Presenter's Name: Brower, Brandon

Additional Author(s): Derouet M, Zhang L, Larsen F, Asfaha S

Abstract Title: Development of an in-vitro Model of Inflammatory Bowel Disease

Abstract:

Introduction: Inflammatory bowel disease (IBD) comprises many chronic intestinal diseases, primarily Crohn's disease (CD) and Ulcerative Colitis (UC). A commonly used strategy to study these diseases in-vitro includes epithelial-only intestinal organoids, but their lack of stromal components limits our ability to study epithelial-mesenchymal/immune cell interactions. In contrast, Air-Liquid Interface (ALI) organoids, first described by Kuo and colleagues, contain all intestinal layers, including both epithelial and stromal tissue. While ALI organoids from tumors and neonatal mouse intestine have been described, ALI organoids derived from healthy and colitis adult colon have not been characterized. Thus, our goal is to use ALI organoids to encapsulate both epithelial and stromal components of the colon to recapitulate healthy and diseased states of the gut.

Methods: Following the previously described Kuo protocol, we generated small intestinal and colonic ALI organoids from healthy neonatal (<1 week) mice. Similarly, ALI small intestinal or colonic organoids were derived from healthy adult (4-6 week old) mice. Finally, adult mice were administered 2.5% dextran sodium sulfate (DSS) in the drinking water for 5 days to induce colitis. 10 days following the cessation of DSS treatment, mice were sacrificed and again colonic ALI organoids generated. Organoids were followed using stereo and bright-field microscopy and assessed by H&E staining of formalin-fixed paraffin embedded sections. To improve our culture efficiency, modifications to the protocol including addition of various stem cell growth factors are currently being examined.

Results: Healthy neonatal and adult ALI organoids appear to encapsulate both epithelial and stromal components consistent with native tissue; however, we have observed a dramatic drop in culture efficiency in ALI organoids derived from adult relative to neonatal tissue. Our preliminary data suggests that addition of Wnt3a to cultures derived from adult mice significantly improves the efficiency of ALI organoid generation. In addition, colonic ALI organoids generated from mice post-colitis also contain both epithelial and stromal components.

Significance: These data suggest that ALI organoids can indeed be generated from the healthy and inflamed adult mouse colon. Therefore, this method holds promise as a model for recapitulating both epithelial and stromal components of the adult colon in health and disease.

POSTER PRESENTATIONS 3
3C: REGENERATIVE & TRANSPLANTATION MEDICINE

Presenter's Name: Collings, Samantha

Additional Author(s): Min W, Zheng X, Liu Q, Won S, Diao H, Haig A

Abstract Title: The role of DLC1β in attenuating cardiac ischemia-reperfusion injury during heart transplantation

Abstract:

Introduction: Heart transplantation is the only known curative step for heart failure. However, the transplantation process is injurious to the donor heart via ischemia-reperfusion (I/R) injury. Pathological changes that occur in a post-I/R injury heart include enhanced hypertrophy of the myocardium, dysregulated angiogenesis, and apoptosis. However, the pathogenesis of cardiac I/R injury is unclear. Past research has displayed that PI3K/Akt1, and RhoA/ROCK pathways may have a role in attenuating cardiac I/R injury. Therefore, the beta-isoform of deleted-in-liver-cancer 1 protein (DLC1β) may be associated with cardiac I/R injury due to its ability to activate Akt1 and regulate RhoA activity. It is hypothesized that downregulating DLC1β will increase the severity of cardiac I/R during transplantation.

