaose (DFLNH); higher concentrations were related to better language and motor scores for maternal non-secretors and lower scores for secretors. Few sex interactions were observed.

Conclusion

This exploratory study contributes to a growing evidence base that select HMOs are related to infant neurodevelopment. Maternal secretor status may play an important role in moderating the relationship between HMOs and neurodevelopment; however, the direction of these effects are not consistent (i.e. higher concentrations and positive secretor status is not always better). More work is needed before translation into practice can occur.

POSTER #140

Mechanisms of Trans-synaptic Control in Synapse Organization

Rebecca E. Twilley[1,2], Nirmala Padmanabhan[1,2], Tabrez J. Siddiqui[1,2,3,4].

[1]Prairie Neuroscience Centre, Kleysen Institute for Advanced Medicine, Health Sciences Centre, Winnipeg, MB R3E 0Z3, Canada. [2]Department of Physiology and Pathophysiology, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba. [3]Program of Biomedical Engineering, University of Manitoba. [4]Children's Hospital Research Institute of Manitoba, 715 McDermot Ave, Winnipeg, MB R3E 3P4

Introduction

Autism spectrum disorder (ASD) is a pervasive neurodevelopmental condition affecting approximately 1% of children worldwide, characterized by social interaction deficits and repetitive behaviors. The clinical severity of ASD can range from mild to profoundly disabling, with no pharmacological interventions currently approved for ASD treatment in Canada. Recent studies have identified de novo mutations in the Leucine-rich repeat transmembrane protein 4 (LRRTM4) and Neurexins (Nrx), cellular adhesion molecules, as potential etiological contributors to ASD.

Methods

To further investigate this, we engineered a LRRTM4 knock-in (KI) mouse model using CRISPR-Cas9 technology, which effectively disrupted the binding interface between LRRTM4, its presynaptic partners Nrx, and Protein Tyrosine Phosphatase sigma (PTPσ). Electron Microscopy was performed to analyze the ultra-structural organization of the hippocampal synapse. Behavioral analysis on mice was performed to understand how the LRRTM4-Nrx- PTPσ complex disruption alters cognitive function and behaviour. Analysis of the Proteome (MAP), an expansion microscopy technique used to increase imaging resolution, has been developed and will be performed to analyze the molecular organization of synapse proteins including the localization, distance, and alignment of pre-and post-synaptic proteins.

Results

We undertook an extensive analysis of the structural and functional changes in the KI mouse model, uncovering compelling evidence of altered synaptic architecture. Specifically, quantitative imaging revealed a significant reduction in excitatory synapses within the dentate gyrus of the KI model. Concurrently, co-immunoprecipitation studies demonstrated a disruption in LRRTM4 binding to Nrx and PTPσ. Electron microscopy further substantiated these findings, reporting a marked decrease in synapse numbers, postsynaptic density, spine head width, vesicle count, and docked vesicles within the dentate gyrus. Analogously, the CA1 region exhibited diminished postsynaptic density and active zone length, indicative of compromised synaptic plasticity and transmission. Importantly, behavioral analyses of LRRTM4 KI mice revealed sex-based deficits. Heightened anxiety-related behavior and a decrease in spatial memory were found exclusively in male mice.

Conclusion

Our research endeavors to elucidate the molecular role of LRRTM4 and the structural complexities of the LRRTM4-Neurexin complex. By understanding the mechanistic underpinnings of this synaptic interaction, potential therapeutic targets to disrupt aberrant binding may be identified, contributing to the development of novel pharmacological interventions for ASD.

POSTER #67

The effects of elevated glucose on islet-derived extracellular vesicles – Implication in type 2 diabetes

Rushie Tyagi and Lucy Marzban

College of Pharmacy, Rady Faculty of Health Sciences, University of Manitoba

Introduction

Type 2 diabetes (T2D) is characterized by reduced beta-cell mass and function. Islet amyloid, formed by aggregation of human islet amyloid polypeptide (hIAPP), contributes to progressive beta-cell loss in T2D. The cellular mechanisms underlying islet amyloid formation are still unclear. In this study, we tested the effects of elevated glucose on islet-derived extracellular vesicles (EV) as a potential mechanism for the clearance of soluble/aggregated (pro)IAPP species from beta-cells and amyloid formation.

Methods

Human islets isolated from cadaveric pancreatic donors (n=4 donors) were cultured in normal (5.5 mM) or elevated (11.1 mM) glucose to form amyloid. EV (exosomes) were isolated from culture medium using classical centrifugation and ultracentrifugation. Purified EV were analyzed by nanoparticle tracking analysis. Western blot analysis and double immunogold transmission electron microscopy were performed to verify the presence of EV markers and (pro)hIAPP species and oligomers (aggregates).

Results

Human islets formed amyloid during culture with elevated glucose which was associated with progressive beta-cell apoptosis. (Pro)IAPP species were detectable in EV released from islets cultured in normal and elevated glucose. The latter markedly increased (pro)IAPP content in islet-derived EV. Interestingly, hIAPP aggregates (oligomers) were present in the majority of EV released from human islets cultured in elevated glucose but were not detectable in islets cultured with normal glucose.

Conclusion

Our data show that (pro)IAPP species are present in islet-derived EV and that elevated glucose increases (pro)hIAPP and its aggregates in EV released from islets. Islet-derived EV may play a key role in the process of amyloid formation in T2D.

POSTER #25

Oxidized Phosphatidylcholine-Protein Kinase C Signalling Axis Desensitizes B2-Adrenoceptors in Airway Smooth Muscle: Mechanism for Bronchodilator Insensitivity

Jignesh M. Vaghasiya[1,3], Nathan Varghese[3], Alaina Bagan[3], Azadeh Dalvand[1,3], Sujata Basu[1,3], Andrew J. Halayko[1-3]

[1]Departments of Physiology & Pathophysiology and [2]Internal Medicine, University of Manitoba, Winnipeg, MB, and [3]Biology of Breathing Group, Children's Hospital Research Institute of Manitoba, Winnipeg, MB.

Rationale

Oxidized phosphatidylcholines (OxPC) are mediators of oxidative stress that accumulate in the airways in response to environment insult to promote asthma pathobiology, including airway smooth muscle (ASM) contraction and inflammation. We showed OxPC impairs β_2-adrenoceptor (β_2AR) induced bronchodilation via protein-kinase C (PKC) signalling. This study tests whether OxPC exposure is sufficient to activate PKC and impair cAMP signaling downstream from β_2AR in human ASM (HASM) cells to underpin bronchodilator insensitivity.

Methods

Cultured HASM cells (N=5) were treated with OxPC (80 µg/mL, 1-24 hrs) or the PKC activator, 12-O-tetradecanoylphorbol-13-acetate (TPA) (0.2 µM, 1 hr). PKC phosphorylation was assayed by immunoblotting using anti-pSer660-PKC. cAMP signaling induced by the β_2AR agonist, isoproterenol (Iso) (1 nM), was assessed by immunoblotting for phosphorylation of the protein kinase A (PKA) substrate, VASP (p-VASP-shift assay), in HASM cells pre-treated with OxPC or TPA. To confirm PKC mediates OxPC effects on β_2AR-signaling, HASM cells were treated with PKC inhibitor (GF-109203x, 10 µM). Isometric force of murine tracheal rings (N=6) was used to evaluate effects of OxPC on Iso-induced relaxation in the presence and absence of PKC inhibitor. Data analysis included one-way ANOVA with Dunnett's post-hoc test or nonlinear-curve-fit.

Results

In cultured HASM cells, OxPC exposure for 1 hr significantly increased PKC phosphorylation by 203±83.6%. This was comparable to induction with the PKC activator, TPA (256±96.8%). OxPC treatment for longer periods (up to 24 hrs) showed sustained PKC phosphorylation above baseline (239±117.0%). For β_2AR-mediated cAMP dependent signalling, OxPC pre-treatment dose dependently reduced Iso-induced PKA specific p-VASP formation, with maximum inhibition of 53% (Table-1). Pharmacological inhibition of PKC prevented the suppressive effects of OxPC on Iso-induced p-VASP formation by 43.3±4.29%, whereas PKC activation with TPA decreased β_2AR-mediated p-VASP formation by up to 27% compared to control. Consistent with studies using cultured HASM cells, OxPC pre-exposure of murine tracheal rings significantly decreased β_2AR-mediated airway relaxation responses (Iso-EC_{50} 4.4 times higher than control), and PKC inhibition was sufficient to prevent OxPC-induced impairment of Iso-induced airway relaxation.

Conclusion

Oxidized phosphatidylcholines cause sustained PKC activation in HASM cells, which impairs β_2AR-mediated cAMP signaling and relaxation of human ASM cells and murine airways, respectively. These data exhibit a mechanism for OxPC induced β_2AR desensitization associated with inflammation and oxidative-stress in asthma.

POSTER #14

Impact of Delayed SARS-CoV-2 Vaccine Dose Interval on B Cell Maturation and Antibody Neutralization.

Jack W. Vallis[1,2], Samantha J. Krosta[2], Paige N. Adams[1,2], Jessica I. Ahmed[2], Sandra A. Kiazyk[1,2], Lyle R. McKinnon[1,3] and Catherine M. Card[1,2]

[1] Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Canada [2]JC Wilt Infectious Diseases Research Centre, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada. [3] Centre for the AIDS Programme of Research in South Africa (CAPRISA), Durban, South Africa

Introduction

Early in the COVID-19 vaccine rollout, Canada implemented a dose-sparing strategy where second doses were delayed, prioritizing wider population access to first doses. A delayed second dose was subsequently shown to reduce infection, hospitalization, and deaths for both Pfizer-BioNTech and Moderna mRNA vaccines when compared to a standard dose interval. A longer interval between doses was also associated with improved antibody titers and breadth for variants. This marked increase in antibody function may suggest maturation of memory B cells during the interval between doses. This study aims to evaluate the effects of an extended dose interval on B cell maturation after the primary dose series and following a third (booster) dose in COVID-19 mRNA vaccine recipients from Manitoba.

Methods

Data on antigen-specific B cell quantity and phenotypes were acquired by flow cytometry using a BD FACS Symphony. Peripheral blood mononuclear cells were collected at different timepoints from participants with either a short- or long-dose interval of the SARS-CoV-2 vaccine and stained with a variety of fluorochrome probes to detect the presence and proportion of antigen-specific B cells over time.

Results

A delayed dose interval leads to a greater increase in anti-Spike and anti-RBD B cells as well as antigen-specific memory B cells 1-2 weeks after the second dose compared to a standard dose interval. The levels of anti-Spike and anti-RBD B cells in the standard dose interval participants increase to match the levels in the delayed dose interval participants 6 months after the second dose is administered. The impact of a third dose in both groups does not appear to result in the same increase in anti-SARS-CoV-2 specific B cells. Analysis of memory B cells and memory B cell-derived antibody neutralization in COVID-19 vaccine recipients is ongoing.

