Group#11

Group #11 Elevator Pitch Video Link: https://youtu.be/crH88d25VZU

Utrophin upregulation via promoter replacement using CRISPR-Cas9

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Abstract

Duchenne Muscular Dystrophy (DMD) is a fatal genetic disease that predominantly affects males caused by different mutations of the DMD gene[1, 2]. Such mutations result in various dystrophin alterations which cause muscle loss, weakness, and eventually death due to cardio-respiratory failure[3]. Upregulation of utrophin A (UA), a homologue of dystrophin[4], is a commonly investigated treatment for DMD. It focuses on increasing its promoter activity with the help of novel transcription factors after genetic manipulation[5] or exogenously delivered proteins[6]. Using a novel approach, we aim to upregulate UA in *mdx* mice by replacing the promoter of the *UTRN* gene, coding for UA, in muscle stem cells—or satellite cells—with that of the highly transcribed *ACTB* gene, encoding actin. CRISPR-Cas9 will excise and replace the original *UTRN* promoter in satellite cells with the *ACTB* promoter. After introducing the modified cells into *mdx* mice, immunofluorescence microscopy against UA and RT-qPCR of UA transcripts will be compared between *mdx*, treated-*mdx* and wild-type mice. Moreover, Creatine Kinase (CK) release will be measured using a Creatine Kinase-SL kit as an indication of muscle degeneration[7]. It is expected that a successful UA upregulation in treated-*mdx* mice muscle cells, would lead to a reduction in CK release when compared to untreated-*mdx* mice, indicating lower muscle degeneration. Therefore, we intend to contribute to the ongoing investigation of UA upregulation as a potential treatment for DMD by investigating the possibility of upregulating UA expression permanently to a level that sufficiently replaces dystrophin.

Keywords: Duchenne Muscular Dystrophy, dystrophin, utrophin upregulation, CRISPR-Cas9, promoter

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