Presenter's Name: Brower, Brandon

Additional Authors: Zhang L, Larsen F, Good H, Shin A, Kakar S, Asfaha S

Abstract Title: Development of an In Vitro Inflammatory Bowel Disease Model

Introduction: Inflammatory bowel disease (IBD) is an umbrella term for a collection of lifelong intestinal diseases, primarily Crohn's disease (CD) and Ulcerative Colitis (UC). The current absence of a biomarker that predicts response to pharmacological treatment forces clinicians to choose medications somewhat arbitrarily. A recent expansion of new medications, including biologics, has increased the breadth of IBD therapeutics, but in doing so, engendered a clinical dilemma: how do we identify the optimal choice of medications for a given patient? An in vitro drug screening platform using patient-derived IBD tissue would offer a novel approach to testing drug response; however, at present, there are no in vitro models of IBD. The advent of intestinal organoids has provided new opportunities for IBD research, including the development of new in vitro model systems, but, currently, the most prevalent intestinal organoid methodology is limited to culture of epithelial cells and thus excludes stromal components such as immune cells. In contrast, another organoid methodology, the Air-Liquid Interface (ALI) system, harbours both epithelial and stromal components. However, ALI organoids have not been used to model IBD; thus, their ability to recapitulate this disease and to serve as a platform for drug testing remain unexplored.

Methods: To generate an organoid model of IBD, we first established ALI organoids from normal neonatal (1 week or younger) mouse small intestinal and colonic tissue as organoid growth is more vigorous with younger tissue. After gradually increasing the age of mice to approximately 6 weeks, we will begin attempting to culture ALI intestinal cultures derived from the colon of mice treated with dextran sodium sulfate (DSS), a colitis inducing agent. We will test both in vitro (ie. treating organoids directly) and in vivo DSS administration. Upon successful culture of ALI organoids from DSS-treated mice, we will attempt to generate ALI cultures from human IBD tissue samples, obtained from consenting patients during endoscopy. As endoscopy samples lack underlying mesenchymal layers, we will additionally test various growth factor combinations to fulfill the role of the absent stromal cells, which would otherwise provide the intestinal stem cells with factors necessary for their survival and differentiation.

Results: We have successfully generated small intestinal and colonic ALI organoids from mice up to 4 weeks of age and are currently examining their stem cell populations with transgenic mice. Additionally, we are currently working to establish ALI organoids from healthy control patients and patients with IBD.

Discussion: Organoids strike a unique balance between ease of use and level of realism, hence, in many respects, elevating them above both traditional 2D cell culture and animal models. Our work thus has the potential to greatly impact IBD research by providing a powerful new in vitro model of IBD which could furthermore be used to not only bolster our understanding of IBD pathophysiology but also provide a platform for drug screening.

POSTER PRESENTATIONS 2
2C: CARDIOVASCULAR BIOLOGY

Presenter's Name: Day, Brooke

Additional Authors: Feng B, Chakrabarti S

Abstract Title: Role of ANRIL in Diabetic Nephropathy

Introduction: Diabetes is a growing health and socioeconomic crisis. Noncoding RNAs (ncRNA) play important roles in diabetic complications, however, the role of these ncRNAs in diabetic kidney fibrosis is not clear. The long ncRNA ANRIL is located at the CDKN2A/B locus, which is a highly susceptible region to human cancers and metabolic disease. Previous work in our lab has reported elevated ANRIL expression in the kidneys of diabetic animals. Here, ANRIL was demonstrated to mediate structural and functional alterations in the kidneys. Further, ANRIL-knockout (ANRILKO) has demonstrated to protect against renal fibrosis. In other systems, including atherosclerosis and cancer, ANRIL is regulated by transcription factors E2F1 and CTCF. A reciprocal relationship between ANRIL and these transcription factors has also been hypothesized. Here, we explored whether ANRIL modulates renal fibrosis in diabetic nephropathy (DN) through the regulation of E2F1 and CTCF transcription factors.

Methods: Renal tissues from ANRILKO and wild-type (WT) mice, both with and without streptozotocin-induced diabetes were used. Real-time quantitative reverse transcription PCR was used to measure mRNA expression of E2F1 and CTCF. The mRNA expression of the extracellular matrix (ECM) protein Collagen 4a1 (Col4a1) was measured as an indicator of renal fibrosis.