Conclusion

Dosing intervals affect antibody responses following vaccination. The finding that longer dose intervals result in greater proportions of anti-SARS-CoV-2 specific B cells suggests that dose interval may impact outcomes of B cell somatic hypermutation. The study will shed light on whether the establishment of long-term memory B cells is also affected, which could have implications for vaccine design.

POSTER #118

p53 pathways associated with colorectal cancer are induced when dietary β-fructan fibers are not fermented in inflammatory bowel diseases

A. Voisin[1], S. Balko[2,3], R. Mahmood[4], O. Fedorova[4], E. Zoungrana Ouali[4], T. Jeanson[4], A. Raouf[2,3], C. Bernstein[4], H. Armstrong[1,2,4]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, MB R3E 3P4, Canada

[2]Department of Immunology, University of Manitoba, Winnipeg, B R3E 3P4, Canada
[3] CancerCare Manitoba Research Institute, Winnipeg, MB, R3E 0V9
[4]Department of Internal Medicine, University of Manitoba, Winnipeg, MB R3E 3P4, Canada

Introduction

Dietary fibers are not digested; they are fermented by gut microbes producing beneficial short-chain fatty acids (SCFAs). However, inflammatory bowel disease (IBD) patients have altered gut microbiota, reduced SCFAs, and can experience sensitivity to a high-fiber diet. We showed unfermented β-fructan fibers induce inflammation and gut damage via TLR2 and NLRP3 inflammasome in IBD patients. Chronic inflammation and gut damage in IBD increases the risk of developing colorectal cancer (CRC). Therefore, we ***hypothesize*** that diet-induced inflammation could create a mutagenic environment that promotes risk of CRC in IBD.

Methods

Bulk RNA sequencing was performed on biopsies taken from IBD patients before and after 6-month consumption of either 15g/day β-fructans or placebo (clinicaltrials.gov NCT02865707). To validate findings, biopsies from non-IBD (n=5) and IBD (n=5) patients were cultured *ex vivo* with β-fructans (inulin and oligofructose) or a combination of oligofructose+SCFAs reflective of levels following fermentation by non-IBD or IBD whole gut microbiota (Fig 3F PMID: 36183751). Changes in cancer pathway markers were examined (microscopy [p53, villin, axin2, NLRP3], mesoscale discovery, ROS production). Cell migration was examined in Caco-2 human gut epithelial cells exposed to β-fructans (scratch wound assay with mitomycin C).

Results

From the clinical trial cohort, the CRC-related genes SLIT2/MAPK ($p<0.0001$) and SOS1 ($P<0.001$) were induced only in IBD patients who displayed reduced fiber-fermenting capacity (stool) and who had relapsed following 6-months consumption of β-fructans. In *ex vivo* cultures, unfermented β-fructans increased expression of axin2 and p53 compared to the no fibre control (densitometry; fold change). β-fructans had no significant impact on cell migration in cell lines ($p>0.05$; one-way ANOVA).

Conclusions

My findings suggest that if β-fructan fibres are not fermented by gut microbiota in IBD, they can induce p53 associated CRC pathways. Our observations suggest that such fibres should be avoided in a subset of IBD patients if their gut microbiota are unable to utilize these fibres.

POSTER #62

Genomic epidemiology of Vibrio cholerae reveals the regional and national spread of eight non-toxigenic Canadian lineages

Taylor Wells[1,2], Sandeep Tamber[3], Swapan Banerjee[3], Celine Nadon[1,2]

[1] Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Canada
[2] Public Health Agency of Canada, National Microbiology Laboratory, Winnipeg, Canada
[3] Bureau of Microbial Hazards, Food Directorate, Health Canada, Ottawa, Canada

Introduction

Vibrio cholerae comprises over 200 serogroups; O1 and O139 have been associated with severe pandemics and epidemics to date. Understanding the characteristics and genomics of non-O1/O139, non-toxigenic *V. cholerae*, are lacking. Non-O1/O139 *V. cholerae* causing mild to moderate gastroenteritis referred to as vibriosis, is typically not a notifiable disease, and thus underreported. From 2010 to 2023, 160 reported cases and one outbreak in Canada caused by non-O1/O139 *V. cholerae* isolates have provoked considerable public health concern.

Purpose

Explore the underlying diversity and phylogenetic relatedness of *V. cholerae* present in Canada.

Methods

We sequenced 246 genomes isolated from patients and the environment in Canada between 1999-2023. Phylogenetic trees using core genome single nucleotide polymorphisms (cgSNPs) were built using Parsnp. Population structure was determined using fastBAPs. Virulence genes were identified with ABRicate. Analyses and visualizations were done in R and Microreact.

Results

Of the Canadian isolates, all non-O1/O139 strains lacked the cholera toxin-producing genes typically harboured by pathogenic O1 and O139. All of the *V. cholerae* O1 isolates in Canada were identified from travel-related cases as members of the toxigenic 7th pandemic (7PET) lineage. Phylogenetic analysis based on cgSNP analysis divided *V. cholerae* into 7 clusters. Amongst the Canadian isolates, 44 (18%) were identified as belonging to eight new lineages of non-O1/O139, non-toxigenic *V. cholerae*, denoted CAD1-8, where CAD stands for 'Canada'. A new lineage was defined as clades formed by three or more Canadian isolates in the phylogeny. CAD-2 was composed of clinical and environmental isolates associated with the 2018 vibriosis outbreak. Lineages CAD-1, CAD-2 and CAD-6 were geographically associated with the central, western, and eastern Canada, respectively. However, several CAD lineages were dispersed geographically and isolated from different years.

Conclusions

Genomic analyses revealed the regional and national spread of eight non-toxigenic serogroup non-O1/ O139 *V. cholerae* lineages that can cause human infection in Canada. Further characterization will be critical to elucidate transmission and virulence features to mitigate this emerging pathogen.

POSTER #117

CRISPR-Cas Detection of Viral Hemorrhagic Fevers at the Point of Care

Kylene Wupori[1,2]

[1]National Microbiology Laboratory Branch, Public Health Agency of Canada, Winnipeg, Canada
[2]Department of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada

Introduction

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) - Cas system used by prokaryotes for defense against foreign pathogens shows promise for use in point of care (POC) diagnostic tests. Viral hemorrhagic fevers such as Ebola and Lassa are public health threats that necessitate the development of a POC test. Current serological and molecular based diagnostic tests such as Polymerase Chain Reaction are not practical for use in the field as a POC test due to limitations including turn around time and cost. There is currently limited research on the use of CRISPR based detection methods for Ebola virus and Lassa fever. This study aims to develop CRISPR-Cas12b and Reverse Transcriptase Loop Mediated Isothermal Amplification (RT-LAMP) as a POC test to detect Ebola virus and Lassa fever in a one tube format.

Methods

A lateral flow test will be used to indicate CRISPR-Cas detection of viral genetic material. Viral RNA will be extracted from samples using HUDSON (Heating Unextracted Diagnostic Samples to Obliterate Nucleases) or magnetic beads and then amplified using RT-LAMP with primers made using the online NEB LAMP primer tool. CRISPR-Cas12b will be used to detect the targeted NP and L regions of Ebola and Lassa virus using synthetic guide RNA. Successful amplification and detection of genetic material will be indicated through identification of the trans cleavage activity by the Cas12b enzyme on the Milenia HybriDetect flow test. The assay will be tested on different matrices, the cross reactivity assessed, and the limit of detection measured.

Results

Successful amplification and detection of Ebola virus and Lassa fever in a one tube format using the CRISPR-Cas12b LAMP assay in different matrices will be indicated by a positive test line on the lateral flow strip.

Conclusion

POC tests are important for public health care yet there are limited studies on the development of POC CRISPR diagnostics for viral hemorrhagic fevers. This study aims to contribute to the development of a CRISPR based POC test for Ebola virus and Lassa fever to help control future outbreaks and improve patient care through fast, accurate diagnostics.

POSTER #58

A Wireless Litzcage RF coil design for human head MRI at 1.5T

Haoqin Zhu[1], Bocheng Xu[2], Melanie Martin[3]

[1] Sino Canada Health Institute Inc., Winnipeg, Manitoba, Canada
[2]Department of Biomedical engineering, The University of Manitoba, Winnipeg, Manitoba, Canada
[3]Department of Physics, The University of Winnipeg, Winnipeg, Manitoba, Canada

Introduction

The research aimed to develop a detunable wireless Litzcage coil for human head MRI at 1.5 T, providing an alternative to conventional wired coils with a high cost and complex structure. The coil was designed to maintain uniform B1+ of the body coil and improve patient comfort during MRI heading scans.

Method

The methods involved designing and testing wireless coils based on Litzcage and standard birdcage designs, with varying detune circuits, to evaluate detuning performance and SNR in MRI experiments using a 1.5 T scanner. The detuning circuit position and coil modeling were crucial aspects of the human head study.

Results

Evaluation of the wireless Litzcage coil showcased its minimal B1+ change, highlighting high RF transparency to the body coil. Axial B1+ maps exhibited negligible differences with and without the wireless coil, measuring less than 1%. Furthermore, SNR results revealed a substantial enhancement of approximately 3.9 times across all phantom areas compared to using the body coil alone.

Conclusions

The 1.5 T detunable wireless Litzcage coil offers a promising alternative to conventional wired coils in human head MRI. It improves image quality, SNR, and patient comfort, enhancing neuroimaging applications like MRI-guided neurosurgery.

Acknowledgements

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POSTER #98

Using Machine Learning and Magnetic Resonance Imaging to Predict Alzheimer's Disease-like Patterns in Patients with Posttraumatic Stress Disorder.

Gabriella Yakemow[1,2], Tiffany A. Kolesar[1,2], Natalie Wright[1,2,3], Iman Beheshti[1,2], Lawrence Ryner[4], Sarah Chaulk[5], Ronak Patel[5], Ji Hyun Ko[1,2,6]

[1]Department of Human Anatomy and Cell Science, University of Manitoba, Winnipeg, MB
[2] PrairieNeuro Brain Research Centre, Kleysen Institute for Advanced Medicine, Health Sciences Centre, Winnipeg, MB
[3]Undergraduate Medical Education, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB
[4]Department of Radiology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB
[5]Department of Clinical Health Psychology, University of Manitoba, Winnipeg MB
[6]Graduate Program in Biomedical Engineering, Price Faculty of Engineering, University of Manitoba, Winnipeg, MB

Introduction

Posttraumatic stress disorder (PTSD) is a mental health disorder caused by witnessing or experiencing traumatic events. PTSD symptoms include intrusive memories, avoidant behaviors, anxiety, flashbacks and negative changes in mood and cognition. Symptom severity can be measured using the clinician-administer PTSD scale for DSM-5 (CAPS-5). Recent studies show that patients with PTSD have an increased risk of developing Alzheimer's disease (AD), but there is currently no way to predict which patients will go on to develop dementia due to AD. The objective of this study is to identify brain anatomical and activity markers in PTSD that resemble AD-like patterns.

