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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.

474

### 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.

475

### 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  $\alpha$ -synuclein ( $\alpha$ Syn) oligomerization using stool cultures from patients with multiple system atrophy (MSA), a rare neurodegenerative disease hallmarked by pathologic  $\alpha$ Syn 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  $\alpha$ Syn 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  $\alpha$ Syn- $\alpha$ Syn interaction indicating oligomerization. Any CM-altering  $\alpha$ Syn 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  $\alpha$ Syn oligomerization, identified by targeted, reductionist studies that selected and tested separately single microbial factors on

$\alpha$ Syn aggregation in vitro and in vivo. From these studies, lipopolysaccharide and bacterial amyloid protein are expected to increase  $\alpha$ Syn oligomerization, while short-chain fatty acids, such as butyrate, are expected to interfere with and decrease oligomerization. As a complementary systemic approach, this study's agnostic methods involving MSA stool culture combined with the proposed dilution-to-extinction method are expected to identify additional MSA stool secretome components modulating  $\alpha$ Syn oligomerization that might otherwise be missed in earlier reductionist approaches. DISCUSSION/SIGNIFICANCE OF IMPACT: Completion of this reverse-translational work will aid in discovering MSA stool secretome components modulating  $\alpha$ Syn oligomerization. Identification of specific factors contributing to pathologic  $\alpha$ Syn behavior might set the stage for patient screenings for identified stool markers and could lead to microbiome-based interventions for MSA.

477

### **The effect of short- and long-term diets on mechanisms of healthy brain aging: A protocol**

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OBJECTIVES/GOALS: The second highest fear of the aging population is cognitive decline. Diet is associated with brain aging; therefore, the objective is to determine the effects of a Western diet (WD) on cognitive decline and the efficacy of a Mediterranean diet (MeDi) fecal microbiota transplant (FMT) in WD-induced cognitive deficit progression in aged rats. METHODS/STUDY POPULATION: For Study 1, 12-month-old Fischer344 rats (NIA Aging Colony) will be randomly assigned to a WD, MeDi, or control (positive control) for 6 or 12 months. Microbiota composition, blood pressure, and body composition (DXA Scan) will be longitudinally assessed. Groups will undergo a battery of neurobehavioral assessments to measure cognitive performance. At the end of the study, mitochondria bioenergetic assays in isolated cerebral microvessels will be used to determine changes in cerebrovascular function. For Study 2, 18-month-old Fischer344 rats (NIA Aging Colony) will be randomly assigned to a WD, MeDi, or control for 6 months. At month 4, the WD+ MeDi-FMT group will receive once weekly MeDi-FMT for two months. Assessments will be performed as described in Study 1. RESULTS/ANTICIPATED RESULTS: It is anticipated that the WD-related gut dysbiosis will increase blood pressure, fat-free mass, neurovascular dysfunction, and induce cognitive impairment relative to a MeDi. When using a MeDi-FMT as an intervention, it is anticipated that there will be measurable improvements in cognitive function relative to a WD through the regulation of gut dysbiosis, blood pressure, fat-free mass, and neurovascular dysfunction. DISCUSSION/SIGNIFICANCE OF IMPACT: These results are expected to have an important positive impact because they will provide insights into the WD-induced gut dysbiosis-associated cognitive impairments, and evaluate the roles and mechanisms of MeDi-FMT in the therapeutic intervention of aged rats.

478

### **Bacterial dysbiosis and its association with pancreatic cancer progression and poor survival**

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OBJECTIVES/GOALS: Bacterial dysbiosis has emerged as an accomplice in the progression of many cancers. The pancreas microbiome changes in pancreatic cancer patients. The mechanisms via which components of the microbiome regulate tumor growth is unclear. We seek to determine if bacterial dysbiosis influences cancer cell behavior thereby promoting tumor progression. METHODS/STUDY POPULATION: We performed immunohistochemistry for lipopolysaccharide and observed bacteria preferentially located in close proximity to cancer cells. We utilized an in vitro cell culture system and in vivo mouse models, in the presence and absence of gut bacteria, to assess the effect of bacteria and bacterial metabolites on pro-tumorigenic signaling and transcriptional changes in the cancer cell. We analyzed cancer cells and epithelial cells using RNA sequencing, flow cytometry, and enzyme-linked immunosorbent assay. We also used targeted metabolomics to identify bacterial and cancer cell produced metabolites. RESULTS/ANTICIPATED RESULTS: We found microbial dysbiosis can induce proliferation, an inflammatory response and an increase in tryptophan metabolism via the kynurenine pathway in the pancreatic cancer cell. Along with upregulated expression of IDO1 in vivo, we observe an increase in nicotinic adenine mononucleotide. Also, we observe an increase in nicotinic acid in vitro and nicotinic adenine dinucleotide within the cancer cell compartment in the presence of bacteria and bacteria conditioned media. Due to the critical role in many vital pathways of cell survival, NAD<sup>+</sup> production is thought to play a significant role in cancer progression. Nicotinic acid can stimulate NAD production to protect cells from cell death. DISCUSSION/SIGNIFICANCE OF IMPACT: Pancreatic cancer is associated with a distinct tumor microbiome and ablation slows disease progression. Our data delineate mechanisms via which microbes modulate the pancreatic cancer cell and provide insight into therapeutic strategies for gut microbial modulation in treating pancreatic cancer.

479

### **Effects of extracellular matrix on pacemaking cardiomyocyte function**

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OBJECTIVES/GOALS: The extracellular matrix (ECM) of the sinoatrial node (SAN) is critical for maintaining automaticity in hiPSC-derived pacemaking cardiomyocytes (PCMs) under cyclic strain.