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Systemic Administration of miR-451 Improves Autophagy Response in an Accelerated Mouse Model of Diabetic Kidney Disease*

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OBJECTIVES/GOALS: Diabetic Kidney Disease (DKD) is a common diabetes complication, often linked to end-stage renal disease in the United States (US). While autophagy and miRNAs are pivotal, miR-451's specific role remains understudied. Our study explores its renoprotective effects in an accelerated DKD mouse model. METHODS/STUDY POPULATION: We assessed the effect of miR-451 mimic treatment on Diabetic Kidney Disease (DKD) in BTBR ob/ob mice, known for their rapid DKD-like renal lesions. Mice were divided into four groups: WT (wild-type), BTBR ob/ob, WT+miR-451 (wild-type with miR-451 mimic), and BTBR ob/ob +miR-451 (BTBR ob/ob with miR-451 mimic). MiR-451 mimics were administered at 2mg/kg body weight once weekly for three consecutive weeks. We collected spot urine and monitored blood glucose levels at each time point. After the treatment period, mice were euthanized for kidney and blood samples. Western blot analysis assessed autophagy-related protein markers. Statistical analysis included Student's t-test and ANOVA ($p<0.05$). RESULTS/ANTICIPATED RESULTS: The study assessed the impact of miR-451 mimic treatment in BTBR ob/ob mice. Albumin:creatinine ratio increased fourfold ($p=0.01$) in BTBR ob/ob mice at 5 weeks. MiR-451 mimic treatment had no impact on body weight. Blood glucose levels were notably higher in both treated and untreated BTBR ob/ob mice at 12 (425±33.1 mg/dL; $p=0.04$) and 13 weeks (383±25.3 mg/dL; $p=0.007$). However, a significant drop occurred from week 13 (554.7±10.8 mg/dL) to week 14 (289±13.3 mg/dL; $p=0.0002$) in BTBR ob/ob miR-451 treated mice. Western blot analysis in whole kidney homogenates showed a 91% reduction ($p=0.02$) in YWHAZ, a predicted miR-451 target, in treated BTBR ob/ob mice and a 95% reduction ($p=0.01$) in WT mice. Furthermore, miR-451 mimic treatment led to a 68% increase ($p=0.01$) in ATG101 and a 44% increase in Beclin-1 in BTBR ob/ob mice. DISCUSSION/SIGNIFICANCE: The study uncovers miR-451-based interventions as a promising avenue to counter Diabetic Kidney Disease by modulating autophagy, potentially introducing novel therapies for at-risk individuals. However, practical DKD treatments will require further research and rigorous clinical validation to harness the full potential of these insights.

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Unraveling the Immunological Basis of Lobular Involution in Breast Cancer Development

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OBJECTIVES/GOALS: Reveal common immune mechanisms in dysregulated age-related lobular involution (ARLI) and post-partum lobular involution (PPLI) to understand their link to increased breast cancer risk, challenging the traditional view of their distinctiveness. Ultimately, to improve breast cancer risk assessment and personalized prevention METHODS/STUDY POPULATION: The Mayo Clinic Benign Breast Disease (BBD) cohort comprises of ~20,000 women with benign biopsies, including ~1000 women with sequential benign biopsies. Lobular involution (LI) status was assessed by selecting perimenopausal women, ages 45-55, with sequential biopsies, comparing acini number and lobule size between initial and subsequent biopsies. NanoString IO360/ BC360 RNA profiling identified differentially expressed genes associated with dysregulated LI. Using multiplex immunofluorescence (mIF), I'll analyze and spatially map immune biomarkers related to dysregulated ARLI and PPLI in BBD tissue from perimenopausal women who did or did not go on to develop breast cancer, assessing the commonality of ARLI and PPLI markers and exploring their potential as risk biomarkers for breast cancer. RESULTS/ANTICIPATED RESULTS: Preliminary findings link patients who display dysregulated ARLI with an increased breast cancer risk and identify vital PPLI biomarkers in perimenopausal women. I expect the biopsies of women who developed post-menopausal breast cancer (PMBC) and post-partum breast cancer (PPBC) to exhibit elevated levels of dysregulated ARLI immune biomarkers and PPLI biomarkers. Spatially mapping these markers promises to provide a more comprehensive understanding of their interactions, potentially revealing common immunological pathways. These findings could transform our current paradigm of ARLI and PPLI as distinct processes and demonstrate their interconnection in shaping breast cancer risk. DISCUSSION/SIGNIFICANCE: PMBC and PPBC dominate majority of breast cancer cases. Both involve activation of the understudied process of lobular involution, which has been shown to have pro-tumorigenic traits. Elucidating these mechanisms will aid more efficient risk stratification and personalized prevention to reduce incidence and mortality of breast cancer.

