Presenter's Name: Abo-Amer, Yamen

Additional Author(s): Charkrabarti S, Feng B

Abstract Title: The Role of miR-9 in the Protection against Diabetic Renal Fibrosis

Abstract:

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

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

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

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

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

POSTER PRESENTATIONS 1
1A: CARDIOVASCULAR, RESPIRATORY HEALTH & METABOLIC DISEASE

Presenter's Name: Bajwa, Gurleen

Additional Author(s): Ni R, Peng T

Abstract Title: Forced Overexpression of CHOP Induces Myocardial Necrosis in a Mouse Model

Abstract:

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

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

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

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

Presenter's Name: Canatan, Aysegul

Additional Author(s): Samuels TN

Abstract Title: Epigenetic Biomarkers of Diabetic Cardiomyopathy: A Translational Scoping Review

Abstract:

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

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

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

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

POSTER PRESENTATIONS 1
1A: CARDIOVASCULAR, RESPIRATORY HEALTH & METABOLIC DISEASE

Presenter's Name: Dhupar, Narisa

Additional Author(s): Khan ZA

Abstract Title: The impact on CXCL12/CXCR4 signaling in target organ dysfunction in diabetes

Abstract:

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

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

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

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

Presenter's Name: Hijazi, Hassan

Additional Author(s): Peng T

Abstract Title: Targeting DDIT3-mediated necroptosis as a potential strategy to reduce diabetic cardiomyopathy

Abstract:

Diabetes, a group of metabolic disorders characterized by inappropriately high levels of blood glucose, is a major threat to global public health. While all organs may be negatively affected by diabetes, substantial morbidity and mortality is seen among patients whose heart becomes severely compromised absent any ischemic, hypertensive, valvular, or congenital heart diseases – a condition that is termed diabetic cardiomyopathy (DC). DC is characterized by cardiomyocyte cell death, impaired systolic and diastolic function, myocardial remodeling, and heart failure. However, the pathogenesis of DC and the cause of cardiomyocyte cell death are only partially understood. ER stress and necroptosis have recently been implicated in the pathophysiology of various cardiovascular diseases. When ER stress is prolonged, cell death may occur via the increased expression of DNA damage inducible transcript 3 (Ddit3), a pro-apoptotic transcription factor. Our first aim is to block necroptosis in both cardiomyocytes and mouse models of diabetes via plasmid overexpression of a truncated viral protein that inhibits complex formation of necroptosis mediators receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and RIPK3. To investigate whether the targeted inhibition of Ddit3 will reduce cardiomyopathy under diabetic conditions, shRNA will be used to determine the role of DDit3 in cardiomyopathy both in cultured cardiomyocytes and mouse models of diabetes. Our next aim is to target the pro-necroptotic functional domain of Ddit3 by peptides in order to inhibit necroptosis in cardiomyocytes and diabetic animal models. In the event that Ddit3's role in the progression of diabetic cardiomyopathy becomes established, treatments may be generated to reduce the cardiac morbidity burden for those suffering from diabetes.

POSTER PRESENTATIONS 1
1A: CARDIOVASCULAR, RESPIRATORY HEALTH & METABOLIC DISEASE

Presenter's Name: Kim, Matthew

Additional Author(s): Kiser P

Abstract Title: Impact of life long western diet consumption on late-term placentae

Abstract:

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

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

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

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

Presenter's Name: Lau, Ethan

Additional Author(s): Fung M, Armstrong JJ, Liu H, Hutnik CML

Abstract Title: Investigating Netarsudil's inhibition of myofibroblast activity using a novel microfluidics model

Abstract:

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

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

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

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

POSTER PRESENTATIONS 1
1A: CARDIOVASCULAR, RESPIRATORY HEALTH & METABOLIC DISEASE

Presenter's Name: Rehman, Iqra

Additional Author(s): Khan ZA

Abstract Title: PPARG Expression and Function in Non-Adipose Tissue

Abstract:

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

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

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

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

Presenter's Name: Tse, Brennan

Additional Author(s): Vanin Moreno S, Hardy D, Arany E

Abstract Title: Omega 3 supplementation's influence on ∆9-tetrahydrocannabinol exposure-induced fetal pancreatic growth restriction

Abstract:

