430

Time restricted feeding in diet induced obesity mouse model reduces aortic stiffness and inflammatory T cells

Claudia Edell[1], Paramita Pati[1], Jackson Colson[1], Patrick Molina[1], David M Pollock[1] and Jennifer S Pollock[1]
[1]University of Alabama at Birmingham

OBJECTIVES/GOALS: Time restricted feeding (TRF) in diet induced obesity (DIO) has several health benefits, including improved metabolic rhythms and inflammation. Our lab has shown that TRF in DIO significantly reduces renal and aortic damage. The main goal of our research is to understand how TRF impacts aortic function, organ damage, and T cell activation in DIO. METHODS/STUDY POPULATION: We will use a 20-week DIO model, where mice will be on 20 weeks of normal fat diet (ND) or high fat diet (HFD). During weeks 18-20, mice will go through TRF intervention where food is restricted to the 12-hour active period or continue ad libitum feeding. At the end of the 2-week TRF intervention or continued ad libitum feeding, aortic stiffness will be measured via pulse wave velocity measurements. We will also collect kidney, aorta, and small intestine at the end of the 20-week protocol for flow cytometric analysis of tissue T cell activation as well as histological assessments. This will allow us to determine the relationship with organ damage, organ function, and the T cell response. We will also analyze tissue and circulating levels of inflammatory T cell-derived cytokines such as interleukin-17A (IL-17A) via ELISA. RESULTS/ANTICIPATED RESULTS: DIO mice showed significantly increased aortic stiffness (measured by pulse wave velocity) compared to mice on ND. Interestingly, TRF intervention in DIO mice reduced aortic stiffness compared to DIO ad libitum. Histological assessments also showed that TRF abolished aortic and kidney fibrosis suggesting a role for the timing of feeding in regulating aortic function and organ damage from chronic HFD. We have several ongoing experiments to determine the T cell response with TRF in DIO mice. We predict that TRF in DIO mice will significantly decrease inflammatory T cells and reduce cytokine abundance in target organs. DISCUSSION/SIGNIFICANCE: Our lab has shown that TRF reduces aortic thickness and aortic and kidney fibrosis, but the driving mechanisms are unknown. We propose that TRF reduces T cell activation in DIO mice leading to reduced organ damage. Our work will provide insight on how TRF in DIO regulates the T cell response and may improve inflammation in the kidney and aorta.

431

Galectin-3 as a Biomarker and Potential Therapeutic Target in Biliary Atresia*

Dor Yoeli[1], Yuhuan Luo[2], Alexander Chaidez[2], Zhaohui Wang[2], Megan A. Adams[2], Christene A. Huang[2], Cara L. Mack[2] and Nalu Navarro-Alvarez[2,3]
[1]University of Colorado Denver, [2]University of Colorado and Childrens Hospital Colorado, Aurora, CO, USA and [3]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Department of Gastroenterology, Mexico City, Mexico.