Results: Our results showed that Col4a1 expression was significantly increased in WT diabetic mice compared to WT nondiabetic mice. Elevated Col4a1 expression was prevented in ANRILKO diabetic mice. E2F1 expression was significantly reduced in ANRILKO nondiabetic and ANRILKO diabetic mice compared to WT nondiabetic mice. E2F1 expression was also reduced in WT diabetic mice compared to WT nondiabetic mice. Further, CTCF expression was significantly increased in ANRILKO nondiabetics compared to WT nondiabetic mice. CTCF expression was also increased in WT diabetic mice compared to WT nondiabetic mice and reduced in ANRILKO diabetic mice compared to ANRILKO nondiabetic mice.

Discussion: Our results demonstrate that ANRIL regulates mRNA expression of ECM components in renal tissues of diabetic animals and that ANRILKO is protective against renal fibrosis. In addition, the results suggest that ANRIL may play a protective role in regulating E2F1 and CTCF expression in renal tissues in a nondiabetic state. However, the ability of ANRIL to regulate E2F1 and CTCF in renal tissues may be altered during diabetes. These results contribute to our understanding of the regulatory networks through which ANRIL contributes to renal fibrosis. A greater understanding of such networks can assist in the identification of early-stage biomarkers for diagnosis and treatment of DN.

POSTER PRESENTATIONS 2
2C: CARDIOVASCULAR BIOLOGY

Presenter's Name: Gomes, Janice

Additional Authors: Janssen B, Dayarathna T, Pasternak S, and McIntyre CW

Abstract Title: Development of an Extracellular Vesicle-based biomarker of hemodialysis induced vascular injury

Introduction: Hemodialysis(HD) is a repetitive and ongoing treatment that creates complications such as hemodynamic instability, microvascular dysfunction, and damage to vulnerable vascular beds, which can lead to irreversible multi-organ injury (including increased risk of cardiovascular disease, stroke, and death). HD associated vascular damage and organ injury can be observed through gold standard imaging techniques such as Echocardiography (Echo), Computed Tomography (CT), Magnetic Resonance Imaging (MRI), and Positron Emission Tomography (PET). However, these techniques are complex, and expensive. What is needed is an inexpensive non-invasive test to guide the application of HD to prevent end organ damage. A biomarker for assay-development that has gained interest and has been identified as being a predictive and prognostic marker of vascular injury, are Extracellular Vesicles (EVs). Although, previous studies do not explain all the parameters that impact EV levels among HD patients. Therefore, we have developed a novel system using nanoscale Flow Cytometry(nFC) which can rapidly count, and characterize EVs found in the blood, based on size and antigen markers present. By using this system, we can investigate some of the parameters and determine whether endothelial and platelet derived EVs can be used as an indicator of HD associated vascular damage.

Methods: To determine the effect of uremic stress on EV release, endothelial cells (HUVEC) were exposed to stressors such as Lipopolysaccharide(LPS), pooled serum from HD patients(n=10), or pooled serum from healthy controls (n=10) for 24 hours. The cell culture media was collected, concentrated, and analyzed on the nFC to determine EV size distribution and characteristics using endothelial antibodies(CD62e, CD146). A cell death assay was performed using trypan blue. To further understand the effects of HD treatment on vascular damage, we developed an in vivo rodent model of HD. We exposed healthy Winstar Kyoto rats(n=6) to a 2-Hour hemodialysis session. During the HD session we completed intravital microscopy imaging to observe visual microcirculatory stress, recorded blood pressure, and collected blood samples hourly for analysis of endothelial and platelet derived EVs.

Results: Our preliminary work showed that HUVEC cells produced EVs of various sizes, and that total number of EVs increased with cell confluency. In vitro work confirms that when HUVEC cells are exposed to LPS, the cells produced more EVs than the control group, especially among smaller sized EVs. Although cells exposed to HD serum or Healthy serum did not show any significant differences in EV levels. On the contrary, our in vivo work showed that animals that underwent HD expressed changes in both total EV levels as well as in the size distribution of EVs over the course of HD.

Discussion: Our in vitro work suggests that uremic stress alone, does not significantly impact EV levels. However, our in vivo work showed that there could be a potential impact of HD treatment itself on vascular damage since EV levels and EV size changed through the course of HD. We plan to apply our EV assay to observational and interventional clinical studies done with HD patients to further assess the clinical applicability of our assay. Overall, our preliminary findings show that EVs might be a promising biomarker of HD induced vascular injury.