Methods: The study aims to: 1) investigate DLC1β's role in cardiac I/R injury, 2) to analyze if DLC1β is a novel target in preventing cardiac I/R, and 3) to explore signaling pathways in which DLC1β mediates I/R injury. The study includes both in-vivo/in-vitro aspects. The in-vivo design utilized DLC1β deficient mice compared with wild-type (WT C57BL/6) mice. Murine heterotopic heart transplantation was performed, and grafts were subject to cold-storage ischemia for 18 hours. Finally, harvested grafts were analyzed. Pathological changes in excised heart tissues (dysregulated angiogenesis, apoptosis, and hypertrophy) will be noted on further analyses. The in-vitro design utilizes HL-1 cells (mouse atrial cardiomyocytes) transfected with DLC1β overexpressing plasmid. Cells will undergo GENbag ischemia, and then will subjected to subsequent experiments.

Results: Like the in-vivo study, differences in injury grade (percentage of the heart effected by I/R injury) were noted. In-vitro, there was a reduction in apoptosis when cells were transfected with DLC1β overexpression plasmid. There also appears to be a reduction in cell death in the DLC1β overexpression plasmid groups. However further analysis is required to improve both techniques and reduce intragroup variability in future.

Discussion: It's of interest analyze potential cardioprotective effects of DLC1β for future therapeutics. It's expected that DLC1β is a negative regulator of I/R injury due to preliminary data. The findings of this experiment are believed to be significant to the field as reducing cardiac I/R injury could potentially enhance the viability of heart transplantation in future.

Presenter's Name: Greasley, Adam

Additional Author(s): Peng T, Zheng X

Abstract Title: Circular RNA HIPK3 as an antagonist of ischemia reperfusion injury

Abstract:

Introduction: Heart transplantation is arguably the only long-term cure for people living with end-stage heart failure. From the estimated 750,000 Canadians living with heart failure, only a small portion will ever be considered as transplant candidates. A large factor in the low number of heart transplantations is graft survival following organ preservation due to ischemia reperfusion injury (IRI). Circular RNA HIPK3 (circHIPK3) is a popular circular RNA in recent years, which has been shown to be involved in injury response and cell growth. Our previous work showed that circHIPK3 is an immediate responder to IRI, with expression increase just 2 h after reperfusion begins. We showed that circHIPK3 knockdown prior to IRI leads to increased cell death and injury. Therefore, we hypothesize that circHIPK3 works as a cell survival signal and an antagonist to cell death, and circHIPK3 over-expression may be an effective tool for reducing injury following organ preservation.

Methods: To increase circHIPK3 expression, circHIPK3 over-expression vectors were constructed using flanking intron sequences. The expression of circHipk3 was measured by qPCR. To simulate IRI after organ preservation in vitro, we used AC16 human cardiomyocytes and induce cold (10°C) hypoxia at 0.5% O2, 5.0% CO2 and 94.5% N2 during storage. To assess whether circHIPK3 can be an effective mediator of IRI, cells were transfected with circHIPK3 over-expression plasmids, or RNA fractions enriched with circHIPK3 by RNase R digestion prior to hypoxia. Following reperfusion, we dynamically measured cell death using an incucyte system and quantified at time points of interest using Annexin V staining. To examine cell injury, we measured LDH to assess damage and mitochondrial membrane potential to examine mitochondrial function following injury. In addition, apoptotic and necroptotic genes such as Bax, Bcl-XL and P-MLKL are measured using qPCR and western blotting.

Results: Our results show that circHIPK3 over-expression can be achieved following transfection and seems to attenuate injury. However, our results suggest plasmid-based expression systems induce cell stress and toxicity during preservation.

Discussion: Although we hypothesize circHIPK3 expression increase to be beneficial for cell survival following IRI, our current plasmid-based systems induce cell toxicity. Therefore, purified and concentrated forms of circHIPK3 must be administered to determine circHIPK3 as an effective therapy.

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3C: REGENERATIVE & TRANSPLANTATION MEDICINE

Presenter's Name: Hsu, Mackenzie

Additional Author(s): Howlett C, Khan ZA

Abstract Title: Molecular mechanisms of ageing related stem cell depletion in the bone marrow

Abstract:

Introduction: Bone marrow is a rich reservoir of hematopoietic and non-hematopoietic stem cells. These regenerative cells are responsible for replenishing blood, connective, and vascular cells. However, the cellular composition of the bone marrow changes with ageing. These changes are generally associated with stem cell depletion. The mechanisms dictating bone marrow cellular composition and depletion of regenerative stem cells are not fully known, and are the focus of the present study.

