using human stool inoculum, centrifuged, and filter sterilized. Intestinal epithelial cells (Caco-2, ATCC) were grown to confluence on 0.4 μm 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.

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Senolytic therapies as treatments for posttraumatic epilepsy*

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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.

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

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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.

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

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https://doi.org/10.1017/cts.2024.1065 Published online by Cambridge University Press

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OBJECTIVES/GOALS: This research aims to identify genetic alterations influencing congenital anomalies of the kidney and urinary tract (CAKUT) and bridge a fundamental gap in understanding the cellular mechanisms underlying kidney development, with the long-term goal of enhancing treatments for congenital renal anomalies. METHODS/STUDY POPULATION: We will use a loss-of-function approach in combination with immunofluorescent microscopy techniques to determine the influence of Dnmbp perturbation on Daam1 localization, actin assembly, and junctional turnover. Additionally, to establish a foundation for delineating the molecular mechanism of DNMBP during kidney development, we will utilize clinical whole exome sequencing data to identify human DNMBP mutations associated with urogenital anomalies. Furthermore, we will determine whether human DNMBP mutations linked to CAKUT lead to disruptions in nephron development through loss-of-function rescue experiments in *Xenopus*. RESULTS/ANTICIPATED RESULTS: Here, we evaluate the dynamics of Dnmbp-mediated transport of Daam1 within the developing kidney and show preliminary data suggesting that Dnmbp and Daam1 directly interact to promote adhesive contact formation between nephron progenitor cells. Furthermore, we propose a model in which Dnmbp functions as a critical regulator of epithelial tissue morphogenesis and provides a functional link between the dynamic processes of actin cytoskeleton regulation, intracellular adhesion, and vesicular transport. Future studies will determine whether Dnmbp interaction with Daam1 facilitates junctional actin assembly by directing Daam1 to cell–cell contact sites via Dnmbp-associated vesicle targeting, enhancing our understanding of the cellular mechanisms influencing tubule morphogenesis. DISCUSSION/SIGNIFICANCE OF IMPACT: This research will establish a previously unknown role for DNMBP in kidney development and provide a comprehensive understanding of the impacts of simultaneously regulating vesicular transport and actin dynamics in nephrogenesis.

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Repositioning monensin: Enhancing anti-cancer activity and immune modulation in breast cancer cells

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OBJECTIVES/GOALS: Monensin is FDA approved for use in veterinary medicine. Recent studies pointed to its potent anticancer activity. Since de novo drug discovery process typically takes 10 to 15 years and requires an investment of approximately $1.3 to $3 billion, drug repositioning can bypass several steps in this process and increase the potential for success. METHODS/STUDY POPULATION: Cell viability assays were conducted on human MDA-MB-231, MDA-MB-468, and MCF10A breast cancer cell lines and mouse EO771 and 4T1 breast cancer cell lines. MDA-MB-231 cell line was used in all the studies unless specified otherwise. Time course levels of Bcl-2, Bak, p62, and LC3II were assessed via Western blotting with GAPDH as a loading control. Proteomics analysis was conducted by the IDEA National Resource for Quantitative Proteomics. Time course levels of major histocompatibility complex (MHC) I and II and calreticulin were evaluated using flow cytometry. At least three biological replicates have been conducted for each experiment. RESULTS/ANTICIPATED RESULTS: Monensin and several of its novel analogs were potent toward human and mouse breast cancer cell lines. Furthermore, they induced apoptotic cell death as evidenced by Annexin V/PI assay, downregulation of Bcl-2, and upregulation of Bak in MDA-MB-231 cells. Proteomics analysis revealed that several molecular pathways related to MHC class I and II antigen presentation were significantly altered following treatment with these compounds. Additionally, monensin and its analogs significantly increased the expression of MHC class I and II. Our studies also showed that monensin and its analogs increase the surface calreticulin levels. Treatment of MDA-MB-231 cells with these compounds also resulted in an increase in p62 and LC3II expression, suggesting a disruption of the autophagic process. DISCUSSION/SIGNIFICANCE OF IMPACT: These results suggest that monensin and its analogs not only exhibit anti-breast cancer cell activity but also modulate immune-related pathways. By disrupting autophagy and enhancing calreticulin levels, these compounds may potentiate antitumor immune responses, providing a promising avenue for drug repositioning in cancer therapy.

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Impact of secretome derived from stool samples of patients with multiple system atrophy in alpha-synuclein oligomerization

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OBJECTIVES/GOALS: This study investigates the contribution of the stool secretome (the soluble factors secreted by microbes into extracellular space) to in vitro α-synuclein (αSyn) oligomerization using stool cultures from patients with multiple system atrophy (MSA), a rare neurodegenerative disease hallmarked by pathologic αSyn aggregates. METHODS/STUDY POPULATION: Stool samples from MSA patients (n = 20), household controls (n = 20), and healthy controls (n = 20) will be cultured using an adapted dilution-to-extinction approach. The goal is to reduce microbial complexity progressively to produce random secretome combinations that may affect αSyn oligomerization differentially. The original inoculant and dilutions will be cultured anaerobically to collect conditioned media (CM) enriched with microbial secretomes. CM will be used to expose a fluorescence resonance energy transfer (FRET) biosensor assay and a Gaussia luciferase protein complementation assay – both modified to quantify αSyn-αSyn interaction indicating oligomerization. Any CM-altering αSyn oligomerization will undergo multiomic characterization to identify potential causative agent(s). RESULTS/ANTICIPATED RESULTS: Specific microbe-produced molecules from the literature are anticipated to modulate αSyn oligomerization, identified by targeted, reductionist studies that selected and tested separately single microbial factors on

https://doi.org/10.1017/cts.2024.1065 Published online by Cambridge University Press