POSTER PRESENTATIONS 2
2C: CARDIOVASCULAR BIOLOGY

Presenter's Name: Greasley, Adam

Additional Authors: Zheng X, Nagpal A

Abstract Title: The Role of CircHipk3 in Ischemia Reperfusion Injury During Heart Transplantation

Introduction: Heart disease remains the second leading cause of death in Canadian men and women and has been increasing each year. The ideal treatment for end-stage heart failure is heart transplantation (HT) however, the low number of eligible donors and complications hinder this ability. One complication of donor eligibility and successful HT is ischemia reperfusion injury (IRI). Ischemia is recognized as a leading complication of HT and although early reperfusion is the standard of care, IRI is increasingly recognized as a significant risk for poor outcomes. There is no effective treatment for IRI. Discovering new molecules and regulators involved in IRI is the key for development of an ideal treatment for IRI. Emerging evidence shows that circular RNAs (circRNA), a new class of endogenously expressed non-coding RNAs produced from back splicing which form a covalently closed loop without free terminals, have the potential to be a novel master regulator for controlling IRI. circRNA can sponge miRNA and directly interact with proteins, rendering circRNA as a master and powerful new type of gene regulator. CircRNA plays critical roles in physiological and pathological processes. CircRNA Hipk3 (circHipk3) generated from the homeodomain-interacting protein kinase 3 (Hipk3) gene has emerged as an abundant circRNA which functions as a potent miRNA sponge. CircHIPK3 plays a crucial role in cell survival in cancer and diabetes, however, its role in IRI remains unstudied.

Hypothesis: We hypothesize that circHipk3 plays a regulatory role in cellular stress response to IRI and contributes to the molecular switch between proliferation and cell death.

Methods: In this study, we use HL-1 cell line, a murine model of cardiomyocytes and a 24 h cold-storage model, 4°C, for organ preservation followed by 12 h warm reperfusion, 37°C. To determine the role of circHipk3 in IRI, real-time cell monitoring using an Incucyte system were used to determine cell death and quantified using flow cytometry at specific time points. CircHipk3 expression patterns across reperfusion time points were analyzed using qPCR. siRNA mediated knockdown was performed to understand circHipk3's role in cell viability during IRI. Fluorescent microscopy was used to circHipk3 cellular localization in response to IRI. To elucidate the mechanism of circHipk3, an RNA-pulldown assays using a biotinylated probe was performed followed by mass-spectrometry and miRNA array.

Results: Our results suggest that circHipk3 expression varies during IRI and can be indicative of cell survival. siRNA knockdown of circHipk3 leads to increase in pro-apoptotic genes such as Caspase 3,8,9 and mitochondrial dysfunction through up-regulation of Bax. In accordance, siRNA treated cells exhibit exacerbated cell death during IRI compared to GL2 siRNA. At the molecular level, siRNA treated cells show limited difference in pro-apoptotic genes between GL2 and circHipk3 siRNA in IRI.

Discussion: Our incucyte and circHipk3 expression data suggest that circHipk3 levels influence the cellular ability to cope with IRI mediated stress and apoptosis. Mechanistically, our results suggest the mechanism of cellular death is through mitochondrial dysfunction and increased expression of caspases following siRNA, leaving the cells more susceptible to IRI. We further hypothesize that circHipk3 over-expression will protect the cells from IRI induced stress and prevent apoptosis by preventing mitochondrial dysfunction.

Presenter's Name: Hong, Megan

Additional Authors: Raveendraraj J, Liang O, Abdolmaleki D, Cameron L

Abstract Title: Effect of glucocorticoids on Th2 cell metabolism and mitochondrial ROS production

Introduction: Reactive oxygen species (ROS) have been associated with asthma severity by promoting T helper 2 (Th2) cell-driven inflammation. This elevation in ROS may be attributed to mitochondria dysfunction observed in asthmatics. We have shown that while glucocorticoids (GC) suppress Th2 cell-driven inflammation, they may also counterintuitively promote the expression of some pro-inflammatory genes. Furthermore, GCs may be less effective in women as estrogen can potentiate the pro-inflammatory effects of GCs. The underlying mechanism(s) for these occurrences are not well understood. However, GCs and estrogen could potentiate mitochondria dysfunction seen in asthmatics by altering cell metabolism and lead to increased ROS production. Despite ROS having an important role in T cell function, the effect of GCs on Th2 cell metabolism remains elusive. We hypothesize that GCs will promote mitochondria dysfunction in Th2 cells by shifting metabolism toward oxidative phosphorylation and result in increased mitochondrial ROS production, an effect that will be potentiated by estrogen.