Methods: To understand cellular and molecular changes in the bone marrow during the normal ageing process, we will examine bone tissues of male and female C57BL/6 mice at different ages. To do this, we will harvest both femur and tibia of mice at 8, 23, and 48 weeks of age. These timepoints correspond to approximately 20 to 50 human years. Harvested bone tissues will be processed for morphometric analyses and immunostaining to examine cell types in the marrows. We will also prepare a single cell suspension from the bone marrows of these mice for gene expression profiling to identify molecular changes that may govern stem cell depletion.

Results: This study is currently ongoing. We have harvested bone tissues from mice and single cell suspension of the bone marrows has been prepared. Based on limited data available from previous human and rodent studies, we expect to see increased adiposity in the bone marrow with advanced ageing. In addition, we anticipate stem cell niche factors to be supressed, leading to reduced number of marrow-resident stem cells. Once candidate factors have been identified, we will explore the effect of neutralizing the change to determine if age-related stem cell depletion can be prevented.

Significance: This research will allow for a better understanding of the age-related changes which occur in the bone marrow. We anticipate that our studies will lead to the identification of targets for the prevention of ageing related deteriorations.

Presenter's Name: Lee, Connar

Additional Author(s): Lu H, Zhang ZX

Abstract Title: The Role of RIPK3 and GLUD1 during TLR3-mediated Necroptosis in Mouse Microvascular Endothelial Cells

Abstract:

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

POSTER PRESENTATIONS 3
3C: REGENERATIVE & TRANSPLANTATION MEDICINE

Presenter's Name: Lim, Samantha

Additional Author(s): Ravichandran S, Luke PPW, Bhattacharjee RN

Abstract Title: Impact of Carbon Monoxide on Ischemia Reperfusion Injury in an Ex Vivo Porcine Model of Donation after Circulatory Death

Abstract:

Introduction: Chronic kidney disease is a condition that affects millions of people around the world. When end-stage kidney failure occurs, kidney transplantation is the best treatment option. Due to a shortage of living donors, donation after circulatory death (DCD) kidneys are often used for transplants. DCD kidneys are particularly susceptible to ischemia reperfusion injury (IRI). IRI is mediated by toll-like receptors (TLRs) which are activated due to tissue injury, leading to the transcription of pro-inflammatory cytokines. The inflammation and cellular damage associated with IRI can cause delayed graft function and graft failure in renal transplants. Previous studies have shown that carbon monoxide (CO) has anti-inflammatory properties. We hypothesize that CO will reduce inflammation and injury in ex vivo renal porcine models under DCD transplant conditions.

Methods: We subjected large, male, Landrace pigs to in situ cross-clamping of the renal pedicle to simulate DCD conditions. We then removed the kidneys and treated them with gaseous CO before putting them on a pulsatile perfusion pump for 12 hours. We then reperfused the kidneys with stressed porcine blood for 4 hours. Tissue, urine, and blood samples were taken. Levels of pro-inflammatory cytokines and kidney damage will be measured using enzyme-linked immunosorbent assay (ELISA) specific for porcine HMGB1, NGAL and IL-6. To assess levels of acute tubular necrosis (ATN) and apoptosis, pathological staining and grading with hematoxylin-eosin and TUNEL stains have been performed. Expression of TLRs will be explored using immunohistochemistry.

Results: Preliminary results indicate that CO reduces levels of ATN and apoptosis in CO-treated kidneys compared to control kidneys. We expect that CO-treated kidneys will express lower levels of pro-inflammatory cytokines and kidney injury markers than their control counterparts. We expect to see many different TLRs expressed on renal porcine tissue.