OBJECTIVES/GOALS: Biliary atresia (BA) is a progressive congenital disease that is characterized by periductular inflammation and fibrosis that leads to bile duct destruction and cholestasis in neonates. Galectin-3 (Gal3) plays a key role in inflammation and fibrosis. The aim of this study was to evaluate plasma Gal3 levels in early and late BA. METHODS/STUDY POPULATION: Samples from our institutional Pediatric Liver Biobank were used for this study. Patients were categorized as early BA (at diagnosis), late BA (at liver transplant), early other cholestatic liver disease (CLD), late other CLD, or controls without cholestasis or structural liver disease. Plasma Gal3 levels were measured by standard ELISA. Inflammatory cytokines were measured in a subset of samples using MSD Proinflammatory Panel 1 multiplex ELISA. Liver fibrosis was categorized as none (Ishak or METAVIR 0), mild (Ishak 1-2 or METAVIR 1), moderate (Ishak 3-4 or METAVIR 2-3), and severe (Ishak 5-6 or METAVIR 4) based on histology. Data are presented as median (IQR) and compared using Kruskal-Wallis test. Spearmans correlation was used to assess the relationship between Gal3 and clinical and inflammatory markers. RESULTS/ANTICIPATED RESULTS: Samples from 10 controls, 26 early BA, 24 late BA, 13 early other CLD, and 8 late other CLD patients were used for this study. Gal3 levels in late BA (20.8 [12.4-30.5] ng/mL) and late other CLD (21.8 [16.9 – 27.2] ng/mL) were significantly higher than in controls (10.2 [7.6 – 14.5] ng/mL, $p < 0.02$) and early BA (11.3 [8.7 – 16.8] ng/mL, $p < 0.01$), but not significantly different from early other CLD (15.7 [11.9 – 21.4] ng/mL, $p > 0.05$). Gal3 positively correlated with fibrosis score (rho 0.3, $p = 0.01$), total bilirubin (rho 0.3, $p = 0.002$), ALT (rho 0.3, $p = 0.01$), AST (rho 0.3, $p = 0.005$), and APRI score (rho 0.3, $p = 0.009$), and negatively correlated with albumin (rho -0.3, $p = 0.01$). Out of the 10 cytokine proinflammatory panel, Gal3 was significantly correlated with IL-6 (rho 0.3, $p = 0.006$). DISCUSSION/SIGNIFICANCE: Gal3 is elevated in late BA and other CLD at time of transplant and correlated with degree of fibrosis, suggesting it may play a role in disease progression to cirrhosis. If targeted in the early disease stage, blocking Gal3 in pediatric cholestatic liver diseases may help delay the progression to cirrhosis and need for transplant.

433

Is miR let-7c protective against Acute Chest Syndrome in Sickle Cell Disease?

James Fan[1], Joanna Gemel[1], Theodore Lang[1], Eric Beyer[1] and MD, Gabrielle Lapping-Carr[1]
[1]University of Chicago

OBJECTIVES/GOALS: We have shown that small extracellular vesicles (exosomes) isolated from patients with a history of ACS disrupt the endothelium in vitro. Sequencing of miRNA contents of these vesicles suggested that miR let-7c was differentially expressed. The current study was designed to determine the relationship between miR let-7c levels and ACS. METHODS/STUDY POPULATION: We identified 16 subjects from the SCD Lungomics biobank at the University of Chicago Comer and La Rabida Childrens Hospitals who had samples obtained at baseline. Among them, 9 had a history of ACS (ACS(+)) and 7 did not (ACS(-)). For all subjects, we reviewed clinical data relevant to their SCD and laboratory data (including hemoglobin, absolute reticulocyte count, white blood cell count) obtained at the same time as the baseline samples. RNA was isolated from the plasma and miR let7c was quantified using quantitative RT-PCR. RESULTS/ANTICIPATED RESULTS: Subjects were similar clinically, except that those with a history of ACS were more likely to be on hydroxyurea (p<0.05) and to have obstructive sleep apnea (p<0.05). Hematologic laboratory values were similar irrespective of

ACS history. The mean miR let-7c level was 2-fold less in subjects with a history of ACS than in those who had never had ACS (p<0.05). A plasma miR let-7c level < 1 (normalized to the control subjects) had a positive predictive value of 0.78 for history of ACS and a sensitivity of 78% and specificity of 71%. Among subjects with a history of ACS, the let7c levels did not correlate with time since the last ACS event. However, among subjects who developed ACS following the baseline samples, higher miR let-7c levels correlated with increased length of time to next ACS event (R=0.8). DISCUSSION/ SIGNIFICANCE: Our results in a group of subjects with SCD show that plasma miR let-7c levels are decreased in subjects with a history of ACS. They suggest that miR let-7c may be protective against development of ACS and that measurement of its levels could be a useful biomarker to assess or predict risk for this complication of SCD.