Methods

We previously developed a machine learning-based AD designation (MAD) algorithm trained to objectively distinguish neural activity between AD patients and Healthy Controls (HC) using neuroimaging modalities. Baseline data was used from a clinical trial of 67 participants. Forty individuals were treatment seeking and diagnosed with PTSD (14M:26F, age =40.0±3.6) and 27 age matched HC were recruited from community at large (11M:16F, age= 35.4±5.2). We assessed grey matter (GM) volume using structural MRI images by Voxel Based Morphometry (VBM). Cerebral blood flow changes were assessed using pseudo-continuous arterial spin labeling(pCASL) images. MAD scores were then calculated using mean CBF scores from pCASL images. Statistical analysis was performed with both groups using SPSS to identify correlations between MAD scores, anatomical results, and CAPS-5 scores.

Results

VBM analysis identified 1 significant cluster of decreased volume in the temporal lobe in the PTSD group when compared to HC. CBF analysis identified 8 significant clusters primarily in the right hemisphere of the brain showing decreased CBF in the PTSD group compared to HC. MAD scores correlated with CAPS-5 scores (r=0.335, p = 0.019) in the PTSD group. CAPS-5 scores also correlated with the GM cluster in the PTSD group (r= -0.427, p= 0.007).

Conclusion

Results show that PTSD patients experiencing a greater degree of symptom severity were more likely to exhibit AD-like patterns and decreased regions of CBF. These results show promising potential for possible early diagnosis of dementia in an at-risk population, in the hopes of allowing for early treatment.

POSTER #80

Effect of serotonin on ventral V3 populations

Muniza M. Zaman[1], Narjes Shahsavani[1], Lucia E. Dominguez-Rodriguez[1], Jeremy W. Chopek[1]

[1] Spinal Cord Research Centre, University of Manitoba, MB, Canada

Introduction

Spinal central pattern generators (CPGs) are responsible for the generation of locomotion characterized by alternation of flexor and extensor muscles and left and right limbs. However, to ensure stable movement, descending input from the brainstem provides on-going excitation to spinal CPGs. For instance, serotonergic descending inputs modulate spinal interneuron excitability. Within the spinal cord, interneurons (INs) can be characterized based on their genetic origins and molecular profiles. One such are V3 INs, having a vital role in regulating balanced motor output. Despite the importance of V3 INs in locomotion, and the importance of descending neuromodulation to initiate and maintain locomotion, it remains unknown how V3 INs respond to descending neuromodulation. We hypothesized that serotonin increases the excitability of ventral V3 INs.

Methods

Using in-vitro whole cell patch clamp electrophysiology, basic intrinsic and firing properties of V3 INs from thoracolumbar spinal cord slices of Sim1Cre; TdTomato mice were recorded from 3 different age groups (p1-p3, p4-p8 and p9+). Comparisons of intrinsic (rheobase, input resistance, threshold potential and afterhyperpolarization) and firing properties of V3 INs were made between pre-bath and bath application of serotonin.

Results

V3 INs demonstrated age-dependent trends where approximately 30% of V3 neurons displayed increase in excitability, approximately 30% had a decrease in their excitability and remaining percentage of neurons showed no change in their excitability after addition of 5-HT in response to current injection ($p>0.05$).

Conclusion

This is a novel approach where an age-dependent study is carried out to find the effects of 5-HT on the ventral V3 INs. In the future, these findings will lead to better understanding of the differential role of serotonin and its neuromodulatory effects on spinal INs.

POSTER #97

Lipidomics Analysis of Cardiogenic Shock

Hannah Zhang[1,2], Ashish Shah[1,2], Amir Ravandi[1,2]

[1]Department of Physiology and Pathophysiology, University of Manitoba
[2]Institute of Cardiovascular Sciences, St. Boniface Research Centre

Introduction

Cardiogenic shock (CS) is a life-threatening state characterized by acutely failing heart function. CS is the most common cause of death in heart attack patients, and despite significant advances in therapy options, CS continues to have an unacceptably high in-hospital mortality rate of 50%. Due to a lack of standardized defining features and stages of CS, clinicians struggle to diagnose and intervene on patients early, which is likely an important component of patients' survival. Current methods of identifying patients are not cardiac-specific, and often are not timely enough to prevent CS progression. The development of high-throughput lipidomic technology has shown us that lipids represent an active pool of plasma compounds that change rapidly in response to human pathology, including cardiovascular diseases.

Methods

We have collected plasma samples and non-invasive hemodynamic measurements for patients presenting with acute myocardial infarction at three timepoints over the course of their hospital stay (pre-intervention, post-intervention, 24hrs post-intervention). We have performed comprehensive lipidomics analysis using liquid chromatography/mass spectrometry (LC/MS), and will stratify the changes based on hemodynamic, clinical and biochemical markers of CS.

Results

There are significant changes in the lipidome over the course of AMI. Triglycerides decrease immediately post coronary angioplasty, with a significant increase by 24 hours, while free fatty acids increase immediately post intervention. Principal component analysis of the lipidome also shows a distinction between the early vs 24hrs post-intervention timepoints. We will correlate the plasma lipidomic shifts to CS-related hemodynamic parameters such as lactate, cardiac output and stroke index, and determine the impact on clinical outcomes.

Conclusion

Discovering early lipid biomarkers of disease can allow physicians to identify which patients have CS early in the disease process and allow early interventions and preventive measures, thereby reducing patient morbidity and mortality.

POSTER #50

Neuregulin-1 therapy promotes brain remyelination in mice with chronic demyelination by harnessing the reparative capacity of microglia

Seyyed Mohyeddin Ziaee[1,2], Shiva Nemati[1,2], Hardeep Kataria[1], Seyed Mojtaba Hosseini[1,2], Pejman Ghelich[3], Elisabet Jakova[1,2], Ali Tamayol[3], Soheila Karimi-Abdolrezaee[1,2,4].

[1] Department of Physiology and Pathophysiology, Spinal Cord Research Centre, Rady Faculty of Health Sciences, University of Manitoba Winnipeg, Manitoba, Canada.

[2] Manitoba Multiple Sclerosis Research Center, Rady Faculty of Health Sciences, Winnipeg, Manitoba, Canada.

[3] Department of Biomedical Engineering, University of Connecticut Health Center, Farmington, CT 06030, USA.

4 Children's Hospital Research Institute of Manitoba

Introduction

Prolonged demyelination in progressive multiple sclerosis (MS) leads to white matter degeneration and increased disability. Currently, minimal regenerative treatments are available for P-MS. Microglia show the capacity to facilitate remyelination by clearance of cholesterol-rich myelin debris in acute demyelinating lesions. However, remyelination fails in chronic lesions despite the abundance of microglia. We previously reported downregulation of neuregulin-1 (Nrg-1), a key myelination factor, in MS lesions. Restoring Nrg-1 levels promoted a pro-regenerative phenotype in microglia in acute demyelinating lesions. Here, we examined whether Nrg-1 availability fosters oligodendrocyte maturation and remyelination in progressive MS by enhancing microglial myelin clearance and cholesterol recycling.

Methods

We induced chronic demyelination by cuprizone (CPZ) diet for 10 weeks in PDGFR-Cre mice that allow tracking new oligodendrocytes. PLX5622 [colony stimulating factor 1 receptor inhibitor] was used in diet to deplete microglia. Nrg-1 treatment was delivered subcutaneously. Immunohistochemistry, electron microscopy, and lipid assessment were performed in CPZ mice. Parallel *in vitro* experiments were conducted in primary microglia and oligodendrocyte progenitor cells (OPCs) on nanofibrous scaffolds to assess myelin phagocytosis, lipid metabolism, and remyelination.

Results

Nrg-1 treatment promoted remyelination in chronic CPZ demyelinating lesions by enhancing oligogenesis and oligodendrocyte maturation. These beneficial effects were microglia dependent as they were diminished in PLX5622 treated mice. Nrg-1 also elevated microglia abundance in chronic demyelinating lesions by attenuating their cell death. *In vitro*, Nrg-1 promoted myelin phagocytosis and intra-cellular cholesterol levels in activated microglia, resulting in increased cholesterol release through upregulation of cholesterol efflux transporter ABCA1. Exposure of OPCs to conditioned media from Nrg-1 treated microglia promoted oligodendrocyte maturation and myelination of nanofibers.

Conclusions

We show, for the first time, the promise of Nrg-1 as a potential treatment for promoting repair and remyelination in progressive MS.

POSTER #46

Loss of Crucial Protein-Protein Interactions in Bowen-Conradi Syndrome

Courtney Sanjenko[1,2,5], Trent Nelson[1,2,5], Danielle Isfeld[1,2,3,6], Courtney L. Geer[1,2,3,6], Hannah M. Ziegler-Blair[1,2,3,6], Rafe Helwer[1,2,3], Carthy Caswell[1,2,3], Gol Roberts[4], and J. Michael Charette[1,2,3]

[1]Department of Chemistry, Brandon University, Brandon, Manitoba, Canada. [2]Children's Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada. [3]Paul Albrechtsen Research Institute at CancerCare Manitoba, Winnipeg, Manitoba, Canada. [4]Department of Biology, Brandon University, Brandon, Manitoba, Canada. [5]Co-first authors [6]Co-second authors

Introduction

Ribosomopathies are disorders associated with reduced ribosome assembly, which in turn affects the translation of the genetic code into proteins. Bowen-Conradi Syndrome is a rare genetic ribosomopathy that directly affects children of the Hutterite population. It is characterized by severely reduced growth and development, and results in death during infancy. The main cause of the disease is a D86G amino acid substitution in the ribosome small subunit assembly protein Emg1, which is responsible for the methylation of an important pseudouridine site in the rRNA of the SSU. We believe that the substitution from a charged aspartate molecule to a small glycine amino acid could facilitate changes in protein structure and disrupt normal protein-protein interactions. Here we show that in Bowen-Conradi Syndrome, Emg1 loses its ability to form important connections with itself as a dimer as well as the nucleolar protein Utp2.

Methods

To investigate this possible loss of protein-protein interactions we performed a series of yeast two hybrid experiments involving both human and yeast versions of wildtype Emg1, the Bowen-Conradi variant of Emg1, and Utp2. We then validated these findings via co-immunoprecipitation of the proteins.

