

3071

### Cell Survival in Corneal Endothelial Dystrophies

Rajalekshmy Shyam<sup>1</sup>, Diego Ogando<sup>2</sup>, Moonjung Choi<sup>2</sup> and Joseph Bonanno<sup>2</sup>

<sup>1</sup>Indiana University School of Medicine and <sup>2</sup>Indiana University Bloomington

**OBJECTIVES/SPECIFIC AIMS:** Purpose - The goal of this study is to understand how loss of the membrane protein SLC4A11 alters endothelial cell metabolism thereby producing Corneal Endothelial Dystrophy. Studies from our lab indicated that glutamine-dependent mitochondrial dysfunction is one of the outcomes of SLC4a11 loss. In the current study, we ask if autophagy and mitophagy pathways and the signaling pathways that regulate these processes are altered in SLC4a11 KO cells. **METHODS/STUDY POPULATION:** Methods – Immortalized mouse WT and SLC4a11 KO cell lines were incubated in DMEM with and without 0.5mM glutamine for 6 hours. In order to assess mitophagy, cells were stained using LysoTracker Red and Mitotracker Green. Colocalization co-efficients of red and green channels were obtained for at least 35 cells using Zeiss-Zen Pro software. Student's t-test was used to determine statistical significance. For Western Blots, antibodies against LC3b, AMPK, pAMPK, and b-actin were used to examine autophagy flux and potential signaling pathways that regulate autophagy. **RESULTS/ANTICIPATED RESULTS:** Results – In the presence of glutamine, the colocalization co-efficient of LysoTracker Red and Mitotracker Green channels was significantly increased in KO cells ( $0.74 \pm 0.18$ ) relative to WT ( $0.58 \pm 0.20$ ) with a p-value  $\leq 0.0024$ . In the absence of glutamine, the colocalization co-efficient was reversed, for KO cells  $0.54 \pm 0.14$  and for WT cells  $0.77 \pm 0.16$  with a p-value  $\leq 0.0001$ , suggesting increased mitophagy by glutamine in KO cells. Western Blots indicated that glutamine increased autophagy flux, as indicated by increased levels of LC3b following bafilomycin A treatment in KO cells. Concomitantly, there was an increase in pAMPK/AMPK levels suggesting a potential mechanism for increased mitophagy. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Conclusion and Future studies –Our data indicates enhanced mitophagy as well as autophagy in SLC4a11 KO cells. Future studies will determine whether these processes regulate cell survival in mouse models of corneal endothelial dystrophies.

3069

### Characterizing the Neural Signature of Metabolic Syndrome

Eithan Kotkowski<sup>1</sup>, Larry R. Price<sup>1</sup>, Crystal G. Franklin<sup>1</sup>, Maximino Salazar<sup>1</sup>, Ralph A. DeFronzo<sup>2</sup>, David Glahn<sup>3</sup>, John Blangero<sup>4</sup> and Peter T. Fox<sup>1</sup>

<sup>1</sup>University of Texas Health Science Center San Antonio: Research Imaging Institute; <sup>2</sup>UTHSA: Diabetes Division, Texas Diabetes Institute; <sup>3</sup>Yale School of Medicine and <sup>4</sup>UTRGV School of Medicine

**OBJECTIVES/SPECIFIC AIMS:** Our objective is to understand the influence of the features comprising metabolic syndrome (central obesity, raised fasting plasma glucose, triglycerides, blood pressure, and decreased HDL cholesterol) on brain structure in men and women. With the understanding that MetS is a strong predictor of gray matter volume loss in specific brain regions, in this study we sought to quantify the influence of each of the metabolic syndrome biometric variables on the structures involved in the neural signature