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Source Localization of Burst Suppression in Post-Cardiac Arrest Patients

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OBJECTIVES/GOALS: Burst suppression is a neurophysiological marker associated with severe hypoxic-ischemic injury following cardiac arrest. The goal of this study is to identify the anatomical regions of the brain associated with burst suppression post-cardiac arrest. METHODS/STUDY POPULATION: 86 comatose patients post-cardiac arrest admitted to the neurological-ICU from Massachusetts General Hospital and Brigham and Women's Hospital were included in this study. EEG data after return of spontaneous circulation were preprocessed and artifact was rejected. Burst segments were extracted for source localization analysis from epochs with burst suppression. Four bursts for each patients were manually selected. The source of the bursts were obtained using the Champagne algorithm and mapped on the Desikan-Killiany atlas. The source for each burst was defined as any region of interest (ROI) with power > = 75th percentile relative to all ROIs. The power of the bursts at each source was correlated with the burden of brain injury measured using apparent diffusion coefficient (ADC) per ROI. RESULTS/ANTICIPATED RESULTS: 48 (56%) patients had burst

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suppression. 5 (10.4%) of patients with burst suppression were independent at the time of hospital discharge. Preliminary analyses was performed on 6 patients (24 bursts in total). ROI's determined to be sources in a majority of the burst (>=13) were bilateral superior frontal, rostral middle frontal, parstriangularis precentral, superior parietal, inferior parietal, right post central, superior temporal, lateral occipital, and left middle temporal ROI. A lower mean ADC intensity was associated with a higher EEG power in the bilateral superior frontal ($r = -0.80$, $p < 0.0001$; $r = -0.677$, $p < 0.001$, respectively), left superior parietal ($r = -0.53$, $p = 0.009$), left middle temporal ($r = -0.43$, $p = 0.042$) ROI. DISCUSSION/SIGNIFICANCE: The source of bursts in patients post-cardiac arrest experiencing burst suppression is not well defined. This study will improve our understanding of how burst suppression is a measure of cortical injury, how it may relate to the burden of injury found on ADC imaging, and patient outcomes.

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Investigation of a translational astrocyte-targeted AAV-mediated gene addition therapy in two models of Vanishing White Matter disease

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OBJECTIVES/GOALS: Vanishing White Matter Disease (VWM), is a childhood neurodegenerative leukodystrophy that presents with motor deficits, neurologic decline, and seizures leading to death.There are no treatments. Herein we investigate adeno-associated virus serotype 9 (AAV9) gene addition therapy for VWM. METHODS/STUDY POPULATION: To serve as a baseline for disease correction, we characterized the severe VWM Eif2b5^{I98M} murine model with clinically relevant readouts including motor function, gait mapping and myelin loss through magnetic resonance imaging (MRI). Molecular characterization through the identification of biomarkers was also investigated. To provide targeted disease correction, we designed four gene replacement constructs to drive the rapeutic EIF2B5 expression in astrocytes—a critical cell type for VWM pathology. We are currently evaluating our AAV vectors in two murine VWM models, Eif2b5^{R191H} and Eif2b5^{I98M}, and are monitoring disease progression using traditional and clinically relevant readouts. RESULTS/ANTICIPATED RESULTS: The I98M mice display significant mobility loss, ataxic gait, and demyelination. Molecular characterization also indicates that the integrated stress response is significantly dysregulated, supporting the classic VWM phenotype. Our previous biodistribution study confirmed our ability to efficiently target astrocytes using varying iterations—including one novel—of the glial fibrillary acidic protein (GFAP) promoter. Our data suggests that targeting astrocytes with gene addition delays disease onset, partially rescues motor function, and attenuates myelin loss. Survival of the AAV9-gfaABC(1)D-EIF2B5 treated I98M mice is also significantly increased ($p<0.0001$), currently with a 2-fold extension in life expectancy. DISCUSSION/SIGNIFICANCE: Overall, we anticipate emergence of a lead astrocyte-targeted gene therapy candidate in which the data will be strengthened through the evaluation of clinically relevant measures in two murine models of disease, allowing fortimely translation to the clinic.

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Detecting Hypofibrinolysis in Clinical Coagulation Testing

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OBJECTIVES/GOALS: The goal of this study is twofold: To develop a method for an ex vivo hypofibrinolytic control and second to analyze patterns in standard and recently developed clinical coagulation assays for the detection of hypofibrinolytic states. METHODS/STUDY POPULATION: We analyzed blood samples from healthy patients first under normal conditions and then laced with human recombinant PAI-1 under three different concentrations. We then analyzed both samples using standard clinical assays (PT, aPTT, D-dimer, Fibrinogen), thromboelastography point-of-care tests (Hemosoncs- Quantra system), and with research assays of clot size and aggregation. Our previous research of diagnostic errors showed the patient group with the highest overall risk of these non-identifiable thrombotic complications was post-menopausal women with chronic diseases. We therefore focused our patient population to healthy post-menopausal women who were not using hormone replacement therapy. RESULTS/ANTICIPATED RESULTS: Research assays showed PAI-1 significantly increased clot size and aggregation. Preliminary results of clinical assays showed no detectable difference in hypofibrinolytic samples at any concentration. We anticipate ongoing testing will show similar results. Results on Quantra tests showed much larger differences between control and hypofibrinolysis samples, and we anticipate ongoing testing will achieving statistical significance. It is still unknown whether the mean value for hypofibrinolysis samples on the Quantra Clot Stability assay will be outside of the "normal" reference range. We theorize that this may be due to hypofibrinolytic changes in the overall structure and core density of the clots. DISCUSSION/SIGNIFICANCE: Cellular stress stimulates a concomitant activation of inflammation and coagulation, including decreased fibrinolysis. Unfortunately, current clinical assays do not assess clot breakdown. This connection would account for the increased rate of thrombosis in patients with chronic inflammation without detectable results on clinical tests.

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Epithelial hypoxia maintains colonization resistance against Candida albicans

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