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PROJECT TITLE: Dystrophin- and utrophin-dependent signal transduction in transcriptome regulation and crosstalk between adjacent and distant cell structures, cells and tissues, and its role in Duchenne muscular dystrophy pathogenesis

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Duchenne muscular dystrophy (DMD), an X chromosome-linked disease affecting 1/5000 boys, is caused by lack offunctional dystrophin. The most evident feature of DMD is the progressive musculature disability underlined by loss of myofibers. With time, the extent of muscle fiber loss is so vast that the patients are forced to use a wheelchair and respiratory system aid to help diaphragm sustain breathing. As of today, the disease is incurable and leads to early premature death. Shadowing progressive skeletal muscular dystrophy and heart problems, DMD patients also suffer from cognitive deficits and psychiatric symptoms that are linked to loss of specific dystrophin isoforms. In the past, DMD research focused mostly on structural properties of dystrophin and its paralog, utrophin, aprotein able to compensate for the loss of dystrophin to some extent. However, apart from their roles in linking the extracellular matrix and the inner cytoskeleton inside the myofibers and stabilizing the myofibers against the contraction-induced injury, more recent data indicate that dystrophin and utrophin serve as important components in signal transmission pathways and crosstalk between various cell types.

Specific aims of this project include: (1) identification of roles of various dystrophin and utrophin isoforms in signal transduction pathways and their contribution to specific biological phenomena including cell proliferation, cell growth, homeostasis maintenance and survival, (2) deciphering the regulatory mode of expression of specific dystrophins/utrophins, as these proteins are tightly regulated in a tissue-specific and often time-restricted manner.

Particular focus will be put on putative feedback looping between dystrophins and utrophins by testing activation of their promoters, alternative splicing of pre-mRNA, epigenetic modifications of mRNA and reciprocal down/up regulation of mRNA through identification of protein and small RNA interaction partners, (3) uncovering the roles of dystrophins/utrophins in crosstalk between adjacent and distant cell structures, cells and tissues, based on their membrane localization and ability to assemble protein complexes with both structural and signaling functions.

Particular emphasis will be put on processes within the multinucleated myofiber, neighboring cells and distantly located tissues.

To address these aims, a well-established experimental model of an *in vivo* muscle regeneration process will be used in murine models of DMD as well as cultured muscle cells. Muscle regeneration consists of several sequential steps including signal transmission and crosstalk between various cell structures in multinucleated myotubes as well as distinct cell types such as stems cells, myoblasts, fibroblasts and myofibers. Additionally, isolated muscle stem as well as neural and cardiac cells will be subjected to proliferation and differentiation protocol in cell culture. A wide range of experimental techniques will be implemented in the project, including cloning and transfections, siRNA delivery to cells and tissues, semi-quantitative and quantitative PCRs, Western blotting and co immunoprecipitation and fluorescence microscopy. Additional methods will include: single nuclei isolation, fluorescence-activated cell sorting, cross-linking of mRNAs with interacting small RNAs and proteins, high-throughput mRNA and small RNA sequencing, direct RNA sequencing and assessment of RNA modifications, alternative splicing evaluation, mass spectrometry of co-

immunoprecipitated proteins, and design and delivery of shRNA-rAAV. The experimental outline for each part, although self-sufficient, complement and fuel each other, creating a scope for interaction between coinvestigators.

Despite primary focus on various stages of formation and maintenance of muscle as well as heart and brain cells, the project has more systemic application, that involves: (1) identification and distinguishing particular signaling outcomes dependent on a given dystrophin/utrophin isoform, (2) expanding the knowledge about new dystrophin/utrophin functions, (3) evaluation of the therapeutic potential of each isoform in biological processes such as cell proliferation, growth, differentiation and survival, (4) predicting an outcome of forced expression of a given isoform in non-native cellular environment, (5) distinguishing primary from secondary pathological processes during the DMD progression, (6) broadening the therapeutic repertoire against DMD, (7) uncovering putative toxic properties of various isoforms, including those that pertain to the competition of exogenously and endogenously expressed dystrophins/utrophins.

Summarizing, the project is intended to broaden our understanding of various dystrophin and utrophin isoform functions and bring us closer to formulate an ultimate, holistic therapeutic approach for Duchenne muscular dystrophy (DMD). The innovative aspects of the project and collaborative network of scientific experts from various disciplines guarantee necessary supervision and publishing the obtained data in prestigious scientific journals.