Results

We observed that there was a loss of protein-protein interactions between both Emg1-Emg1 and Emg1-Utp2 in Bowen-Conradi Syndrome yeast cells compared to wildtype cells. This was validated by our co-immunoprecipitation results. We believe the formation of an Emg1 dimer is crucial to the catalytic activity of the protein while Utp2 plays an important role in the nucleolar localization of Emg1.

Conclusion

These findings thus strongly suggest that Bowen-Conradi Syndrome is in part due to a loss of critical protein-protein interactions that aid in the formation of the ribosome SSU. This insight has furthered our understanding of the disease and will allow us to move towards evaluating how these interactions may be possibly regenerated or repaired.

POSTER #177

Improving Pediatric Readiness Scores of General Emergency Departments: A Systematic Review

Hoda Badran[1,2], Banke Oketala[1], Clara Tam[1], Kaitlin Hogue[2], Abosede Orebanjo [1], Terry P. Klassen[1,2], Alex Aregbesola [1,2]

[1]The Children's Hospital Research Institute of Manitoba, Winnipeg, MB, Canada
[2]Department of Pediatrics and Child Health, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada

Introduction:
Child mortality, with 7.7 million annual deaths worldwide, underscores the urgent need to enhance pediatric care in general emergency departments (EDs). In North America, children represent one-fifth of all ED visits, yet many EDs lack sufficient readiness for pediatric emergencies. The National Pediatric Readiness Project, utilizing Weighted Pediatric Readiness Scores (WPRS), aims to address this deficiency. However, the effectiveness of interventions like in-situ simulations in enhancing WPRS remains uncertain.

Aims:
This systematic review summarized available evidence regarding the impact of interventional measures aimed at improving the weighted pediatric readiness score (WPRS) of the EDs.

Method:
A comprehensive systematic review searched major databases and grey literature up to November 2023. We included controlled studies in English that were conducted in EDs serving children and utilized intervention measures aimed at improving WPRS. Studies not meeting these criteria were excluded. Screening of eligible studies and quality assessment were performed independently by two reviewers, with discrepancies resolved by a third reviewer. Data extraction, risk of bias assessment and quality assessment were performed using ROBINS-I and GRADE-pro.

Results:
Out of 3,658 papers initially identified through Covidence, four met the study's eligibility criteria. One additional paper was found through a manual search. All five studies were conducted in the United States and employed before-and-after study designs. This review identified six interventions, including customized reports, online resources, simulations, expert consultations, and a pediatric toolkit, that improved WPRS in the EDs. Performance reports were common across all studies and simulations featured in four. The WPRS increased from 12.9% to 17.1% across all the included studies. This improvement was observed from a minimum baseline WPRS of 53.8% to a maximum achieved score of 80%. Evidence certainty was rated as moderate across the studies.

Conclusion:
Our review underscores the efficacy of strategies like customized performance reports and in-situ-simulations to enhance pediatric care in general EDs. The quality of the evidence presented was moderate, emphasizing the need for rigorous randomized controlled trials that can provide valuable insights for future quality improvement initiatives

POSTER #172

Understanding the Impact of Drug Use on the Microbiome and Sexually Transmitted and Blood Borne Infections (STBBI)

Mariana Herrera[1,2], Yoav Keynan[2], Catherine Card[3], Sandra Gonzalez[3], David Alexander[4], Angela Copete[2], Heather Armstrong[5], Zulma Rueda[2]

[1]Postdoctoral Fellow, CIHR Research Excellence, Diversity, and Independence (REDI) Early Career Transition Award., [2]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Canada., [3]JC Wilt Infectious Diseases Research Centre, National Microbiology Laboratories, Public Health Agency of Canada., [4]Cadham Provincial Laboratory., [5] Internal Medicine, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada.

Context

People living with HIV (PLHIV), people who experience homelessness (PWEH), and people who use drugs (PWUD) are overlapping populations who commonly face social and structural inequalities and barriers that affect their health and well-being. Injection drug use can significantly increase the risk of HIV/STBBI transmission if people share injection supplies or due to other factors, such as heavy alcohol consumption and sexual behaviors.

The microbiome is an essential aspect of understanding HIV/STBBI acquisition. Still, few studies have longitudinally examined microbiomes composition in PLHIV and PWEH. In general, the connection between drug use, the microbiome, and HIV/STBBI (risk of infection, treatment outcomes, progression) is poorly understood. The **goal** is to understand whether drug use is associated with changes in the gut, genital, and upper respiratory tract microbiome of women, men, and gender diverse PLHIV and PWEH, the risk of new STBBI infections, and the outcomes of antiretroviral treatment.

Methodology

In collaboration with Dr. Zulma Rueda, I will invite women, men, and gender diverse PLHIV (n=345) and PWEH (n=338) to participate in two cohorts in Winnipeg starting in July 2024. We will diagnose 14 STBBIs (including HIV). I will collect sociodemographic, behavioral (drug, cigarettes, tobacco, and liquor use, number of partners, etc.), and clinical information. I will also collect information about variables that could modify the microbiomes, like diet, contraception methods, and comorbidities. At baseline and during the follow-ups (months 3, 6, 9, and 12), I will collect genital, respiratory, and fecal samples from participants. I will characterize the microbiome using PCR amplification of the 16S rRNA gene 515f-806rB (V4) region.

The prevalence and incidence of drug use, HIV/STBBI and the HIV treatment outcomes will be reported disaggregated by year, age, gender, sex at birth, etc. We will use multivariable models to evaluate the association of the microbiome diversity (alpha y beta) and the groups, and the relation between drug use and antiretroviral treatment outcomes.

Expected Impacts

Improve adherence to antiretroviral therapy and the antiretroviral treatment outcomes in PLHIV who use drugs. Decrease the frequency of HIV/STBBI acquisition in key populations. Microbiota-based therapies as an innovative area of research with great potential for HIV/STBBI prevention.

POSTER #174

Dysregulation of Neuregulin-1 induces brain demyelination and cognition decline: implications for progressive multiple sclerosis.

Elisabet Jakova [1,2,] Shiva Nemati [1,2], Seyyed Mohyeddin Ziaee [1,2], and Soheila Karimi-Abdolrezaee [1,2,3.]

[1]Department of Physiology and Pathophysiology, Spinal Cord Research Centre, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba., [2]Manitoba Multiple Sclerosis Research Center, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba., [3]Children's Hospital Research Institute of Manitoba.

Introduction

Multiple Sclerosis (MS) is a complex disease where the immune system attacks the protective myelin sheath that covers neurons in the central nervous system (CNS) causing neurological impairments including cognitive declines and anxiety. In early MS, remission happens through myelin repair (remyelination) by neural precursor cells (NPCs). As disease progresses, remyelination fails due to a decline in the number and capacity of NPCs that lead to neurodegeneration. We have previously reported that Neuregulin-1 (Nrg-1) protein level is downregulated in chronic human MS lesions as well as in animal models of MS. Nrg-1 is a key regulator of NPCs and an important factor for myelination. Therefore, we hypothesize that depletion of Nrg-1 contributes to progressive neurodegeneration and a decline in remyelination in chronic MS. Here, we aimed to uncover the impact of Nrg-1 dysregulation on MS progression through a loss of function approach.

Materials

In a tamoxifen-inducible $Nrg1^{fl/fl}$:Cre^{ERTM} conditional knockout (cKO) C57/BL6 J mouse model, we performed time course longitudinal neurobehavioral tests including Y-Maze and Novel Object Recognition for hippocampal-dependent spatial working memory, Open-Field and Light/Dark Box general locomotor activity and anxiety. In parallel *in vivo* and *in vitro* studies were also performed to determine the impact of Nrg-1 deletion on neurodegeneration, inflammation, demyelination, and NPC properties in the brain.

Results

Nrg-1 cKO mice showed significant spontaneous brain demyelination and neuroinflammation at 16 weeks post tamoxifen injections compared to the Cre-negative wild type mice. These pathological changes in Nrg-1 Cre cKO mice were also accompanied by hippocampal spatial and working memory deficits. These mice spent more time in the corners of the Open-Field and the dark chamber of the Light/Dark box test suggestive of increased anxiety. Interestingly, NPCs isolated from the Nrg-1 Cre cKO mice showed lower self-renewal, proliferation and stem cell activity as compared to the wildtype mice.

Conclusion

Our findings signify a functional impact for the dysregulated levels of Nrg-1 that we have observed in chronic MS lesions and its contribution to remyelination failure in P-MS. These results also underscore the potential of Nrg-1 treatment in promoting myelin repair and cognition recovery in progressive MS.

POSTER #173

Resting Brain Network Changes Following 12-Week Dual-Task Training in Parkinson's Disease—Preliminary Findings from an Ongoing Clinical Trial

Tiffany A. Kolesar,[1,2] Kavisha Mehta,[3] Cherry Kit-Fong Hin,[3] Eun Hyung Choi,[1,2] Neil Junankar,[1,2] Jarrad Perron,[2,4] Tony Szturm,[3,4*] Ji Hyun Ko[1,2,4*]

[1]Department of Human Anatomy and Cell Science, University of Manitoba, Winnipeg, MB., [2]PrairieNeuro Research Centre, Health Science Centre, Winnipeg, MB., [3]College of Rehabilitation Sciences, University of Manitoba, Winnipeg, MB., [4]Graduate Program in Biomedical Engineering, University of Manitoba, Winnipeg, MB.

Introduction

Gait/balance and cognitive impairment in Parkinson's disease (PD) can be debilitating symptoms that inconsistently respond to anti-Parkinsonian medication. Cognitive reserve hypothesis suggests that gait function unusually relies on the prefrontal brain network in PD to compensate for impaired basal ganglia circuitry, which makes it more difficult for people with PD to perform dual task (DT; i.e., mechanical walking while performing other cognitive tasks such as navigation, conversation, or videogaming). This can exacerbate freezing of gait and/or falls. We previously demonstrated that DT-training (videogaming while treadmill walking) significantly improves gait and cognition in PD. However, we do not yet know the underlying neurophysiology associated with these improvements. In this ongoing randomized clinical trial, we are implementing a 12-week DT training program to simultaneously target gait and cognition in people with PD, and to assess the underlying neural mechanisms of this treatment using neuroimaging.

Methods

People with PD (H&Y stages II-III) are randomly assigned to control or DT groups. Both groups undergo training three times per week for 60 minutes. The DT group practices treadmill-walking while playing cognitively challenging videogames with a head-mounted, remote-controlled mouse. Conversely, the PD control group completes a single task at a time (i.e., playing videogames while standing, followed by treadmill-walking only). Various neuroimaging data is collected at baseline and following the 12-week exercise program to include positron emission tomography (PET) data, and functional magnetic resonance imaging (fMRI) data to investigate neural function during tasks, and at rest. Neuropsychological and motor/gait data are also collected at baseline and follow-up assessments. Age-matched healthy controls will be recruited to compare baseline functioning.