The recent rise in popularity of cannabis use has driven the need for a more complete understanding of its implications. Recent reports suggest up to 7% of pregnant women use cannabis. Our lab and others previously showed in a rat model that exposure to ∆9-tetrahydrocannabinol (∆9-THC) in utero caused fetal growth restrictions and altered pancreas development, which caused glucose intolerance, decreased pancreatic islet density and β-cell mass, and peripheral insulin resistance at 5 months after birth. Previously, our laboratory showed that similar fetal defects caused by gestational diabetes were rescued by the supplementation of dietary olive oil via activation of PPARs. Therefore, we hypothesize that the dietary supplementation of the PPAR ligands omega-3 fatty acids will prevent fetal and pancreatic growth restrictions caused by maternal ∆9-THC exposure. To test this, a pregnant rat model was used. Subjects were given daily intraperitoneal injections of either 3 mg/kg ∆9-THC or vehicle from gestational day 6 until birth. Those who received the treatment were fed a chow diet that was or was not supplemented with omega-3 fatty acids. We collected pancreata at 3 weeks of age and at 3 months when glucose tolerance was tested to investigate if glucose impairment was detected earlier than our previous research demonstrated. Preliminary data showed no significant glucose impairment at 3 months in any of the treatment or control groups. Next another group at 5 months will be tested. Currently, immunohistochemistry is being conducted to analyze endocrine morphological changes by examining pancreatic islet density and α/β-cell mass. A decrease in β-cell mass and pancreatic islet density in the ∆9-THC group is expected. These changes will be eventually rescued by the addition of omega-3 fatty acids. The results of this study will further drive cannabis research and bring awareness to the potential dangers of cannabis use in pregnancy.

POSTER PRESENTATIONS 1
1A: CARDIOVASCULAR, RESPIRATORY HEALTH & METABOLIC DISEASE

Presenter's Name: Wang, Eric

Additional Author(s): Feng B, Chakrabarti S

Abstract Title: miR-9 prevents endothelial to mesenchymal transition in diabetic retinopathy

Abstract:

Background: Diabetic retinopathy (DR) is a common complication of diabetes and a significant cause of visual loss in working-aged adults. Elevated blood glucose penetrates and damages cells. Endothelial cells are particularly vulnerable to hyperglycemic damage due to their inability to dynamically regulate glucose uptake. Glucose-induced endothelial damage and dysfunction an important early occurrence in DR. Endothelial dysfunction in DR can come in the form of endothelial to mesenchymal transition (EndMT), a process where endothelial cells lose their endothelial characteristics acquire a mesenchymal phenotype. EndMT in the retina is driven by glucose, mediated by TGFβ and inflammatory signalling, and ultimately regulated by epigenetic processes. microRNA (miR) 9 is a small non-coding RNA and a mediator of epigenetic regulation that is significantly inhibited in the retina in DR. miR-9 reportedly affects both TGF and inflammatory signalling. We hypothesized that miR-9 downregulation facilitates EndMT in early DR.

Methods: Human retinal endothelial cells (HRECs) were used to model the behaviour of the retinal endothelium in DR. HRECs were harvested after 48-hour treatments with normal (5mM) or high (25mM) concentrations of glucose, and RNA expressions of EndMT-related genes were assessed using qPCR. miR-9 mimic was used to rescue glucose-induced EndMT in HRECs. Mouse models were used to validate the findings in vivo. Diabetes was induced in 8-week-old mice using streptozotocin, age-matched controls were given mock injections. Retinal tissues were harvested after 2 months, and RNA and protein expressions of EndMT-related genes were analyzed using qPCR and ELISA respectively. Specially generated endothelial-specific miR-9 overexpressing mice were used to validate the ability of miR-9 overexpression to prevent EndMT.

Results: Glucose significantly inhibited miR-9 expression in HRECs. Glucose-induced inhibition of miR-9 correlated with the occurrence of EndMT. Transfection with miR-9 mimics prevented EndMT in HRECs. Similar results were reproduced in animals; EndMT was detected in the retinas of normal diabetic mice, and not in those miR-9 overexpressing diabetic mice.

Discussion: We showed that glucose-induced miR-9 inhibition facilitates EndMT in the retina, and that overexpression of miR-9 can prevent glucose-induced EndMT in DR. miR-9 may represent an opportunity for targeted RNA-based gene therapy against DR.