Creation of a Duchenne Muscular Dystrophy Myoblast Cell Culture Model by Knocking Out the Dystrophin Gene Using CRISPR/Cas-9 for High Throughput Drug Screening

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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 a wheelchair in their early teens, followed by 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. Treatment of DMD has primarily focused on reduction of inflammation with corticosteroids with only limited improvement in strength and lifespan with many side effects including osteoporosis and diabetes. One potential treatment is to upregulate expression of utrophin which is fetally expressed but structurally similar to dystrophin. Upregulation of utrophin in mouse models has shown to act as a surrogate, compensating for the lack of dystrophin. This project aims to create a human muscle cell culture model to test treatments for DMD. Primary human myoblast cells were co-transfected with a CRISPR/Cas-9 dystrophin knockout plasmid and HDR plasmid using matrigel coated plates for high transfection efficiency of nearly 100%. This allows for the knockout of the dystrophin gene as well as the insertion of a puromycin antibiotic resistance gene. Creation of the cell model allows for the testing of potential treatments. Differentiated myotubes were treated with L-arginine and utrophin expression was analyzed using SDS-PAGE/Western blot. The treatment group showed a qualitative increase in utrophin protein expression. Future Studies include selection of positive clones with dystrophin gene knockouts. This model can be used to test treatments for DMD as well as model different DMD mutations.