Utrophin and Dystrophin Localization in Dystrophic Myotubes After Transduction With AAV rh10 Vectors Containing Microdystrophin Targeted to Striated Muscle Cells; Treatments for Duchenne Muscular Dystrophy

Miller, Sarah (School: Satellite High School)

Duchenne Muscular Dystrophy (DMD) is an inherited X-linked disease with an incidence of 1:3,600. Patients become weak at 2-3 years old with progression to respiratory failure and death in their 20-30s. The absence of dystrophin causes contraction-induced injury to the muscle which results in muscle inflammation and degeneration. Historically, treatment of DMD has primarily focused on reduction of inflammation with corticosteroids with only limited improvement in strength with many side effects. New approaches include gene therapy in which a truncated version of the dystrophin gene is introduced into skeletal muscle cells through a viral vector but is limited in its packaging size. Due to dystrophin's large size (427 kDa) several dystrophin minigenes utilizing a truncated version are in development, but complications have arisen due decreased function as well as displacement of endogenous utrophin (a dystrophin-like protein) which localizes at the sarcolemma. This project aims to localize and quantify endogenous utrophin and exogenous microdystrophin in both wild type and diseased (DMD) skeletal muscle cells transduced with dystrophin minigenes. A dystrophin minigene was successfully transduced into DMD differentiated myotubes using AAV and expressed at levels detectable by immunofluorescence microscopy. Decreased expression of utrophin was qualitatively observed in these cells suggesting downregulation of utrophin. Future studies will focus on utilizing micropattern hydrogels both for the purpose of analysing cellular microstructure, utrophin distribution and functional analysis of tensile strength. The ability to screen different minigenes for function in a cell culture model will facilitate maximizing utility of these truncated dystrophin constructs.

Awards Won: