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.

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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.

https://doi.org/10.1017/cts.2019.60 Published online by Cambridge University Press