434

In silico ADMET optimization and preliminary biologic activity of novel spermine oxidase inhibitors as neuroprotective agents

Amelia B Furbish[1], Pieter Burger[1], Yuri K Peterson[1] and Patrick M Woster[1]
[1]Department of Drug Discovery and Biomedical Sciences, Medical University of South Carolina

OBJECTIVES/GOALS: The goal of this project was to conduct a preliminary assessment of in vivo feasibility early on in the drug-discovery process in an effort to expedite the translation of novel drug scaffolds to potential clinical candidates. The data gathered in this study will be used to direct analog synthesis of our current lead compounds through rational drug design. METHODS/STUDY POPULATION: Based on virtual and physical high-throughput screening efforts and subsequent similarity searching, we identified a set of potent and selective spermine oxidase (SMOX) inhibitors adhering to a common structural scaffold. In order to address potential barriers to in vivo use, we then conducted a robust optimization analysis in an effort to identify analogs with improved drug-like characteristics. Docking simulations to predict binding were performed and visualized using molecular modeling software (MOE and PyMol). ADMET properties were calculated using a variety of software resources including SwissADME and CDD Vault. RESULTS/ANTICIPATED RESULTS: Through these optimization efforts, we were able to successfully identify analogs with improved drug-like characteristics, including increases in predicted CNS penetration, isosteric replacement of metabolically labile functional groups, increased lipophilicity, and elimination of structural attributes suggestive of off-target activity. Analogs were ranked according to predicted binding and properties of in vivo feasibility. Compounds achieving the highest scores were then selected as scaffolds to guide analog synthesis. DISCUSSION/SIGNIFICANCE: Despite evidence implicating induction of SMOX as a mechanism contributing to neuronal pathology, the lack of potent and selective inhibitors with profiles conducive for in vivo use has significantly impeded clinical investigation of this target. In this presentation, rational drug design focusing on translational optimization will be discussed.

435

Age-dependent Regulation of Follicle-Stimulating Hormone N-glycosylation in Female Gonadotrope Cells

Rosemary McDonald[1], James Eudy[2], Siddesh Southekal[2] and Babu Gadu[3] and T. Rajendra Kumar[1]
[1]University of Colorado Anschutz Medical Campus Department of Obstetrics and Gynecology, [2]University of Nebraska Medical Center DNA Sequencing Core and [3]University of Nebraska Bioinformatics and Systems Biology Core

OBJECTIVES/GOALS: Age-specific N-glycosylation occurs on follicle-stimulating hormone (FSH) in pituitaries of post-menopausal women and results in a higher ratio of fully glycosylated to hypoglycosylated FSH. Our goal is to identify in vivo the N-glycosylation pathway enzymes and the regulatory mechanisms in gonadotropes of young and old female mice. METHODS/STUDY POPULATION: Pituitaries were isolated from female mice (at 4m and 8m; n=5 per group) carrying an Fshb-Cre transgene on a Rosa mT/mG genetic background and the GFP-tagged gonadotropes were purified by FACS. RNA-Seq analysis, and subsequent qPCR assays were performed on GFP+ cells from pituitaries of female mice at 4m (reproductively young), 8m (reproductively mid age) and â‰¥ 12m (reproductively old) of age. To identify the role of progesterone signaling in age-dependent N-glycosylation in gonadotropes, a gonadotrope-specific knockout of Pgr was achieved. Gonadotropes from these mutant mice at 4-, 8-, and â‰¥12 months of age (n=5 per group) were isolated for qPCR analysis of N-glycosylation enzyme gene expression. Predicted progesterone receptor (PR) promoter binding sequences was performed using JASPAR. RESULTS/ANTICIPATED RESULTS: RNA-seq identified 28 differentially expressed N-glycosylation enzyme-encoding mRNAs in gonadotropes of female mice at 4- and 8-months. Three genes showed significant differences between ages (Man2a1, Man1c1, and B4galt5.), and further qPCR analyses revealed six out of eight genes analyzed showed age-dependent expression, including Man2a1, Man1c1, and B4galt5. The promoters of all N-glycosylation enzyme genes showed strong predicted binding sequences for PR. Further qPCR analysis showed age-and genotype-dependent differences in N-glycosylation enzyme expression in Pgr cKO females, with the most striking differences observed at 13 months, where B4galt5, Man1a2, Mgat5, and Man2a1 were downregulated in Pgr cKO gonadotropes compared to controls. DISCUSSION/SIGNIFICANCE: We identified changes in the N-glycosylation machinery in female mouse gonadotropes and confirmed the age- and Pr-dependent regulation of the corresponding mRNAs. Our results provide insights into the mechanisms at the level of the pituitary by which old age-specific FSH glycoform regulates osteoporosis and weight gain in post-menopausal women.