signaling downstream of Smad 2/3. Additionally we anticipate downregulation of osterix and osteocalcin, two essential genes for osteoblast differentiation and activity. We anticipate that hyperglycemia will potentiate the negative effects of myostatin on osteoblastogenesis. DISCUSSION/SIGNIFICANCE OF IMPACT: We have demonstrated that myostatin can directly act on osteoblastic cells. As myostatin is a negative regulator or bone mass, its direct effects on bone cells can be detrimental to the bone health of patients with elevated myostatin levels and/or activity. There is evidence suggesting that myostatin is elevated in Type 1 diabetes, and its effects might be potentiated in a hyperglycemic environments. Future experiments will be evaluating the role of myostatin on a diabetic animal model and in humans. Our experiments provide an additional mechanism by which muscle-bone interactions could be contributing to the development of diabetic bone disease.

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Tissue Engineered Nigrostriatal Pathway as a Test-Bed for Evaluating Axonal Pathophysiology in Parkinson's disease

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OBJECTIVES/SPECIFIC AIMS: Selective loss of long-projecting neural circuitry is a common feature of many neurodegenerative diseases, such as the vulnerable nigrostriatal pathway in Parkinson's disease (PD). Current in vitro approaches for studying disease development generally do not mimic complex anatomical features of the afflicted substrates such as long axonal pathways between stereotypical neural populations. Such exquisite features are not only crucial for neural systems function but may also contribute to the preferential vulnerability and pathophysiological progression of these structures in neurodegenerative disease. We have previously developed micro-tissue engineered neural networks to recapitulate the anatomy of long-projecting cortical axonal tracts encased in a tubular hydrogel.1 Recently, we have extended this work to include the first tissue-engineered nigrostriatal pathway that was anatomically-inspired to replicate the structure and function of the native pathway.2 Notably, this tissue-engineered brain pathway possesses three-dimensional (3D) structure, multicellular composition, and architecture of long axonal tracts between distinct neuronal populations. Therefore, in the current study we apply this system as a biofidelic test-bed for evaluating axonal pathway development, maturation, and pathophysiology. METHODS/STUDY POPULATION: Dopaminergic neurons from the ventral mesencephalon and medium spiny neurons (MSNs) from the striatum were separately isolated from rat embryos. Tissue-engineered nigrostriatal pathways were formed by initially seeding dopaminergic neuron aggregates at one end of hollow hydrogel micro-columns with a central extracellular matrix, collectively spanning up to several centimeters in length. Several days later, tissue-engineered MSN aggregate was seeded on the other end and was allowed to integrate. Immunocytochemistry (ICC) and confocal microscopy were used to assess health, cytoarchitecture, synaptic integration, and mitochondrial dynamics with stains that label cell nuclei (Hoechst) and mitochondria (MitoTracker Red) and antibodies that recognize axons (anti-β-tubulinIII), neurons/dendrites (anti-MAP2), dopaminergic neurons/axons (anti-tyrosine hydroxylase; TH), and MSNs (anti-DARPP-32). RESULTS/ANTICIPATED RESULTS: Seeding tubular micro-columns with dopaminergic neuronal aggregates resulted in unidirectional axonal extension, ultimately spanning >5mm by 14 days in vitro. For constructs also seeded with Tissue-engineered, ICC confirmed the presence of the appropriate neuronal sub-types in the two aggregate populations, specifically TH+ dopaminergic neurons and DARPP-32+ MSNs. Moreover, confocal microscopy revealed extensive axonal-dendritic integration and synapse formation involving the dopaminergic axons and MSN somata/dendrites. Collectively, these constructs mimicked the general cytoarchitecture of the in vivo nigrostriatal pathway: a discrete population of dopaminergic neurons with long-projecting 3D axonal tracts that were synaptically integrated with a population of MSNs. Mitochondria structure along axonal tracts was also observed using MitoTracker staining, revealing dynamic intra-axonal mitochondrial motility in this system. Ongoing studies are evaluating real-time mitochondrial dynamics and axonal physiology in this tissue-engineered nigrostriatal pathway in vitro, under both baseline conditions as well as following the addition of exogenous α-Synuclein fibrils to model synucleinopathy in PD. DISCUSSION/SIGNIFICANCE OF IMPACT: This tissue-engineered nigrostriatal pathway provides an anatomically-inspired platform with neuronal-axonal architecture that structurally and functionally emulates the nigrostriatal pathway in vivo. We are applying this paradigm as a powerful in vitro test-bed for understanding mitochondrial activity and inter-axonal energetics pathways under homeostatic as well as PD pathological conditions. Successful demonstration will serve as proof-of-concept that this technique can be used to study mitochondria pathology in personalized constructs built using cells derived from PD patients in order to evaluate pharmacological therapies targeted at improving mitochondrial resiliency and fitness so as to delay and/or prevent dopaminergic axonal/neuronal degeneration in tailored to specific PD patients.