Results

While data collection is ongoing, preliminary fMRI results (n = 18) suggest that DT-training in the DT group results in increased neural function within the resting brain in regions necessary for motor/somatosensory function. Importantly, this increase was not observed in the PD control group.

Conclusion

Early results indicate increased resting neural activity within motor/somatosensory regions, following 12-weeks of simultaneous challenging motor and cognitive tasks, but not when these tasks were completed separately. Exercise treatment programs that tax multiple domains may provide the most clinical benefit in PD.

POSTER #169

How effective are dietary interventions in the management of metabolic acidosis in adult chronic kidney disease? A systematic review and meta-analysis of randomized controlled trials

Sepideh Mahboobi[1,2], Rebecca Mollard [1,2], Navdeep Tangri[2,3], Nicole Askin[4], Thomas Ferguson[2], Tahmina Rahman[1], Rasheda Rabbani[5], Ahmed M. Abou-Setta[6], Dylan Mackay[1,7]

[1]Department of Human Nutritional Sciences, Faculty of Agriculture and Food Sciences, University of Manitoba, Winnipeg, MB, Canada., [2]Chronic Disease Innovation Center, Seven Oaks Hospital, Winnipeg, Manitoba, Canada., [3]Department of Internal Medicine, Max Rady Faculty of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada., [4]Neil John Maclean Health Sciences Library, University of Manitoba, Winnipeg, Manitoba, Canada., [5]George and Fay Yee Centre for Healthcare Innovation, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada., [6]Department of Community Health Sciences, Max Rady Faculty of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada., [7]Department of Internal Medicine, Section of Endocrinology, Max Rady Faculty of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada.

Introduction

Metabolic acidosis defined as serum bicarbonate concentrations <22 mmol/L is one of the first complications of kidney failure which results in further disease progression. Alkali therapy has been used to treat metabolic acidosis for decades. However, some concerns have been raised regarding its safety and long-term tolerability. Existing data suggest that dietary interventions can be beneficial in the management of chronic kidney disease (CKD). This systematic review and meta-analysis aim to summarize findings from studies comparing dietary interventions focused on adding base via plant-based diets or dietary interventions focused on lowering acid load, versus placebo/usual care/no treatment in the management of metabolic acidosis in outpatient adults with CKD.

Methods

Inclusion criteria were randomized controlled trials, with adult participants with CKD and estimated glomerular filtration rate (eGFR) between 15 and 40 ml/min/1.73 m^2 and serum bicarbonate levels of 14-24 mEq/L. Our primary outcome measure was change in serum bicarbonate. Any dietary intervention looking to manipulate dietary acid load was considered as an intervention. Medline, Embase, Cochrane Central, CINAHL, and Web of Science Core Collection were searched. Data screening and extraction were performed by two independent reviewers. Random effects meta-analysis was performed to pool data.

Results

Eight studies were included in our narrative synthesis and 6 studies (n=635) were included in the meta-analysis. Dietary interventions resulted in a clinically significant improvement in serum bicarbonate (mean difference (MD):2.98, 95% CI: [0.77, 5.19]; I^2: 91%) and higher eGFR levels (MD:3.16, 95% CI: [0.24, 6.08] (MD: 3.19, 95%CI: [0.24, 6.08], I^2: 67%) in follow up, compared to controls. Serum potassium, albumin and body mass index and need for renal replacement therapy remained unchanged. Subgroup analyses indicated a superiority of plant-based interventions over non-plant-based interventions in the improvement of acid-base balance and eGFR.

Conclusion

Our findings support the beneficial effects of dietary interventions aimed at reducing acid or adding base in the management of metabolic acidosis, kidney-related markers, blood pressure, calcium and phosphate with no adverse effects on serum potassium and nutritional status. Well-designed clinical trials looking at the treatment of metabolic acidosis with plant-based interventions are required.

POSTER #171

Specific Dietary Protein Sources Reduce Hepatic Steatosis Independently of Body Weight

Fadi H J Ramadan [1,2], Luis Cordero-Monroy [2], Samritha Raman Sivakumar [2], Peter Zahradka [2,3], and Carla Taylor [1,2,3].

[1]Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada., [2]Canadian Centre for Agri-Food Research in Health and Medicine, St. Boniface Hospital Research Centre, Winnipeg, MB R2H 2A6, Canada., [3]Department of Physiology and Pathophysiology, University of Manitoba, Winnipeg, MB R3E 0J9, Canada.

Introduction

Nonalcoholic fatty liver disease (NAFLD) or hepatic steatosis is characterized by triacylglycerol (TAG) accumulation exceeding 5% of liver weight. Given the liver's crucial role in metabolism, NAFLD is closely linked to insulin resistance. Currently, reducing liver fat content through weight loss is the only feasible approach for managing NAFLD and its associated conditions. High-protein (HP) diets have gained significant attention among researchers for their claimed ability to improve metabolic states and reduce body weight, and evidence suggests they may have the potential to decrease hepatic fat content. The aim of this study was to evaluate the effect of HP diets containing animal and/or plant protein sources on NAFLD development in *fa/fa* Zucker rats.

Methods

Obese *fa/fa* Zucker rats received HP diets containing egg white, plant (soy + pea protein, 1:1), mixed (egg white + soy + pea protein, 2:1:1), or casein (HPcasein) as the protein source, or a normal protein (NPcasein) diet for 8 weeks. Body weight was obtained weekly. Body composition and oral glucose tolerance testing were performed at week 8. At the end of the study, livers were removed, weighed, and liver TAG content was analyzed. Serum glucose and insulin were measured.

Results

The HP plant and mixed diets led to a significant 1.2-fold weight gain compared to HPcasein. There were no differences among the groups for fat and lean body composition. HP diets containing egg white, plant, or mixed sources reduced the liver-body weight ratio, and liver TAG by 2.3, 2.1, and 3.6-fold, respectively, compared to HPcasein, and this was associated with decreased insulin resistance.

Conclusions

HP diets containing egg white or plant sources were effective in reducing hepatic steatosis and insulin resistance independently of weight gain. Interestingly, the mixed diet, which has a significant amount of egg white protein, was also successful in reversing steatosis. This suggests that egg white protein could be a good option for improving NAFLD in humans and this requires further testing in a clinical trial.

POSTER #175

Changes in cyclooxygenase 2 levels following hypoxia exposure result in reduced immunoprivilege of allogeneic mesenchymal stem cells

Niketa Sareen, Ejlal Abu-El-Rub, Hania I. Ammar, Weiang Yan, Glen Lester Sequiera, Meenal Moudgil, Elika Verma, Sanjiv Dhingra

Department of Physiology & Pathophysiology, Institute of Cardiovascular Sciences, St. Boniface Hospital Albrechtsen Research Center, University of Manitoba, Winnipeg, Canada

Introduction

Mesenchymal stem cells (MSCs) from allogeneic sources have the potential to treat multiple disorders. However, according to recent animal studies and clinical trials immunogenicity and poor survival of transplanted MSCs impairs their efficacy for regenerative applications. MSCs are immunoprivileged under in vitro conditions, however, after transplantation, MSCs are targeted by the host immune system in the ischemic tissues. We performed in vitro and in vivo (rat myocardial infarction [MI] model) studies to elucidate the mechanisms responsible for change in MSCs from immunoprivileged to immunogenic state.

Methods

MSCs were cultured under normal or hypoxic conditions (0.4%O_2, 24hr), with or without proteasome inhibitor (MG132) and siCSN5 RNA. Following this, levels of immunosuppressive molecules cyclooxygenase-2 (COX2) and prostaglandin E2 (PGE2) were assessed using western blot and ELISA, respectively. Activation of immune response was evaluated using flow cytometry analysis after co-culturing leukocytes with MSCs lacking, or over-expressing COX2. Finally, COX2 over-expressing MSCs were transplanted in rat MI model to assess their survival and immunophenotype in vivo.

Results

We found that PGE2 levels decreased in MSCs following exposure to hypoxia. Further, we found that proteasome-mediated degradation of COX2 (rate limiting enzyme in PGE2 biosynthesis) in hypoxic MSCs is responsible for PGE2 decrease and loss of immunoprivilege of MSCs. While investigating the mechanisms responsible for COX2 degradation in hypoxic MSCs, we found that in normoxic MSCs, COP9 signalosome subunit 5 (CSN5) binds to COX2 and prevents its degradation by the proteasome. However, exposure to hypoxia leads to a decrease in CSN5 levels and its binding to COX2, rendering COX2 protein susceptible to proteasome-mediated degradation. This subsequently causes PGE2 downregulation and loss of immunoprivilege of MSCs. Maintaining COX2 levels in MSCs preserves immunoprivilege in vitro and improves the survival of transplanted MSCs in a rat model of MI.

Conclusion

This data provides novel mechanistic evidence that PGE2 is downregulated in hypoxic MSCs causing post-transplantation rejection of allogeneic MSCs. This suggests that new strategies that target CSN5-COX2 signaling may improve survival and utility of transplanted allogeneic MSCs in the ischemic heart.

POSTER #176

Plasmid transmitted small multidrug resistant (SMR) efflux pumps differ in gene regulation and expression profiles under exposure to stress

Carmine J. Slipski[1], Denice C. Bay[1]

[1] Medical Microbiology and Infectious Diseases, University of Manitoba, Canada.

Introduction

Antimicrobial resistance (AMR) is an increasing health threat globally. The majority of AMR research focuses on antibiotics, while surface disinfectants and antiseptics, such as Quaternary Ammonium Compounds (QACs), are often overlooked. QACs and disinfectants are far less regulated than antibiotics and used in 100 times the annual tonnage in North America. QAC resistance is primarily conferred by efflux pumps with unique selectivity for these compounds, such as the Small Multidrug Resistance (SMR) family. Therefore, understanding mobile regulation of these genes is critical for monitoring resistance and may provide important environmental markers.

Methods

The upstream 500nt region of plasmid-borne SMR sequences were surveyed across several databases. Plasmid constructs were engineered with upstream promotors (Ptac, Pc, or Pq) with a *lacZ* reporter gene for monitoring expression levels after stress exposure. Constructs were transformed into *Escherichia coli (E. coli)* BW25113 and grown established either planktonically (18 Hrs) or as biofilms (24 Hrs). Exposure to each stress condition was performed for 3 Hrs before measuring expression levels under each promotor by Miller assay quantification of *lacZ* expression. Stress conditions included 37°C (control), 42°C (heat-shock), 1 mM H_2O_2 (oxidative stress) and sub-inhibitory QAC exposure. Each promotor construct had 3 biological replicates and 8 technical replicates for statistical analysis with empty vector (no induction) and 0.05 mM IPTG induction of Ptac promotor as controls across each condition.

Results

SMR clades are divided into primarily Qac (qacE, qacEΔ1, qacF, qacG, qacH) and Gdx (or sugE (p)) members. By surveying the upstream 500nt region, it was determined that mobile Gdx sequences were regulated by guanidinium riboswitch (primarily class II) and Qac sequences regulated by Class I integron promoters Pc and Pq. Beta-galactosidase assays for planktonic and biofilm growth exposed to stress conditions heat, peroxide, and QAC, demonstrated that Pq and Pc have varying expression under different bacterial growth conditions. Sub-inhibitory QAC exposure generated the highest expression levels in both Pc and Pq.

Conclusions

This study revealed the mechanisms for plasmid-encoded SMR gene regulation and that promotors Pq and Pc vary in their expression levels under exposure to specific stress conditions in *E. coli* that differ when grown planktonically or as biofilms.

POSTER #170

Germanane quantum dots suppress inflammation by promoting metabolic reprogramming of immune cells towards regulatory T cells

Abhay Srivastava[1,2]†, Alireza Rafieerad[1,2]†, Weiang Yan[1,2], Keshav Narayan Alagarsamy[1,2], Niketa Sareen[1,2], Leena Regi Saleth[1,2], Michael Aliani[2], Sanjiv Dhingra[1,2],*

[1]Regenerative Medicine Program, Department of Physiology and Pathophysiology, Rady Faculty of Health Sciences, Biomedical Engineering Program, University of Manitoba, Winnipeg, Manitoba, R3E 0W2, Canada

[2]Institute of Cardiovascular Sciences, St. Boniface Hospital Albrechtsen Research Centre, University of Manitoba, Winnipeg, Manitoba, R2H 2A6, Canada

† These authors are co-first authors and contributed equally to this work.

Introduction

Recently, nanomaterials have emerged as a tool in developing novel therapies against inflammatory diseases. Metabolic changes in immune cells direct the phenotype and function of host immune system. Therefore, next generation immunomodulatory biomaterials should be designed to target metabolic pathways and trigger specific changes in immune cells to direct their fate toward anti-inflammatory phenotype. Current study reports fabrication and first application of germanane quantum dots (GeHQDs) to modulate inflammation in cell culture and in vivo mouse model.

Methods

Using rational design and synthesis strategies, our GeHQDs leverage the intrinsic anti-inflammatory properties of germanane to provide a novel nanoplatform to trigger metabolic reprogramming of immune cells toward anti-inflammatory phenotype. This was tested by activating immune cells in vitro using PHA/CD3Ɛ-CD28 and evaluating their response using flow cytometry, cytokine analysis, gene expression and metabolomics. We further tested our hypothesis in LPS treated septic mouse model by injecting GeHQDs through tail vein.

Results

These GeHQDs are spontaneously uptaken into the immune cells and trigger a switch in their phenotype toward regulatory T (Treg) cells. Metabolomic analysis suggested a downregulation in glycolytic flux and upregulation in fatty acid oxidation with an increase in mitochondrial-respiration in GeHQDs treated group, which is typical signature of Treg cells. GeHQDs regulated immune cells' interactions to create an anti-inflammatory environment. In an in vivo mouse model of systemic inflammation, GeHQDs treatment upregulated the circulating Treg cell number, corrected metabolomic profile and downregulated inflammation.

Conclusion

Current study presents a new paradigm in targeting inflammatory diseases by modulating immune cell metabolism using next generation nanomaterials.

POSTER #182

Incidence and outcomes of meningiomas in the province of Manitoba.

Jaydon Gan[1], Nikunj Patil[2], Jai Shankar[3], Saranya Kakumanu[2], Namita Sinha[4]

[1]Med 2 Medical Student (Class of 2026), Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada [2]Division of Radiation Oncology, Department of Radiology, CancerCare Manitoba, University of Manitoba, Winnipeg, Manitoba, Canada [3]Department of Radiology, University of Manitoba, Shared Health, Winnipeg, Manitoba, Canada [4]Department of Pathology, University of Manitoba, Shared Health, Winnipeg, Manitoba, Canada

Introduction:
Meningioma is the most common primary brain tumour in adults that originates from the meninges. Meningiomas constitute around 26% to 38% of all primary intracranial tumours and are graded by the World Health Organization (WHO). Although most meningiomas are benign (WHO grade I), up to 35% of meningiomas can be atypical (WHO grade II), and < 2 % are classified as malignant or anaplastic meningioma (WHO grade III). Pathological evaluation is the mainstay for grading tumors. However, surgical intervention is required for sample/specimen collection. While most meningiomas are observed conservatively, symptomatic meningiomas undergo surgical resection with or without radiotherapy. The outcome of patients with meningiomas is dependent on their pathological grade and treatment. Our study aims to assess the incidence of meningiomas in the province of Manitoba and their outcome.

Methods:
A retrospective analysis was performed on all patients with surgically restricted, pathologically proven meningioma diagnosed at Health Sciences Center, Manitoba, between 2011 and 2021. The demographic data, neuropathological information, treatment, and follow-up details were collected. The descriptive variables were tabulated, and the incidence rates were analysed.
Furthermore, cox/Poisson regression analysis will also be used to find significant features that predict patient outcomes.

Results:
Over the 10 years of study period, we had 508 patients with pathologically proven meningioma in our institution. Meningiomas were found to be more common in females than males (315 vs 193). Most meningiomas were grade 1(n=347; 68.3%), followed by grade 2 (n=121; 23.8%) and grade 3 (n=9; 0.02%). The grading was not available for 31 patients, primarily due to the absence of brain tissue to evaluate for brain invasion. Based on this, the calculated incidence was 51 patients per year with pathologically proven meningioma and the incidence rate of meningiomas in our province was 0.046 (95% CI- 0.042- 51) cases per 1000-person year. The average age of these patients was 59.98 (±13.96) years. The outcome of these patients is still being evaluated.

Conclusion:
Pathologically proven meningiomas are relatively rare in our population, with an incidence rate of 0.046 cases per 1000-person year. These are more common in females, and the majority of them are grade 1 tumors.

POSTER #183

Targeting Apoptosis Resistance in Glioblastoma: Efficacy of ARTS Mimetics with Autophagy Inhibitors as Novel Therapy

Esha Kaul[1], Emma Martell[2,3], Vinith Yathindranath[4], Helgi Kuzmychova[2], Harshal Senthil[2], Ujala Chawla[2], Sheila K Singh[5,6], Donald Miller[4], Tanveer Sharif[2]

[1]Faculty of Science, University of Manitoba; [2]Department of Pathology, Max Rady Faculty of Health Sciences, University of Manitoba; [3]Department of Human Anatomy & Cell Science, Max Rady Faculty of Health Sciences, University of Manitoba; [4]Department of Pharmacology and Therapeutics, Max Rady Faculty of Health Sciences, University of Manitoba; [5]Department of Surgery, Faculty of Health Sciences, McMaster University; [6]Department of Biochemistry and Biomedical Sciences, Faculty of Health Sciences, McMaster University

Introduction:

Glioblastoma (GBM) is the most common malignant brain tumor in adults, characterized by its resistance to treatment and poor prognosis. The current standard of care for GBM patients involves maximal surgical resection followed by chemotherapy and radiation. However, despite aggressive treatment, the average 5-year survival rate for GBM patients remains dismal at approximately 5%. One of the main reasons for the poor therapy response in GBM is the resistance to programmed cell death, or apoptosis. Resistance to apoptosis in GBM is often mediated by the upregulation of inhibitors of apoptosis proteins (IAPs), which hinder the action of pro-apoptotic proteins and promote cell survival even under conditions of severe DNA damage.

Methods:

We aimed to investigate the efficacy of utilizing ARTS mimetics as a novel strategy to overcome apoptosis resistance in GBM treatment. We utilized patient-derived GBM cells with exogenous gain-of-function (GOF) overexpression to introduce full-length ARTS protein, as well as pharmacological small peptide mimetics of ARTS, to evaluate the response of GBM cells following the promotion of ARTS activity. Additionally, we employed a patient-derived orthotopic xenograft mouse model to test novel combinatorial strategies in vivo. We used a nanoparticle drug delivery system to ensure treatment penetration across the blood-brain barrier (BBB).

Results:

Our results showed that patient-derived GBM cells were highly resistant to ARTS-induced apoptosis. Further molecular investigation unveiled a novel role of ARTS in the upregulation of autophagy as a resistance mechanism. A combination of ARTS GOF or mimetic peptides with autophagy inhibitors mitigated resistance to ARTS-mediated apoptosis and drastically decreased GBM cell proliferation. This combination was further tested in the patient-derived xenograft mouse model, which demonstrated the successful delivery of ARTS mimetic peptides and autophagy inhibitors through the BBB via nanoparticle lipid carriers. Animals treated with a combination of the autophagy inhibitor Spautin-1 and ARTS mimetic peptides demonstrated significantly prolonged survival, confirming the therapeutic potential of such a combinatorial approach for GBM treatment.

Conclusion:

Altogether, these findings uncover a previously unknown role of ARTS in autophagy regulation and provide evidence that the combination of IAP-antagonist mimetics with autophagy inhibitors can be an effective strategy against highly aggressive GBM brain tumors.

POSTER #186

Sex Determines Bronchodilator Effectiveness of Albuterol in a Murine Model of Allergic Asthma

Sparshita Mishra[3,4,5], Sujata Basu[1,3,4,5] & Andrew J Halayko[1,2,3,4,5]

Department of Physiology and Pathophysiology[1], Internal Medicine[2], Biology of Breathing Group[3], Childrens Hospital Research Institute of Manitoba[4], University of Manitoba, Winnipeg, MB[5].

Introduction

Globally 4.9 million children under the age of 18 have asthma, including 12% boys and 7% girls. Inhaled bronchodilators are primary therapeutic tools; however, some patients exhibit insensitivity. In this study, we optimized a protocol to assess bronchodilator responsiveness in a murine model of allergic asthma and compared effects in male and female mice.

Methods

Female and male BALB/c mice (8 week) were challenged with intranasal house dust mite (HDM) (25µg/35µL) 5 days/week for 2 weeks. Mice were subjected to lung function testing (Scireq flexiVENT) with nebulized methacholine (Mch) (25, 50 and 100mg/mL) alone or after albuterol (0.1mg/mL).

Results

Percent increase of bronchoconstriction in total lung resistance (Rrs) with Mch alone from baseline by 25, 50 and 100 mg/mL was increased in females by 72, 81 and 85% compared to 62, 70 and 72% in male mice. Percent inhibition of bronchoconstriction by albuterol was evident, as it prevented the increase in Rrs caused by 25, 50 and 100mg/mL Mch, by 55, 52 and 47%, in female mice. In contrast, albuterol improved Rrs by 20, 8 and 9%, respectively in male mice. Similarly, albuterol reduced Newtonian resistance – a measure of airway reactivity – by 46, 31 and 8 %, in females, but was without effect in male mice. A similar pattern was seen for measures of small airway function (tissue damping) and lung stiffness, with albuterol reducing responses to all concentration of Mch by more than 100-200% in female mice.

Conclusions

Albuterol improves overall lung function in allergen challenged mice. There is a sex-dependent response to albuterol, being significantly greater in female mice. These findings indicate that sex is a determinant of bronchodilator response in murine models of allergic asthma.

POSTER #181

Investigating the therapeutic potential of interferon lambda during neutrophil-driven inflammation in the female genital tract

Claudia Camila Oda Jallime[1,4], Marina Costa Fujishima[1], Deesha Nayar[1], Nicole Thompson[2], Morgan Taverner[3], Hubert Sidarta[1,4], Warren Wakarchuk[2], Deanna M. Santer[1] and Thomas T. Murooka[1, 3]

[1]University of Manitoba, Rady Faculty of Health Sciences, Department of Immunology, Winnipeg
[2]University of Alberta, Faculty of Science, Department of Biological Sciences, Edmonton
[3]University of Manitoba, Rady Faculty of Health Sciences, Department of MMID, Winnipeg
[4]University of Manitoba, Faculty of Science, Winnipeg

Introduction

The mucosal surface of the female genital tract (FGT) consists of physiochemical, immunological, and microbial components that play a pivotal role in the defense against foreign pathogens. Disruption of the FGT barrier has been associated with increased acquisition risk of sexually transmitted infections (STIs) such as HIV and HPV. A *Lactobacillus*-dominant (optimal) vaginal microbiome is associated with favorable health outcomes, whereas the presence of a diverse community of anaerobes species (or non-optimal microbiome) is associated with high inflammatory signatures and adverse disease outcomes. Epidemiological data from >700 women from the CAPRISA004 cohort show that women with a non-optimal microbiome were at significantly higher risk of sexual HIV acquisition (HR=2.0), which strongly correlated with biological signatures associated with vaginal epithelial barrier disruption, inflammation, and neutrophil accumulation. To better understand the immunological basis of host-pathogen interactions within the lower FGT, our laboratory has published that mucosal neutrophils are the main contributors to barrier breakdown in the presence of non-optimal species using a murine bacterial challenge model. We hypothesized that interferon lambda (IFN-λ), a potent gut anti-inflammatory factor, would similarly decrease inflammation and restrict neutrophil infiltration to restore vaginal barrier health in our *in vivo* model.

Methods

Female Balb/c mice were intravaginally inoculated with either PBS or *Mobiluncus mulieris* (non-optimal), with vaginal treatment with either PBS + 0.1% BSA or 2-5ug recombinant IFN-λ3 over 7 days (4 total applications). Vaginal tissues were collected for immunohistochemical analyses to measure neutrophil accumulation (anti-Ly6G), and vaginal epithelial thickness and integrity, the latter using a fluorescent marker applied into the vaginal lumen prior to tissue harvest.

Results

IFN-λ3 treatment restored vaginal barrier function in the presence of inflammatory *M. mulieris*, which was associated with a dramatic reduction in neutrophil influx into the vaginal epithelial layer. Short-course vaginal IFN-λ3 treatment was well tolerated with no adverse reactions observed.

Conclusion

Our pilot study shows that vaginal application of IFN-λ3 has therapeutic benefit by resolving neutrophil-driven inflammation and restoring vaginal barrier integrity. These observations underscore the therapeutic potential of IFN-λs in dampening chronic inflammation in women with a non-optimal vaginal microbiome and reduce STI acquisition risk.

POSTER #185

Neuroligin-1 interaction and modulation of mGluR2 *in trans*

Hanok H. Tadele, Shayan Amiri, and Henry A. Dunn

Department of Pharmacology and Therapeutics, University of Manitoba
Division of Neurodegenerative Disorders, St. Boniface Hospital Albrechtsen Research Centre

Introduction:

Recent advancements in G protein-coupled receptor (GPCR) neurobiology have identified GPCRs in trans-synaptic complexes with synaptic adhesion molecules; however, the consequences of these interactions on GPCR pharmacology are predominantly uncharacterized. Interestingly, neuroligin-1 (NLGN1) soluble ectodomain was found to interact with and modulate metabotropic glutamate receptor 2 (mGluR2): suggestive of the potential for a trans-synaptic complex between two known proteins associated with autism spectrum disorder (ASD). Additionally, 6 genetic variants have been found in the NLGN1 ectodomain of autistic patients; however, their contribution to mGluR2 binding remains to be explored. We propose to investigate the nature of mGluR2 and NLGN1 trans-synaptic complexes: with the hypothesis that some autism associated NLGN1 variations may alter their trans-synaptic relationship.

Methods:

Using separate cell populations, we transiently transfected either empty plasmid or myc-NLGN1 and mixed these cell lysates with cells transiently expressing human mGluR2. We also used myc antibody for immunoprecipitation, and mGluR2 antibody for Western blotting to determine binding efficacy between the 2 molecules. For expression level comparison between autistic mutant P89L-NLGN1 and WT-NLGN1 we performed titration experiments to determine the cDNA conditions whereby NLGN1 and P89L expression levels were comparable.

Results:

Full-length NLGN1 was able to co-immunoprecipitate mGluR2 from another cell population: suggestive of their interaction *in trans.* We also demonstrate that autism mutant P89L-NLGN1 exhibits >85% reduction of expression levels compared to “wildtype” NLGN1: predominantly in the mature (glycosylated) form. Titration experiments of different levels of NLGN1 DNA determined the condition where NLGN1 and P89L-NLGN1 expression were comparable and determined DNA needed to be provided in a 2:5 ratio to begin functional characterizations.

Conclusion:

Under these standardized conditions, we plan to compare the ability of P89L-NLGN1 to bind to mGluR2 in comparison to “wildtype” NLGN1, and their functional consequence on mGluR2 pharmacology using a recently developed transcellular GPCR signalling assay platform. In conclusion, our study should provide a greater understanding of trans-synaptic interactions between NLGN1 and mGluRs in synaptic neurobiology and begin to explore the potential of mGluR2 as a pharmacological target for autistic patients harbouring genetic variations in NLGN1.

POSTER #180

Application of the Delphi Consensus Building Method to Formulate Genetic Assistant Competencies

Tkach, Kailey[1,2]; Krutish, Angela[2,3]; Hartley, Jessica N.[2,3]

[1]Faculty of Science, University of Manitoba, Winnipeg, Manitoba, Canada. [2]Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Manitoba, Canada. [3]Childrens Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada.

Introduction:

Genetic assistants (GAs) have been implemented in the genetics workforce to take on tasks regularly assigned to genetic counsellors (GCs) that do not require their Masters' level training, allowing GCs to spend more time with patients.

Methods:

This project aimed to create a generalizable list of competencies required for GAs with one year of experience, appropriate training, and supervision by a higher-level practitioner to successfully complete tasks assigned in a variety of settings. Consensus was built through three rounds of surveys using a classic Delphi method among individuals with extensive first-hand or research experience with GA positions.

Results:

In total, 37 individuals expressed interest in the study and, of those between 29-31 completed each iteration and 24 completed all iterations (65% retention rate) while three individuals did not complete any. Through the first iteration of the study, 32 sub-competencies were drafted based on a task list our team previously developed. After the second and third iteration surveys, 28 of these sub-competencies reached consensus for inclusion (based on a predetermined threshold of 70%) while four did not reach consensus. Three of the four competencies not meeting consensus were research-based and were noted by participants to be more applicable for research assistants than GAs.

Conclusion:

The sub-competencies developed in this project contribute to standardization of the GA role across multiple work settings and specialties, which will eventually allow for easier implementation of GA positions in genetics services, and development of content for training and continuing education.

POSTER #179

Examination of RTT-associated phenotypes in a mouse model of Rett Syndrome

Brian Toor[1], Khatereh Saei Arezoumand[1], Abbas Rezaeian Mehrabadi[1], Arsalan Alizadeh[1], Ghanan Bin Akhtar[1], Marjorie Buist[1], and Mojgan Rastegar[1]

[1]Department of Biochemistry and Medical Genetics, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada

Introduction

Rett Syndrome (RTT) is a childhood neurodevelopmental and neurometabolic disorder that typically affects females with symptoms presenting as early as 6-18 months and diagnosis between 2-4 years of age. RTT is presented by various symptoms such as gait abnormalities, motor impairment, stereotypical hand-wringing, loss of speech, and breathing abnormalities. As there is no cure, metabolic drugs such as statins and metformin are emerging as therapeutic strategies for brain and neurological disorders; therefore, these treatments are also promising therapies for RTT. As a result, in this study, we explore the effect of simvastatin in mouse models with the most common RTT-causing mutation in the *MECP2* gene: the missense T158M mutation in the methyl-binding domain.

Methods

We assess the body weight and different phenotypic scoring data such as activity/mobility, gait, hind-limb clasping, tremor, general condition, and breathing to analyze potential improved symptoms in untreated and treated (vehicle and 100 mg/kg simvastatin) RTT female and male mice, while also exploring potential sex-dependent effects.

Results

We established that untreated male mutant mice are lighter than wild types with similar weight gain. In contrast, female mutants were more obese, although the female mice had minimal weight change, suggesting a potential sex-dependent effect for RTT. Male and female mutants also had significantly worse phenotypes for RTT in most scoring criteria. We observed that simvastatin-treated male mutants had a different trend of treatment impact on their weight compared to female mutant mice, suggesting a possible sex-dependent effect. We lastly observed various scoring criteria differences between simvastatin-treated male and female mutants, serving as evidence for a potential biologically dependent impact of RTT-like phenotype presentation.

Conclusion

These findings help support the potential applicability of simvastatin as a therapeutic drug for Rett Syndrome.

POSTER #184

Deciphering the Oxidized Phospholipid-Protein Kinase C Signalling Axis in Human Airway Smooth Muscle

Nathan B. Varghese[3], Jignesh Vaghasiya[1,3], Azadeh Dalvand[1,3], Andrew J. Halayko[1-3]

[1]Departments of Physiology & Pathophysiology and [2]Internal Medicine, University of Manitoba, Winnipeg, MB, and [3]Biology of Breathing Group, Children's Hospital Research Institute of Manitoba, Winnipeg, MB.

Introduction

According to an Asthma Canada report, approximately 317 Canadians, including children, are diagnosed with asthma daily, and a significant number of them are refractory to current therapies. The mechanism of refractory asthma remains unclear. We showed that oxidized phosphatidylcholines (OxPC), a mediator of oxidative stress, exacerbates asthma pathobiology (i.e. increased inflammation, bronchodilator insensitivity) via pathways that involve Protein Kinase C (PKC). The present study aims to develop protocols to test whether OxPC exposure is sufficient to activate PKC.

Methods

Cultured Human Airway Smooth Muscle (HASM) cells (n= 5 donors) were treated with OxPAPC (the most common variants of OxPC in the lungs) (80 µg/mL) for 1 to 24 hours. We used untreated cells as a negative control and cells treated with the PKC activator 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) (0.2uM, 1 hour) as a positive control. After treatment, cell lysates were collected, proteins were separated by 8% SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose by electroblotting, and PKC phosphorylation was detected using polyclonal anti-pSer660-PKC. Using β-actin as a loading control, pPKC bands were quantified by densitometry using AlphaEase FC software and calculated as % p-PKC increased from baseline.

Results

OxPAPC (80 µg/mL) exposure for 1 hour in HASM cells significantly induced PKC phosphorylation (203% ±187 increase from baseline), which was comparable to induction with the PKC activator, TPA (256% ±216). Interestingly, OxPAPC (80 µg/mL) treatment for longer periods showed that OxPAPC exposure leads to a sustained increase in PKC phosphorylation (i.e. 222%±169 at 3 hours, 233%±195 at 6 hours, 239%±261 at 24 hours).

Conclusion

Oxidized phosphatidylcholine causes sustained PKC phosphorylation in HASM cells. This finding confirms the existence of an oxidized phospholipid-protein kinase C signalling axis, furthering our understanding of asthma pathobiology involving mediators of oxidative stress.

MSHRF 2024 Poster Competition Judging Schedule

Group	Surname	Given Name	Poster	Time
Group-1	Ranatunga	Sriyani	1	9:00
	Malhotra	Danish	2	9:20
	Asemi Rad	Azam	3	9:40
	Sattarifard	Hedieh	4	10:00
	Llanes-Cuesta	M. Alejandra	5	10:40
	Maisha	Jeba Atkia	6	11:00
	Saltibus	Eola	7	11:20
	Liu	Ruotong	8	11:40
	Tough	Riley	9	13:00
	Huang	Shiqi	10	13:20
	Gork	Elise	11	13:40
	Gingras	Shanelle	12	14:00
Group-2	Sabzevary-ghahfarokhi	Milad	13	9:00
	Vallis	Jack	14	9:20
	Khan	Mohd Wasif	15	9:40
	Ogunsola	Samuel	16	10:00
	Ryz	Jillian	17	10:40
	Hosseini	Seyed Mojtaba	18	11:00
	Roberts	Chris-Tiann	19	11:20
	Layug	Paul Jerard	20	11:40
	Shen	Tiffany	21	13:00
	Saei Arezoumand	Khatereh	22	13:20
	Shanmugam Anandhan	Santhosh	23	13:40
	Siddiqui	Mohd Sarim	24	14:00
Group-3	Vaghasiya	Jigneshkumar	25	9:00
	Helwer	Rafe	26	9:20
	Ferreira	Olivia	27	9:40
	Onwah	Somtochukwu Stella	28	10:00
	Rajani	Huda	29	10:40
	Hesampour	Fatemeh	30	11:00
	Orchard	Taylor	32	11:40
	Taguiam	Erwin	33	13:00
	Olanipekun	Tobi	34	13:20
	Afshari	Havva	35	13:40
	O'Reilly	Peter	36	14:00

MSHRF 2024 Poster Competition Judging Schedule (Continued)

Group	Surname	Given Name	Poster	Time
Group-4	Tshikudi	Diane	37	9:00
	Frederick	Christina	38	9:20
	Ong	Gideon	39	9:40
	Cerato	Júlia	40	10:00
	Loeb	Kristi	41	10:40
	Maghsoudi	Saeid	42	11:00
	Bhatia	Yeshika	43	11:20
	Lukawy	Chelsea	44	11:40
	Obi	Patience	45	13:00
	Ziegler-Blair	Hannah	46	13:20
	Acharya	Sushank	47	13:40
	Khan	Eefa	48	14:00
Group-5	Abrar Basha	Mohammed	49	9:00
	Ziaee	Amir	50	9:20
	Ogungbola	Olamide	51	9:40
	Akinola	Pelumi Samuel	52	10:00
	Matlabi	Mahnoosh	53	10:40
	Omole	Tosin	54	11:00
	Farooq	Faiza	55	11:20
	Le	Toby	56	11:40
	Chauhan	Sanjana	57	13:00
	Xu	Bocheng	58	13:20
	Jamilchelvan	Rubendren	59	13:40
	Awada	Abraham	60	14:00
Group-6	Akhtar	Ghanan Bin	61	9:00
	Wells	Taylor	62	9:20
	Abulannaz	Omaymah	63	9:40
	Ninalaya	Marcelo	64	10:00
	Berk	Ahmet Burak	65	10:40
	Alagarsamy	Keshav Narayan	66	11:00
	Tyagi	Rushie	67	11:20
	Klassen	Levi	69	13:00
	costa fujishima	marina	70	13:20
	Heilmann	Ashley	71	13:40
	Taverner	Morgan	72	14:00

MSHRF 2024 Poster Competition Judging Schedule (Continued)

Group	Surname	Given Name	Poster	Time
Group-7	Shaji	Suraj	73	9:00
	Kuzmychova	Helgi	74	9:20
	Hedley	Adam	75	9:40
	Lowry	McKay	76	10:00
	Khodabandehloo	Narges	77	10:40
	Hayes	Chad	78	11:00
	Shirinbakhshmasoleh	Mina	79	11:20
	Zaman	Muniza Mehrin	80	11:40
	Crandall	Molly	81	13:00
	Chattopadhyaya	Sikta	82	13:20
	Bernier	Kate	83	13:40
	Pallikkara Suresh	Rohini	84	14:00
Group-8	Lu	Jueqin	85	9:00
	Caron	Aurelien	86	9:20
	Mota	Ryan	87	9:40
	Sanghai	Nitesh	88	10:00
	Crooks	Megan	89	10:40
	kannan	Kamali	90	11:00
	Ren	Yujie	91	11:20
	Turner	Sarah	92	11:40
	Houenagnon	Floriane	93	13:00
	Nausheen Zaman	Ramiza	94	13:20
	Balasko	Allison	95	13:40
	Marshall	Courtney	96	14:00
Group-9	Zhang	Hannah	97	9:00
	Yakemow	Gabriella	98	9:20
	Chisholm	Madison	99	9:40
	De Luca	Domenica	100	10:00
	Miao	Deanne Ni1ie	101	10:40
	Rozovsky	Tim	102	11:00
	Okeke	Obinna	103	11:20
	Storm	Jasmyne	104	11:40
	Lawal	Samuel Adefisoye	105	13:00
	Crooks	Sara	106	13:20
	Loiselle	Genevieve	107	13:40
	Kadar Shahib	Ashraf	108	14:00

MSHRF 2024 Poster Competition Judging Schedule (Continued)

Group	Surname	Given Name	Poster	Time
Group-10	Soliman	Lea	109	9:00
	Pipella	Jasmine	110	9:20
	Safa	Mira	111	9:40
	Ma	Heqing	112	10:00
	Humphreys	Christian	113	10:40
	Baskerville	Kaden	114	11:00
	Thingnam	Raneeta	115	11:20
	Ozerklig	Berkay	116	11:40
	Wupori	Kylene	117	13:00
	Voisin	Athalia	118	13:20
	Robert	Erin	119	13:40
	Aguilar	Jaypee	120	14:00
Group-11	Martens	Brielle	121	9:00
	Ahmed	Samah	122	9:20
	Amarasinghe	Ovini	123	9:40
	Siddik	Abu Bakar	124	10:00
	Byun	Michael	125	10:40
	Topolnitska	Diana	126	11:00
	Leclerc	Nicolas	127	11:20
	Junankar	Neil	128	11:40
	Bernacki	Annika	129	13:00
	Kantroo	Meher	130	13:20
	Greenslade	Riley	131	13:40
	Saleth	Leena Regi	132	14:00
Group-12	Ha	Ryan	133	9:00
	Parvin	Afroza	134	9:20
	Chhabra	Manik	135	9:40
	Quinlan	Kaitlyn	136	10:00
	Manson	Anne	137	10:40
	Taefehshokr	Sina	139	11:20
	Twilley	Rebecca	140	11:40
	Li	Katherine	141	13:00
	Pidsadny	Paula	142	13:20
	Kaur	Amandeep	143	13:40
	Jebreili Rizi	Delaram	144	14:00

MSHRF 2024 Poster Competition Judging Schedule (Continued)

Group	Surname	Given Name	Poster	Time
Group-13	Ghiyamihoor	Farshid	145	9:00
	Abed	Amin	146	9:20
	Anderson	Melissa	147	9:40
	Alexiuk	Mackenzie	148	10:00
	Senthil	Harshal	149	10:40
	Kostal	Kayla	150	11:00
	Rahman	Azizur	151	11:20
	Everton	Amanda	152	11:40
	Pascual	Danielle `	153	13:00
	Perron	Jarrad	154	13:20
	Grover	Sanjana	155	13:40
	Abual'anaz	Besher	156	14:00
Group-14	Nuhu	Faisal	157	9:00
	Narayanan Sudhakar	Sadhana Rithi	158	9:20
	Obtial	Mon Francis	159	9:40
	Her	Yina	160	10:00
	Petrelli	Berardino	161	10:40
	Anonna	Shamima Nasrin	162	11:00
	Copete	Angela	163	11:20
	Chawla	Ujala	164	11:40
	Donahue	Julie	165	13:00
	Olumade	Testimony	166	13:20
	Lemaille	Candice	167	13:40
	Rutherford	Kailee	168	14:00
Group-15	**Post Doctoral Fellows**			
	Mahboobi	Sepideh	169	9:00
	Srivastava	Abhay	170	9:20
	Ramadan	Fadi	171	9:40
	Herrera Diaz	Mariana	172	10:00
	Kolesar	Tiffany	173	10:40
	Jakova	Elisabet	174	11:00
	Sareen	Niketa	175	11:20
	Slipski	Carmine	176	11:40
	Badran	Hoda	177	13:00
Group-16	**Undergraduate Students**			
	Toor	Brian	179	9:00
	Tkach	Kailey	180	9:20
	Oda Jallime	Claudia Camila	181	9:40
	Gan	Jaydon	182	10:00
	Kaul	Esha	183	10:40
	Varghese	Nathan	184	11:00
	Tadele	Hanok	185	11:20
	Mishra	Sparashita	186	11:40