SACI Office of Society Academia Collaboration for Innovation
KYOTO UNIVERSITY

Exon skipping by CRISPR-Cas9 editing for Duchenne muscular dystrophy

We are looking to out-license the technology for its commercialization

Background

Duchenne muscular dystrophy (DMD) is a rare, severe genetic disease characterized by progressive muscle wasting. It is caused by mutations in DMD gene that prevent the production of the muscle isoform of dystrophin protein. The symptom onset is usually in early childhood and life expectancy is ~30 years. Elevidys is currently the only gene therapy approved for DMD, which is designed to deliver a gene coding for a shorter version of dystrophin. This approach was taken because delivery of the whole DMD gene is challenging as it is the largest human gene. Antisense oligonucleotide medications for exon skipping (exons 45, 51, and 53) to produce a shorter yet functional dystrophin gene have been approved, which require periodic administration. None of the drugs is suitable for all DMD patients. More treatment solutions are urgently needed.

Technical Summary

The present invention is CRISPR-Cas9- mediated genome editing by Cas9 mRNA and in vitro transcribed sgRNA delivery in myoblasts derived from DMD patient iPS cells. The researchers first obtained myoblasts from iPSCs derived from a DMD patient with a deletion of exon 44 in the DMD gene. They then designed and tested sgRNAs to skip exon 45 to restore the open reading frame and produce a shorter but functional dystrophin protein (Ifuku et al. 2018, Fig.1).

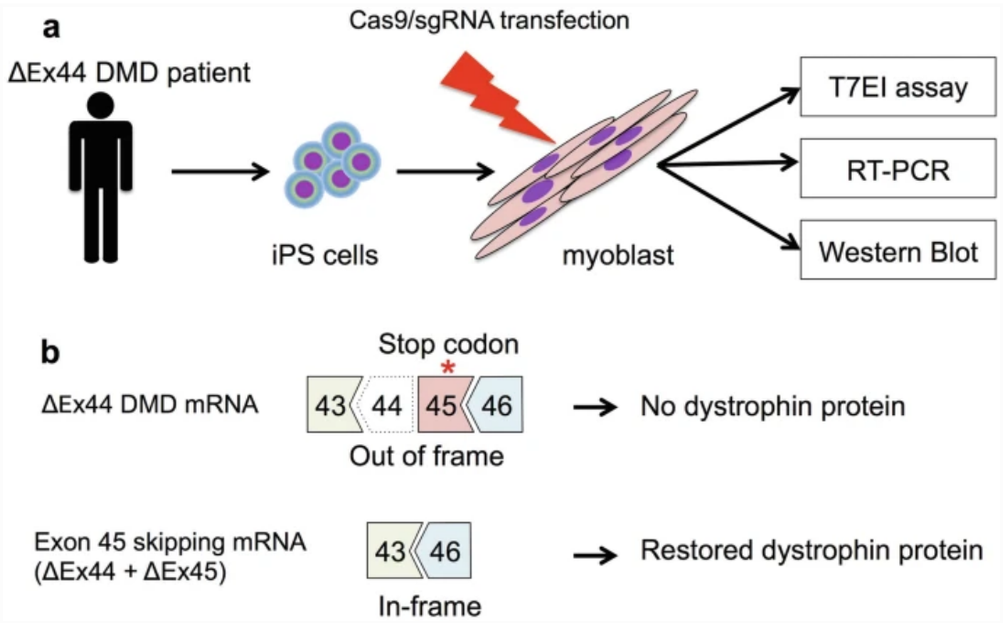

Figure 1. Schematic of experimental procedures to induce and assess the efficiency of exon skipping in DMD patient-derived myoblasts.

This protocol is a step-by-step manual for successful genome editing and can serve as a useful platform to screen candidate sgRNA sequences to effectively induce exon skipping for DMD. Although AAV-delivered CRISPR-Cas9 editing has been tested in DMD model organisms, its therapeutic translation is hindered by the limited packaging capacity of viral particles and possible immune response to them. Moreover, AAV transgenes tend to be expressed for an extended period of time, oftentimes years, which increases off-target mutagenesis risk. Instead, the CRISPR-Cas editing system described here can be delivered with either NanoMEDIC method, subject of another patent, or LNP (lipid nanoparticles) for pre-clinical testing and clinical applications. The NanoMEDIC gene editing delivery system is an associated technology, details can be found in the corresponding Technology Summary.

Technology Readiness Level

- 2

Approach has been validated in DMD patient-derived iPSCs

Potential Applications

- DMD disease modeling
- Drug discovery and screening
- Gene therapy for DMD

Possible Collaboration Mode(s)

- R&D collaboration
- Licensing
- IP Acquisition
- Other

Patent No

WO2018179578A1

Publication(s)

Ifuku M, Iwabuchi KA, Tanaka M, Lung MSY, Hotta A. Restoration of Dystrophin Protein Expression by Exon Skipping Utilizing CRISPR-Cas9 in Myoblasts Derived from DMD Patient iPS Cells. *Methods Mol Biol* 2018; 1828: 191–217.

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