A new approach to introduce specific point mutations in the gene coding for dystrophin: PRIME editing technology.

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Abstract

Mutations in the dystrophin gene lead to neuromuscular dis¬orders such as Duchenne Muscular Dystrophy which is a lethal X-linked hereditary disease with the prevalence of 19.8 per 100 000 males' birth. Currently available clinical therapies with corti¬costeroids or with morpholino antisense oligomer injections pro¬vide limited phenotypic improvement. Our study aimed to mea¬sure the PRIME editing technology efficiency. This technology uses a PRIME editor plasmid (PE2 or PE3) coding for a Moloney murine leukemia virus reverse transcriptase fused with the Cas9 H840A nickase, and a plasmid coding for a pegRNA containing a primer binding sites (PBS) and a reverse transcriptase template (RTT). It permits specific nucleotide substitutions, deletions or insertions in the genome. We designed different pegRNAs target-ing several hDMD exons (9, 20, 35,43, 51, 55, and 61) to intro¬duce a STOP codon by modifying a single nucleotide. HEK293T cells were harvested from DMEM culture media three days after being simultaneously transfected with the PE2 and pegRNA. Ex¬ons were PCR amplified and sequenced using the Sanger method. Results were analysed using the EditR program to estimate the ed¬iting percentage. We confirmed that PRIME editing permits the specific C to T and G to T substitutions in the DMD gene with an editing efficiency between 6 to 11 % (PE2) and 21% (PE3). Repeated transfections 6 days after the first one showed up to 15 % (PE2) edition in exons 9 and 35. An additional mutation in PAM sequence (exon 35) improved a PE2 result to 38% for a single transfection. Thus, PRIME editing permits the specific sub¬stitutions in the DMD gene and might be used to correct point mutations in the DMD gene to lead to dystrophin expression.

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