3174

Tumor suppressors p53 and ARF control oncogenic potential of triple-negative breast cancer cells by regulating RNA editing enzyme ADAR1

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OBJECTIVES/SPECIFIC AIMS: Triple-negative breast cancer (TNBC) accounts for one-fifth of the breast cancer patient population. The heterogeneous nature of TNBC and lack of options for targeted therapy make its treatment a constant challenge. The co-deficiency of tumor suppressors p53 and ARF is a significant genetic signature enriched in TNBC, but it is not yet clear how TNBC is regulated by this genetic alteration. METHODS/STUDY POPULATION: To answer this question, we established p53/ARF-defective murine embryonic fibroblast (MEF) to study the molecular and phenotypic consequences in vitro. Moreover, transgenic mice were generated to investigate the effect of p53/ARF deficiency on mammary tumor development in vivo. RESULTS/ANTICIPATED RESULTS: Increased transformation capability was observed in p53/ARF-defective cells, and formation of aggressive mammary tumors was also seen in p53-/-ARF-/- mice. RNA-editing enzyme ADAR1 was identified as a potential mediator for the elevated oncogenic potential. Interestingly, we found that the overexpression of ADAR1 is also prevalent in human TNBC cell lines and patient specimen.

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Using short hairpin RNA (shRNA) to reduce ADAR1 expression abrogated the oncogenic potential of human TNBC cell lines, while non-TNBC cells are less susceptible. Different levels of RNA editing of known ADAR1 targets were detected in shRNA-treated human TNBC cell lines, suggesting that ADAR1-mediated RNA editing contributes to TNBC pathogenesis. DISCUSSION/SIGNIFICANCE OF IMPACT: These results indicate critical roles played by the tumor suppressors p53 and ARF in the pathogenesis of TNBC, partially through affecting ADAR1-mediated RNA editing. Further understanding of this pathway could shed light on potential vulnerabilities of TNBC and inform the development of personalized therapies based on patients' genetic signiatures.

3213

Unraveling the role of Phospholamban (PLN) in humans via the characterization of Induced Pluripotent Stem Cell (iPSC) Cardiomyocytes (CM) derived from carriers of a lethal PLN mutation

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OBJECTIVES/SPECIFIC AIMS: To study the biology of Phosholamban (PLN) in a human relevant model. METHODS/STUDY POPULATION: State of the art stem-cell technologies using iPSC-CMs derived from carriers of a lethal PLN mutation. RESULTS/ANTICIPATED RESULTS: Our preliminary data demonstrate that this particular PLN mutation (L39) results in reduced expression and mis-localization of PLN as well as increased incidence of early after depolarization in isolated iPSC-CMs. DISCUSSION/SIGNIFICANCE OF IMPACT: Phospholamban (PLN) is a critical regulator of Ca++ homeostasis yet many uncertainties still remain regarding its role in humans. Our study will provide unique insights into the pathophysiology of this protein in HF.