Methods: Immortalized CD4+ T cells (CCRF-CEM) exhibiting a Th2 cell phenotype were treated with dexamethasone, hydrocortisone and/or estrogen receptor alpha agonist, propyl pyrazole triol, for 24 h (n=3-6). Total intracellular ROS was quantified using a CM-H2DCFDA ROS probe with flow cytometry for detection. Expression of mRNA for genes involved in metabolism and redox homeostasis (GSTM3, CYC1, CAT, TXNIP, MYC, LDHA, and SLC38A1) were measured using RT-qPCR.

Results: ROS production was increased in response to GC and potentiated by estrogen. GC decreased mRNA expression of LDHA and MYC that promote glycolysis. Whereas GC increased the expression of TXNIP mRNA that negatively regulates glycolysis. The level of CYC1 mRNA, a gene encoding for complex III of the electron transport chain, decreased in response to GC. The effect of GC on LDHA, TXNIP, and CYC1 mRNA levels was enhanced by estrogen.

Discussion: These results suggest that GCs may increase mitochondrial ROS production by promoting the use of oxidative phosphorylation over glycolysis and/or impairing the efficiency of electron transfer along the electron transport chain. Estrogen was shown to potentiate the metabolic effects of GCs. These findings suggest a possible mechanism of mitochondria dysfunction resulting in increased ROS production from changes in T cell metabolism. This mechanism may explain the paradoxical nature of GC therapy and suggests that estrogen levels may influence the efficacy of GCs.

POSTER PRESENTATIONS 2
2C: CARDIOVASCULAR BIOLOGY

Presenter's Name: Hu, Xiangtian (Shawn)

Additional Authors: Diao H, Liu Q, Min WP

Abstract Title: DLC1 beta-mediated Regulation of Cardiac Ischemia-Reperfusion Injury in Heart Transplantation

Introduction: Heart transplantation is the gold standard therapy for end stage cardiac diseases. However, donor hearts are susceptible to ischemia-reperfusion (I/R) injury, which may lead to surgical failure and mortality of the recipient. Increased apoptosis, hypertrophy, and deregulated angiogenesis are primary manifestations of I/R injured hearts. Injury progression is directed by PI3K/Akt1, ROCK/Rho kinase pathways; for instance, increased RhoA activity is associated with increased expression of apoptotic proteins. Detailed signalling mechanisms in these pathways remain uncharacterized. One such multi-domain protein, DLC1 beta, is downregulated after I/R injury. This study will investigate the signaling pathways and role of DLC1 beta in cardiac I/R injury. We hypothesized that overexpression of DLC1 beta will reduce the severity of I/R injury.

Methods: H9c2 rat myocardium cell lines will be cultured in vitro with DMEM and FBS; then, DLC1 beta adenoviral vectors will be transfected for DLC1 beta overexpression. GENBAG Anaerobics will be used to simulate ischemia/reperfusion environment by inducing hypoxia. qPCR, Western blot and SYTOX green nucleic acid stain will be used to determine protein expression and cell death under hypoxia.

Results: Our results show cells overexpressing DLC1 beta under hypoxia had decreased apoptosis. It is expected to see decreased RhoA activity in cells overexpressing DLC1 beta.

Discussion: These findings suggest overexpression of DLC1 beta provides protection for cardiomyocytes in hypoxic environments by reducing apoptosis. Reduced apoptosis is likely attributed to DLC1 beta's RhoGAP activity.

Significance: For the first time, this study will define the role and signaling mechanism of DLC1 beta in I/R injury. The results may lead to the exploration of therapeutic potentials of DLC1 beta-targeted I/R therapy.

Presenter's Name: Jackson, Ashley

Additional Authors: Luke P, Bhattacharjee R

Abstract Title: Development of an in vitro Model of Ischemia Reperfusion Injury in Kidney Transplant

Introduction: Kidney transplantation is the gold standard treatment for patients with end-stage kidney failure, providing increased patient survival and quality of life compared to dialysis. To address the shortage of transplantable organs, kidneys from donation after circulatory death (DCD) donors are being used more frequently. These organs are particularly susceptible to damage during the transplantation process known as ischemia reperfusion injury (IRI). IRI is characterized by a lack of oxygen and nutrients during warm ischemia within the donor and cold storage followed by reintroduction of oxygen upon transplantation. IRI promotes cell death and inflammation within the transplanted organ leading to poor graft function. Our group is focused on organ preservation with one strategy being to use existing clinically approved drugs to reduce the damage from IRI during organ storage. However, a relevant in vitro model is required to assess the utility and understand the mechanism of these drugs in the context of IRI. As existing in vitro models of IRI do not accurately mimic the clinical situation of DCD kidney transplantation, we propose a model to encompass the collective damage from warm and cold ischemia and reperfusion as seen in the clinical setting.

Hypothesis: We hypothesize that human kidney proximal tubular epithelial (HK-2) cells subjected to our hypoxia and reoxygenation conditions will exhibit increased cell death and expression of damage markers and pro-inflammatory cytokines characteristic of IRI.

Methods: To mimic in vivo conditions, HK-2 cells will be subjected to hypoxia for 1hr at 37°C followed by 24hr in cold storage at 4°C. Reperfusion will be simulated with 24hr reoxygenation at 37°C. Cell viability after treatment will be determined using flow cytometry. Known markers of injury and inflammation associated with IRI will be quantified using ELISA. Once the model has been established, the mechanism of inflammation will be explored using multiplex ELISA and drug candidates will be tested in the model.

Results: Results from flow cytometry showed a reduction in cell viability from 94% to 70% in our hypoxia model compared to control cells. Additionally, we detected a significant increase in markers of cell damage (HMGB-1) and inflammation (IL-6) in hypoxia-treated cells.

Significance: Using our model as a foundation, we hope to identify an existing drug that can limit damage from IRI in kidney transplantation. The ultimate goal of this research is to improve organ storage thereby improving post-transplant renal graft outcomes.

POSTER PRESENTATIONS 2
2C: CARDIOVASCULAR BIOLOGY

Presenter's Name: Kum, Jina

Additional Authors: Howlett CJ, Khan ZA

Abstract Title: High glucose disrupts transforming growth factor-β signalling to enhance adipogenic differentiation of marrow-derived progenitor cells

Introduction: Enhanced bone marrow adiposity and skeletal fragility are chronic complications of diabetes. We have shown that high glucose exposure skew marrow-derived progenitor cells towards adipogenesis, while inhibiting osteoblastogenesis. Additionally, our laboratory has shown that diabetes depletes regenerative stem cells in the marrow. These findings suggest that changes in the marrow composition, with increased adipogenesis and suppressed osteogenesis, may underlie the depletion of regenerative stem cells and impairment of endogenous tissue repair in diabetes. Our screening for potential mediators of this skewed differentiation showed suppressed levels of transforming growth factor-β (TGF-β) signalling in primary human marrow cells exposed to elevated glucose levels. Hence, I hypothesize that hyperglycemia alters TGF-β1 signalling to favour adipogenesis in the marrow. This signalling change alters the stem cell niche, ultimately depleting the regenerative stem cells.

Methods: I challenged the marrow-derived cells with exogenous TGF-β1 in adipogenic induction media to assess for cellular and molecular alterations. Furthermore, marrow-derived progenitor cells were exposed to various inhibitors of downstream proteins of the TGF-β signalling pathway to further dissect the intracellular signalling proteins mediating the changes.

Results: My results show that marrow cells activate canonical SMAD1/5 pathway when induced to differentiate into adipocytes. Interestingly, TGF-β1 addition normalizes SMAD1/5 activation and suppresses adipogenic differentiation of marrow cells. TAK1 has been previously reported to negatively regulate SMAD1/5 and inhibit precocious differentiation. In support of these findings, I found that inhibition of the non-canonical TAK1-JNK axis reverses the effect of TGF-β1 and normalizes adipogenic differentiation.

Discussion: My findings show that restoring TGF-β1 signalling activates TAK1-JNK axis and disrupts SMAD1/5 activation, ultimately hindering adipogenic differentiation in marrow cells. My study suggests that diabetes may fine-tune the balance between canonical and non-canonical TGF-β pathways in marrow cells to favour adipogenic differentiation. I am currently investigating the effects of pharmacologically inhibiting PPARγ, the master regulator of adipogenesis, in a diabetic mouse model.