of metabolic syndrome. **METHODS/STUDY POPULATION:** We conducted multiple linear regression analyses on a cross-sectional sample of 800 individuals from the Genetics of Brain Structure (GOBS) image archive (352 men and 448 women). GOBS is an offshoot of the San Antonio Heart Study involving an extended pedigree of Mexican Americans from the greater San Antonio area. Its goal is to localize, identify, and characterize genes/quantitative trait loci associated with variations in brain structure and function (Winkler, 2010). The archive has continuously added participants from approximately 40 families since 2006. Neuroanatomic (T1-weighted MRI scans obtained on a Siemens 3T scanner and processed using FSL), neurocognitive, and biometric phenotypes have been obtained for each subject (including blood lipids). Linear regressions were run using SPSS and incorporated biometric and gray matter volume values obtained from 800 GOBS participants. **RESULTS/ANTICIPATED RESULTS:** Linear regressions incorporating metabolic syndrome variables as dependent variables and gray matter volume from regions involved in the neural signature of metabolic syndrome as predictors show significant predictive patterns that are largely similar between men and women, with some differences. Another linear regression conducted with gray matter volume from the neural signature of metabolic syndrome as the dependent variable and metabolic syndrome variables as predictors show that waist circumference and triglycerides are the greatest predictors of gray matter volume loss in men, and fasting plasma glucose and waist circumference are the greatest predictors of gray matter volume loss in women. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Significant sex differences in the relationships between metabolic syndrome variables and gray matter volume changes between brain regions comprising the neural signature of metabolic syndrome were identified. waist circumference, fasting plasma glucose, and triglycerides are the most reliable predictors of gray matter volume loss. The variance in gray matter volume of the neural signature of metabolic syndrome in men is more significantly explained by waist circumference and triglycerides (when accounting for age) and in women is more significantly explained by waist circumference and fasting plasma glucose (when accounting for age). A model of metabolic syndrome that emphasizes a risk of neurodegeneration should focus on waist circumference for both men and women and weigh the remaining variables accordingly by sex (triglycerides in men and fasting plasma glucose in women).

3375

### Chronic inflammation promotes intestinal macrophages to become modulators of the Notch pathway

Eliseo Castillo

University of New Mexico Clinical and Translational Science Center

**OBJECTIVES/SPECIFIC AIMS:** The purpose of this research was to investigate how chronic inflammation promotes the generation of proinflammatory intestinal macrophages and if macrophages contribute to intestinal inflammation through Notch activation. **METHODS/STUDY POPULATION:** We utilized two animal models of chronic colitis, the chronic DSS-induced colitis mouse model and the spontaneous enterocolitis development in IL-10-deficient mice to investigate the role of chronic inflammation in the generation of proinflammatory intestinal macrophages and its influence in notch signaling. Bone marrow-derived monocytes were collected from each group and differentiated into macrophages (BMM) for gene and protein analysis. Ex vivo phenotypical and functional

analysis of colonic macrophages was assessed as was the presence of goblet cells and mucosal T cells. In addition, we analyzed the development of goblet cell differentiation in colonoids in a co-culture system with proinflammatory macrophages. RESULTS/ANTICIPATED RESULTS: Our chronic inflammation models revealed an increase in proinflammatory macrophages present in the lamina propria and that these cells expressed significantly higher levels of notch ligand, Jagged1. Jagged 1 has been shown to enhance TH1 differentiation and T cells isolated from the mucosa of both chronic colitis models display strong TH1 skewing compared to controls. Chronic inflammation also contributes to intestinal barrier defects, enhanced permeability and bacterial translocation. We believe this enhanced intestinal permeability and subsequent bacterial translocation promote Jagged1 expression in intestinal macrophages. To support this concept, we show TLR stimulation induces the upregulation of Jagged1 in BMM. Additionally, the generation of BMM from our chronic DSS-induced colitis mice or age matched controls, revealed BMM derived from a host of chronic inflammation were skewed to a proinflammatory state prior to stimulation showing increased gene expression of several proinflammatory molecules including IL-1 $\alpha$ , IL-1 $\beta$ , IL-12 and TNF- $\alpha$ . This would suggest monocytes migrating to the intestinal mucosa have more potential to become proinflammatory instead of traditional anti-inflammatory macrophages. Furthermore, proinflammatory notch ligand-positive macrophages co-cultured with colonoids, derived from unperturbed mice, significantly decreased the number of mucus producing goblet cells. In support of this observation, notch activation in intestinal stem cells promote absorptive (i.e. colonocytes) cell differentiation and prevents secretory cell (i.e. goblet cells) differentiation. DISCUSSION/SIGNIFICANCE OF IMPACT: Taken together, our results strongly suggest chronic inflammation modulates macrophages role in maintaining intestinal homeostasis through possible notch activation in both T cells and the intestinal epithelial barrier.