Discussion: This study will demonstrate whether CO has anti-inflammatory properties in ex vivo renal porcine models under DCD transplant conditions. This indicates that CO may reduce IRI-mediated damage in transplanted human kidneys, and lead to the long-term graft survival of more DCD kidneys. TLR expression profiles on porcine kidneys will strengthen its use as a model for human transplant kidneys and will help with selection of cell-lines for future in vitro studies of TLR-mediated inflammation.
Presenter's Name: Lu, Haitao

Presenter's Name: Lu, Haitao

Additional Author(s): McLeod P, Huang X, Zhang ZX

Abstract Title: CaMKs interactively regulate Drp1 in mediating necroptosis and inducing heart transplantation rejection

Abstract:

Background: Mitochondrial dysfunction is associated with organ transplantation rejection and poor allograft prognosis. Activated Drp1 is one of important contribution to damaged mitochondria by inducing mitochondrial fragmentation through excessive fission. During fission, elevated Ca2+ intake and decreased ATP showed the relationship with CaMKs family proteins. It was demonstrated that CaMK2 was activated by RIPK3 to mediate cell death signals; in addition, CaMK1 and /or CaMK4 had functions on activation of CaMK2. Therefore, we hypothesized that interactions among CaMK1/2/4 regulate Drp1 as necroptosis and induce heart transplantation rejection.

Methods: First, In vitro, we confirmed the role of Drp1 and CaMKs in mediating necroptosis with the use of their inhibitors Mdivi1 and KN93 respectively. The number of cell death can be measured by Incucyte ZOOM machine. Next, we determined the activation of Drp1 by CaMK1 or CaMK2 by Western Blotting (WB) with camk1 and camk2 silencing. Moreover, protein-protein interaction assays were done to show proofs for the interaction between CaMK1 and CaMK2. Besides, we noticed that either CaMK1 or CaMK4 has effects on CaMK2 activation, we detected CaMK4 expression level by WB. Then we also determined the phospho- Drp1 in various groups (sicamk1, sicamk2, sicamk1+4, and sicamk1+2+4) for confirming the interaction of CaMK1/2/4. Moreover, we study whether PGAM5 is activated by CaMKs and thereby having effects onto Drp1. Finally, Immunocytochemistry was used to detect p-CaMK1 and p-CaMK2 during necroptosis.

In vivo, first, we tried to confirm the KN93 saves mice in transplantation and lengths survival time. Then we detected anti-p-camk1 and anti-p-camk2 in transplanted heart by immunostaining, observed by H&E.

Results: Mdivi1 and KN93 effectively protected cell death. WB supported inhibitions of cell death had lower phospho-Drp1 expression. In addition, both CaMK1 and CaMK2 activate p-Drp1. Revolutionarily, we found CaMK2 might be activated by either CaMK1 or CaMK4. Activated CaMK4 appeared when CaMK1 reduction. Moreover, CaMK2 interacts with PGAM5 during necroptosis.In vivo results are on waitlist to be detected by the Pathology Center in University Hospital.

Conclusions: CaMKs interactively activate each other and the down-stream PGAM5 and Drp1 to induce mitochondrial damage and necroptosis. Inhibition of CaMKs or Drp1 protects cell death and may be therapeutically used to prevent transplant rejection

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Presenter's Name: Ravichandran, Sevanthi

Additional Author(s): Bhattacharjee RN, Luke PPW, Sener A, Gunaratnam L

Abstract Title: Efficacy of antidiabetic drugs in the management of renal ischemia reperfusion injury

Abstract:

Introduction: Due to a perpetual need for kidneys for transplantation, we must expand on the pool of eligible donors. This includes organ donations after circulatory death (DCD). During the stages of IRI, there are several events taking place. Multiple inflammatory pathways are induced which release a cytokine storm and reactive oxygen species like superoxide and hydrogen peroxide. While machine perfusion has been found to reduce IRI in organs during preservation, there is still a considerable gap. We hypothesize that the in vitro mouse model will show increased inflammation and cell death under hypoxia-anoxia-reoxygenation (HAR) injury conditions compared to normally grown cells. With that, drug repositioning of certain approved anti-diabetic drugs can be used in ameliorating the effects of ischemia reperfusion injury using a HAR injury model.

Methods:
1. Develop a human kidney (HK2) cell culture line under a HAR injury model.
2. Characterize ATP, NFkB binding, IL6, TNFalpha in HK2 HAR model.
3. Comparison of expression of aim 2 post-incubation of a number of antidiabetic drugs on in vitro mouse model.
4. Comparison of effects of IRI via renal pedicle clamping with in vivo mouse model post-incubation of 3 shortlisted drugs.

Results: Experiments are currently ongoing. We expect to see that treatment of HAR to cells will induce an inflammatory response, resulting in increased cytokines and pro-inflammatory markers, along with signs of necrosis and apoptosis. This will be our baseline. Meanwhile, a list of antidiabetic drugs are currently being compiled, referencing to previous studies and some rationale. We expect that some drugs will have a protective effect from ischemia reperfusion injury, showing reduced levels compared to the baseline. 3 shortlisted drugs will be translated to the in vivo model. As inlammatory pathways are conserved between humans and mice, we should expect to see reduced inflammation in mice subjected to ischemia reperfusion injury post-incubation of the shortlisted drugs.

Discussion: We hope to identify at least one drug that can be translated toward clinical trials.

Presenter's Name: Won, Sohyun

Additional Author(s): Collings S, Liu Q, Diao H, Min W

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

Abstract:

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

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

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

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

POSTER PRESENTATIONS 3
3C: REGENERATIVE & TRANSPLANTATION MEDICINE

Presenter's Name: Xu, Laura

Additional Author(s): McLeod P, Huang X, Zhang ZX

Abstract Title: Acidic pH Environment Alters Cell Death and Regulates AIF Translocation in Endothelial Cells

Abstract:

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. Necroptosis is a therapeutic target in ischemia-reperfusion injury, which features an acidic microenvironment that can affect the viability of transplanted organs. Apoptosis-inducing factor (AIF) is implicated in caspase-independent cell death and DNA damage by translocating into the nucleus from the mitochondria. However, its role in acidic conditions during ischemia-reperfusion injury has not yet been established.

Methods: Mice microvascular endothelial cells (MVECs) were isolated for experiments. Cell death was quantified by real-time imaging using media adjusted and stabilised to pH 7.4 and 6.5 and the following treatments: TNF-alpha [T]; TNF-α and second mitochondrial activator of caspase (SMAC) [TS]; TNF-α, SMAC, and IETD [TSI]; TNF-α, SMAC, and necrostatin-1s [TSN]; and TNF-α, SMAC, IETD, and necrostatin-1s [TSIN]. Immunochemistry staining for AIF was performed and counterstained with DAPI. Nuclear translocation of AIF was quantified with cell counts of fluorescent images. Nuclear fragmentation 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: The highest levels of cell death at pH 7.4 were in TS and TSI groups, which induce apoptosis and necroptosis respectively. However, at pH 6.5, TSI displayed a protective effect against cell death compared to the TS group, suggesting that cell death modality is altered at a lower pH. Nuclear translocation of AIF was increased at pH 6.5 in all treatment groups and nuclear fragmentation displayed laddering in all treatment groups at pH 6.5 which was absent at pH 7.4.

Discussion: Cardiac transplantation is the best treatment option for patients with end-stage heart failure. However, prolonged ischemia during the transplantation procedure due to the cessation of blood flow induces anaerobic metabolism, lactic acid accumulation, and intracellular acidosis. Thus, it may be beneficial to examine the mechanisms underlying ischemia and the acidification of the cellular environment to improve cardiac transplantation success.