Presenter's Name: Wang, Tan Ze

Additional Authors: Warsi A, Rong K, Greasley A, Li S, Lin K, Zheng X

Abstract Title: Differential expression of circular RNAs in septic peripheral blood mononuclear cells

Sepsis is the leading cause of death in intensive care units (ICU), characterized by a dysregulation of the immune system in response to infection. Sepsis is associated with alterations in genomic expression in leukocytes, which can potentially be the basis for novel interventions. Circular RNAs (circRNAs) are a class of highly stable, covalently closed single-stranded RNA that is implicated to alter gene expression and contribute to many diseases. Previous research has established the upregulation of circRNAs in blood leukocytes of sepsis patients compared to healthy controls, yet specific circRNAs that contribute to the development of sepsis remain unknown.

The current study seeks to identify circRNAs in peripheral blood mononuclear cells of sepsis patients that are differentially expressed between ICU admission and discharge, and to investigate their biological functions. PBMCs were isolated from three sepsis patients before and after intensive care. Total RNA was extracted from PBMCs with Trizol and subjected to RNA sequencing. Differentially expressed circRNAs were identified using circRNA annotation programs. We found 94 up-regulated and 3 down-regulated circRNAs in PBMCs collected at ICU admission compared with ones at discharge. Gene ontology analysis revealed that the altered circRNAs were associated with immune cell activation and regulation, specifically the Fas and RAS pathways. Looking at the ratio of circRNA to linear RNA expression, we found 56 up-regulated and 13 down-regulated circRNAs after normalization to the expression of their linear counterparts. We then validated 4 of the most significantly altered circRNAs by qRT-PCR. It was found that circular RNA S100A9 (circS100A9, which is back-spliced from the S100A9 gene) is the most differentially expressed circRNA in PBMCs of sepsis patients between ICU admission and discharge, and significantly upregulated at ICU admission, which is consistent with the RNA sequencing results. The precise role of circS100A9 in sepsis is under investigation. Furthermore, we compared different circRNA annotation programs used to analyze our RNA-seq data. We found that different programs generated significantly different expression profiles. The results generated by bowtie and STAR were more consistent with qRT-PCR results.

In conclusion, circRNA expression profiles are strikingly different in septic PBMCs between ICU admission and discharge, suggesting that circRNAs may participate in sepsis. These findings support the role of circRNAs as potential targets for novel genome-based treatments for sepsis.

POSTER PRESENTATIONS 2
2C: CARDIOVASCULAR BIOLOGY

Presenter's Name: Wang, Eric

Additional Authors: Feng B, Chakrabarti S

Abstract Title: The role of miR-9 in epigenetic regulation of EndMT in diabetic retinopathy

Background: Diabetic retinopathy (DR), a highly prevalent chronic complication of diabetes seen in nearly all type 1 diabetics and a majority of type 2 diabetics, is the leading cause of vision loss in working-aged adults. High glucose in diabetes causes endothelial damage and dysfunction in the retina, leading to microaneurysms, hemorrhages, capillary occlusion, ischemia, and neovascularization, ultimately resulting in vision impairments in the patient. One component of early DR-related endothelial dysfunction is a process known as endothelial to mesenchymal transition (EndMT), whereby endothelial cells (ECs) transdifferentiate into mesenchymal-like cells. EndMT can be driven by TGF-β and inflammation, and leads to reduced expression of EC adhesion molecules such as CD31 and CD144, coupled with increased expression of mesenchymal markers such as SM22, FSP, and α-SMA and increased production of extracellular matrix (ECM) proteins. Recent studies have found that epigenetic regulation has a significant role in EndMT, and that diabetes disrupts this regulation. Epigenetic regulation encompasses a variety of processes that result in changes in gene expression without changes in the underlying genomic sequence. One class of epigenetic regulators are microRNAs (miRs), which are short RNA molecules that do not encode protein. miRs exert post-transcriptional regulation by binding to complementary sequences on mRNAs and inducing degradation of the transcripts. miR-9 is one such miR, and has been reported to regulate TGF-β signalling and NF-κB (a proinflammatory transcription factor). miR-9 has also been reported to interact with the long non-coding RNA MALAT1 (another class of epigenetic regulator, one that doesn't directly target mRNAs for destruction, but rather interacts with proteins and miRs in order to regulate gene expression) which we have shown to regulate inflammation in DR. We hypothesized that miR-9 regulates EndMT in DR.

Methods: Human retinal endothelial cells (HRECs) were used for in vitro investigations, and streptozotocin-induced mouse model of diabetes was used for in vivo experiments. Changes in EndMT-related genes were analyzed via qPCR for mRNA, and Western Blot for protein. miR-9 overexpression was used to establish causal relations between miR-9 and EndMT-related genes. miR-9 overexpression in HRECs was done via transfection of miR-9 mimic. EC-specific miR-9 overexpression in mice was induced through miR-9 transgene driven by TIE2 promoter.