3508

### Ciclopirox Olamine Demonstrates Inhibitory Effects on Esophageal Tumor Cells

Randi Ryan<sup>1</sup>, Shrikant Anant<sup>2</sup>, Prabhu Ramamoorthy, PhD<sup>2</sup>, Dharmalingam Subramaniam, PhD<sup>2</sup> and Scott Weir, PharmD, PhD<sup>2</sup>  
<sup>1</sup>University of Kansas Frontiers and <sup>2</sup>University of Kansas Medical Center

OBJECTIVES/SPECIFIC AIMS: Drug repositioning has the potential to accelerate translation of novel cancer chemotherapeutics from bench to bedside. The goal of this study was to determine the effects of ciclopirox olamine (CPX) on esophageal tumor cells. METHODS/STUDY POPULATION: We tested the effect of CPX on four esophageal cancer cell lines, assessing cell proliferation and viability by hexosaminidase and clonogenicity assay, respectively. We analyzed the effects of CPX on three-dimensional (3D) esophageal tumor cell spheroids. We also analyzed effects on cell cycle by flow cytometry. For mechanism, we performed western blots for proteins involved in cell cycle regulation, apoptosis and the Wnt/ $\beta$ -catenin pathway. For in vivo effects, we performed a murine xenograft model with intraperitoneal administration of CPX (100 mg/Kg body weight daily). RESULTS/ANTICIPATED RESULTS: CPX inhibited growth of all cell lines in a time and concentration-dependent manner. CPX also inhibited growth of esophageal spheroids. Cell cycle analysis demonstrated G0/G1 arrest in cells treated with CPX. Western blot analyses

demonstrated decreased expression of cyclinD1, CDK4, CDK6, and transcriptionally active  $\beta$ -catenin, supporting the role of CPX in cell cycle inhibition and decreased  $\beta$ -catenin activity. Finally, treatment of nude mice with CPX significantly decreased tumor xenograft volume. DISCUSSION/SIGNIFICANCE OF IMPACT: CPX demonstrates anti-tumor properties in esophageal cancer cell lines. The current results justify further research into the mechanism of this inhibition. Additionally, given its established safety in humans, CPX is a potential candidate for repositioning as an adjunct treatment for esophageal cancer.

3389

### Coagulation Factor XII-mediated contact system and its role in adaptive and innate immune cell movement

Emily Beltran<sup>1</sup>, Tukisa Smith<sup>1</sup>, Beth Graczyk<sup>1</sup>, Jan Breslow<sup>1</sup> and Manish Ponda, M.D.<sup>1</sup>

<sup>1</sup>Rockefeller University

OBJECTIVES/SPECIFIC AIMS: The objectives of this study are to 1) expand upon the paradigm of HK-D5 accelerated immune cell chemotaxis; 2) characterize the role of FXII in murine models of immune-mediated disease using FXII KO mice and a small molecule inhibitor of FXIIa. METHODS/STUDY POPULATION: To test whether the addition of HK-D5 peptide would accelerate C-C chemokine receptor type 2 (CCR2)-mediated chemotaxis in vitro, a real-time transwell chemotaxis assay was developed utilizing human THP-1 monocyte cell line (Fig 1). For in vivo studies, both pharmacologic FXIIa antagonism and FXII KO mice were used. Genotyping, histopathological review, FXII protein expression, and active partial thromboplastin time (aPTT) measurements were used to phenotypically characterize FXII KO mice compared to C57BL/6 wild type mice (Fig 2). Subsequently, the FXII KO mice were studied in varied models of immune-mediated disease, including LPS-induced sepsis and experimental autoimmune encephalitis (EAE), to see if and how the absence of FXII can mitigate disease severity. The EAE model involved active immunization with myelin oligodendrocyte glycoprotein (MOG) and measurement of established clinical disease severity scores. The LPS sepsis model involved an intraperitoneal injection of LPS followed by 48-hour monitoring of core body temperature using subdermal temperature transponders as a proxy for inflammatory events related to septic shock (Fig 3). RESULTS/ANTICIPATED RESULTS: HK-D5 significantly accelerates CCR2-mediated chemotaxis compared to chemokine alone ( $p = 0.001$ ) similar to HK-D5's ability to accelerate CCR7-mediated chemotaxis as previously established. The FXII KO mice were backcrossed to the C57BL/6J background and confirmed by genotyping and complete absence of FXII protein in plasma. Compared to the control, FXII KO mice have a significantly prolonged aPTT without evidence of bleeding abnormalities, which confirms the expected phenotype previously described and recapitulates what is observed in Factor XII deficiency in humans. KO mice showed no significant gross or histopathological differences in secondary lymphoid structures compared to the control. Immunohistochemistry confirmed well-organized lymphoid structures with intact B- and T-cell populations. FXII KO mice are protected in LPS-induced septic shock and EAE models. Regarding the EAE model, FXIIa inhibition significantly reduced disease severity compared to control. In the LPS model, FXII KO mice recover within 24 hours after LPS-challenge measured subjectively and objectively by core body temperature measurement. DISCUSSION/SIGNIFICANCE OF IMPACT: The current study