

using human stool inoculum, centrifuged, and filter sterilized. Intestinal epithelial cells (Caco-2, ATCC) were grown to confluence on 0.4 μ m polystyrene transwell inserts using a DMEM + 10% FBS medium and allowed to differentiate for 21-days. Highly differentiated monolayers were treated with a 1:4 dilution of fermenta with medium in triplicate. The cell experiment was conducted twice. Cell layer integrity was measured using transepithelial electrical resistance (TEER) 24- and 48-hours after treatment. RESULTS/ANTICIPATED RESULTS: Dietary intake data from the What We Eat in America database indicated that the top 3 fruit and vegetable exposures for infants with Mexican or Hispanic ethnicity were banana, apple, and carrot. Commercial baby food purees of these fruits and vegetables, in addition to baby foods with blueberry and spinach (Natural for Baby, Gerber Products Company) were acquired for digestion and fermentation experiments. Caco-2 cell experiments with these foods are ongoing. We expect Caco-2 monolayer incubated with fermenta from human milk and fruit or vegetables will have greater TEER values due to increased integrity of the cell layer as compared to those with breast milk alone. We also expect that exposure to fruit and vegetable fermenta will increase gene expression of tight junctions compared to exposure to media and human milk. DISCUSSION/SIGNIFICANCE OF IMPACT: Using an in vitro digestion and fermentation system coupled with cell culture studies, we are identifying cellular mechanisms that link individual fruits and vegetables to gut barrier function. This will support translational work focused on mitigating obesity development in vulnerable populations.

470

Senolytic therapies as treatments for posttraumatic epilepsy*

Max Stevenson¹ and Mark Burns²

¹Georgetown-Howard Universities Center for Clinical and Translational Science and ²Georgetown University Patrick Forcelli, Georgetown University

OBJECTIVES/GOALS: Increased numbers of senescent cells have been detected in both traumatic brain injury and epilepsy, suggesting them as targets for therapeutic intervention for treating posttraumatic epilepsy (PTE) and underscoring the need for innovative methods to identify and target senescent cells as a means of alleviating pathology. METHODS/STUDY POPULATION: C57BL/6 mice will receive a single controlled cortical impact (CCI) before having their brains removed at 1 week, 2 weeks, 4 weeks, 1 month, 2 months, and 4 months post injury (n = 5 per time point). Brain sections will then be co-labelled for glial and senescent markers to observe which cells begin to express senescent markers at various time points. We will also perform single-cell RNA sequencing to observe genetic changes associated with both TBI and epileptogenesis. Mice will also be treated with navitoclax, a BCL2 inhibitor being investigated as a senolytic agent, to determine if treatment results in decreased senescence and epileptogenesis, as well as improved behavioral outcomes. RESULTS/ANTICIPATED RESULTS: Preliminary data revealed that senescent microglia begin to arise in the mouse hippocampus as early as 1 week post injury and continue to increase in concentration over the course of the following month, with up to 25% of microglia expressing p16, a known marker of senescence. We anticipate that further staining will reveal senescent astrocytes and neurons in a similar time-dependent manner. Further, we hypothesize that the single-cell sequencing of microglia from injured mice will reveal alterations to the expression of genes associated with neuronal

excitability, inflammation, and/or synaptic modeling, features known to be associated with epilepsy. Finally, we anticipate treatment with navitoclax will alleviate the senescent phenotype, resulting in decreased epileptogenesis and improved behavioral outcomes. DISCUSSION/SIGNIFICANCE OF IMPACT: Considering the lack of any studies examining senescent cell prevalence in PTE, these data will be the first to identify these cells as etiological factors in PTE onset, as well as druggable targets for improving pathological outcomes in PTE patients.

471

Defining proteomic and cellular elements of the pancreatic ductal adenocarcinoma (PDAC) tumor microenvironment with mass spectrometry imaging

Caroline Kittrell¹, Blake Sells², Lyndsay Young¹, Peggi Angel² and Richard Drake¹

¹Medical University of South Carolina and ²Washington University School of Medicine in St. Louis,

OBJECTIVES/GOALS: Currently, a lack of screening markers and targeted therapies prevent clinicians from successfully treating PDAC. Precision medicine may allow oncologists to better combat this disease. To personalize care, knowledge of tumor protein post-translational modifications, extracellular matrix makeup, and infiltrating immune cells is imperative. METHODS/STUDY POPULATION: Matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) was employed to characterize the N glycosylation state, the ECM composition, and immune cell populations present within 10 formalin fixed paraffin embedded PDAC patient samples. Molecular dry spray of PNGase F and Collagenase III followed by enzymatic digestion allowed for the release of N glycans and ECM peptides from the tissue. Multiplex immunohistochemistry with photocleavable, mass-tagged probes was also performed on each tissue. This analysis produced a spatial map of N glycans, ECM peptides and immune cells with their distribution and abundance color-coded as a heat map of each tissue. RESULTS/ANTICIPATED RESULTS: This analysis produced a unique N-glycan signature associated with specific tumor regions (necrosis, invasive margin, etc.) and immune cell clusters. Additionally, immune cells within the PDAC tumor microenvironment were found to be organized into immature tertiary lymphoid structures composed primarily of CD20+ B cells. Finally, a distinct distribution of ECM peptides within and surrounding tumor tissue was visualized, and putative identifications have been assigned to these stromal elements. DISCUSSION/SIGNIFICANCE OF IMPACT: In the future, insights from this hypothesis-generating study may be leveraged to identify diagnostic and prognostic biomarkers for PDAC to improve early diagnosis and treatment response rates. The N glycan signature, ECM composition, and immune activation state in liquid biopsies including serum and PBMCs will be compared to data from this study.

472

Deciphering the role of Dnmbp in kidney development: Implications for CAKUT

Brandy Walker, Vanja Krneta-Stankic^{2,3} and Rachel K. Miller^{1,2,4}

¹Epigenetics, MD Anderson Cancer Center UTHealth Graduate School of Biomedical Center UTHealth Graduate School of Biomedical Sciences Program in Genetics and Epigenetics, MD Anderson Cancer Center UTHealth Graduate School of Biomedical; ²Sciences, TX; ³2 Department of Pediatrics, Pediatric Research