Results: We have found that high glucose reduced miR-9 expression in HRECs, and that this reduction in miR-9 expression correlated with decreased expression of endothelial markers and increased expression of mesenchymal markers and ECM proteins. We also found that miR-9 overexpression in HRECs rescues some of the effects of high glucose on EndMT-related gene expression. Retinal tissue from miR-9 overexpressing mice has been collected and preliminary data mirror our cell-based findings.

Discussion: Our results show that miR-9 is likely protective against EndMT in the early stages of DR. These findings provide a better understanding of the early stages of the pathogenesis of DR, and may represent a novel opportunity for targeted therapy against DR and DR-induced vision impairment.

POSTER PRESENTATIONS 2
2C: CARDIOVASCULAR BIOLOGY

Presenter's Name: Xu, Laura

Additional Authors: McLeod P, Zhang ZX

Abstract Title: Mechanism of AIF-Regulated Nuclei Damage

Introduction: Cell death plays a critical role in organ injury and transplant rejection. Necroptosis is a caspase-independent necrotic pathway and shares features with accidental cell death such as organelle swelling, plasma membrane rupture, cell lysis, and leakage of intracellular components leading to a secondary inflammatory response. The process is initiated by caspase-8 deficiency or a lack of X-linked inhibitors of apoptosis proteins. Necroptosis starts with the ligation of TNFR1 and is regulated by receptor-interacting protein kinases 1 and 3. This leads to a necrosome complex and MLKL (mixed lineage kinase domain like) phosphorylation, promoting MLKL oligomerization, plasma membrane pore opening, and danger-associated molecular patterns. Necroptosis is a therapeutic target in ischemia/reperfusion injury. The mitochondrial apoptosis-inducing factor AIF translocates to the nucleus and induces chromatinolysis. It is possible that AIF participates in necroptosis, promoting chromatin condensation and DNA degradation.

Methods: Mice microvascular endothelial cells (MVECs) were isolated and grown using 1 g/L glucose Dulbecco's Modified Eagle Medium supplemented with 10% FBS and 1% penicillin/streptomycin. Cell death was quantified by real-time imaging with the following treatments: TNF-alpha, TNF-α and second mitochondrial activator of caspase (SMAC), TNF-α, SMAC, and IETD; TNF-α, SMAC, IETD, and necrostatin-1s. A parallel study examined the effects of acidic pH (6.0 and 6.5) and a death assay was performed as described above. Immunochemistry with primary rabbit anti-mouse AIF antibody, biotinylated goat anti-rabbit IgG secondary antibody (H+L), and streptavidin-PE was performed and counterstained with DAPI. DNA was quantified with a fluorescent assay and visualized by agarose gel. MVECs were transfected with siRNA targeting AIF and confirmed using real time PCR and western blots.

Results: From the assay above, treatment of MVECs with human TNF-α increased cell death compared to untreated cells by inducing cell death. The addition of SMAC mimetic further increased cell death by suppressing inhibitor of apoptosis proteins, leading to apoptotic death. Caspase-mediated apoptosis was inhibited through IETD (Z-Ile-Glu-Thr-Asp-Fluoromethylketoe), a caspase 8 inhibitor. RIPK1 inhibitor Nec-1s was added to block necroptosis. There is no AIF translocation. The parallel study involving acidic pH found translocation of AIF into the nucleus in all pH 6 groups except for TSIN (TNF-α, SMAC mimetic, IETD, necrostatin-1s). In the pH 6.5 groups, translocation was seen in the T (TNF-α), TS (TNF-α, SMAC mimetic), and TSI (TNF-α, SMCA mimetic, IETD) groups.

Discussion: Necroptosis has been induced in MVECs and inhibited with necrostatin-1s. Furthermore, RIPK1 inhibition attenuated cell death at acidic pH. In Canada in 2019, around 200 patients underwent a heart transplant. Approximately 2300 Canadians are living with a transplanted heart in 2019 and 86.7% of patients survived at least 5 years. Cardiac allograft vasculopathy is a leading contributor to post-transplant failure and can lead to inadequate circulation due to vessel blockage. It is hoped that this work can improve cardiac transplantation outcomes and reduce rejection rates.