Correction of frameshift and point mutations in *DMD* gene using CRISPR Cas9 prime editing

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Abstract

Duchenne Muscular Dystrophy (DMD) is a hereditary disease responsible for burden, pain and damage to patients and their families. Existing experimental treatments provide limited phenotypic improvement. There is no permanent treatment for the disease. Our study aimed to experiment the CRISPR-Cas9 Prime editing as permanent therapeutic approach.

This technology uses a PRIME editor plasmid (PE) coding for a Moloney murine leukaemia virus reverse transcriptase fused with the Cas9 H840A nickase, and a plasmid coding for a pegRNA containing a primer binding site (PBS) and a reverse transcriptase template (RTT). We designed different components targeting DMD exons (6, 9, 20, 35, 43, 52, 55, 59 and 61) and splice sites of exons 51 and 53 responsible for DMD. HEK293T and myoblast cells were harvested three to five days post-treatment with prime editors. Parts of myoblasts were transformed into myotubes to obtain proteins for western blot analysis. Targeted sequences were amplified, sequenced by Illumina method and analysed using CRISPResso.

Prime editing (PE) permitted the C>T, A>G, G>C, G>A and G>T substitutions with editing efficacy of up to 8% in HEK293T cells. Different optimization systems permitted to achieve up to 80% modification. The PE permitted the insertion and deletion of one to six nucleotides in exon 51 and 53 splice sites amenable exon 51 and 53 skipping for the correction of deletion 52 and deletion 45-52 of up to 32% and 41% in HEK293T cells and, 5.5 and 28% in patient myoblasts. The exon skipping was confirmed by RT-PCR and the dystrophin restoration by western blot. The correction of point mutations in patient myoblasts exons 6 and 59 showed up to 28% correction with dystrophin restoration detected by western blot analyses.

Thus, Prime editing permits the correction of mutations in the DMD gene and might be considered for the permanent correction DMD mutations.