3241

Using infant exertion to tailor treadmill intervention

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OBJECTIVES/SPECIFIC AIMS: This research examined 3 aims to address the need to understand and quantify exertion in infants. Aim 1: Develop a schema to identify and code exertional behaviors in infants during treadmill stepping. Aim 2: Establish feasibility for the schema's use with clinical populations. Aim 3: Pilot the schema in a study designed to induce infant exertion. METHODS/STUDY POPULATION: Aims 1 and 2 were achieved using existing treadmill stepping data. The data used in Aim 1 included eight typically-developing infants (age 7-10 months) who were able to sit independently, but not walk. The data used in Aim 2 came from two separate data sets from infants who took more than 10 steps in a 30-second trial: Data set A included six typically-developing infants (age 2-5 months) who were unable to sit independently (developmentally comparable to atypical populations who might receive treadmill interventions). Data set B included six infants with Spina Bifida (age 3-10 months). Aim 3 was addressed with a prospective study using an exertion model. Pre-walking, typically developing infants (age 8-10 months) underwent five total stepping trials. Trial 1 determined the infant's individualized maximum stepping speed; trials 2-5 were each 60 seconds and alternated between a baseline stepping speed of.20 m/s and the infant's maximum stepping speed determined in trial 1. All video data were coded for step type, step frequency, and exertional behavior. RESULTS/ANTICIPATED RESULTS: Aim 1: Two behaviors were identified and determined to capture infant exertion: foot dragging and leg crossing. Aim 2: The feasibility of capturing exertion with these two behaviors was established for young infants and infants with neuromotor delays, with exertional behaviors increasing with stepping exposure ($p < 0.05$). Aim 3: Total exertion (foot dragging + leg crossing) was higher in the maximum speed trials compared to baseline trials ($p = 0.005$). DISCUSSION/SIGNIFICANCE OF IMPACT: Exertion in infants can be quantified. The exertion schema developed with this study will support the development of dosing guidelines for infant treadmill intervention. The next step in this line of research is to examine the correlation between infant exertion and heart rate, in effort to move from behaviorally-informed protocols to more precise, individualized protocols based on the physiological response of the infant.

Biomedical Informatics/Health Informatics

3354

Biomedical Informatics/Health InformaticsA Preliminary Study of Glaucoma: The Intersection of Genetics and Survey Data from the Health and Retirement Study

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OBJECTIVES/SPECIFIC AIMS: Glaucoma is a leading cause of irreversible blindness worldwide; in the United States alone, over 2.7 million individuals are affected. Various risk factors for glaucoma are known and include age, race/ethnicity, genetics, and ocular measures. Despite numerous studies, molecular and environmental factors that contribute to glaucoma remain elusive. Our objective was to conduct a genome-wide association for glaucoma among black and white HRS respondents, and to determine the feasibility for future analyses examining shared genetic markers between glaucoma and other comorbidities, behaviors, and environmental risk factors. METHODS/STUDY POPULATION: The University of Michigan Health and Retirement Study (HRS) is a longitudinal survey of a representative sample of Americans over the age of 50. Supported by the National Institute on Aging and the Social Security Administration, the HRS is designed to provide reliable data on the decisions, choices, and behaviors of people as they age and respond to changes in public policy, the economy, and health. The study obtains information every two years about income and wealth, health and use of health services, work and retirement, and family connections. Through its unique and in-depth interviews, the HRS provides an invaluable and growing body of multidisciplinary data that researchers can use to address important questions about the challenges and opportunities of aging. Because of its innovation and importance, the HRS has become the model and hub for a growing network of harmonized longitudinal aging studies around the world. Saliva was collected on half of the HRS sample each wave starting in 2006 and respondents were genotyped on the Illumina Human Omni2.5-Quad (Omni2.5) BeadChip at the NIH Center for Inherited Disease Research. We accessed survey results to evaluate prevalence of

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