αSyn aggregation in vitro and in vivo. From these studies, lipopolysaccharide and bacterial amyloid protein are expected to increase αSyn 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 αSyn 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 αSyn oligomerization. Identification of specific factors contributing to pathologic αSyn behavior might set the stage for patient screenings for identified stool markers and could lead to microbiome-based interventions for MSA.

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

Rebecca Solch-Ottaiano, Elizabeth B. Engler-Chiurazzi, Gregory Bix, M Demetrius and Maraganore
Tulane University School of Medicine

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.

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

Holly Attebury, Ann Arbor, Dominik Awad, Katelyn Donahue, Ahmed Elhossiny, Tim Frankel, Tom Schmidt, Costas Lyssiotis, Marina Pasca di Magliano and Donnele Daley
University of Michigan

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+ 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.

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Effects of extracellular matrix on pacemaking cardiomyocyte function

Brian Howard, Regan Smithers, Kaitlin Van Brusselen, Hillary K.J. Kao and Deborah K. Lieu
University of California, Davis Department of Internal Medicine, Division of Cardiovascular Medicine and Institute for Regenerative Cures

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.

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