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NanoMEDIC gene editing delivery system

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

Background

Gene therapy holds great potential for treatment of genetic diseases. However, delivery to specific tissues is still a major bottleneck. Although AAV-delivered CRISPR-Cas9 editing is being developed for multiple diseases, 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, which increases off-target mutagenesis risk. Extracellular vesicles (EV) is another option and, when combined with ribonucleoprotein (RNP) delivery of CRISPR-Cas9, reduces off-target effects. The structural polyprotein Gag is typically used to package cargo into EVs, which nevertheless limits the EV packaging capacity. The present invention is an all-in-one EV delivery system termed NanoMEDIC (nanomembrane-derived extracellular vesicles for the delivery of macromolecular cargo). It is a transient EV-based CRISPR-Cas9 editing delivery system which ensures efficient genome editing in various cell types.

Technical Summary

➢ Particle construction and production

First, the researchers designed NanoMEDIC delivery system which relies on two homing mechanisms to package Cas9 protein and sgRNA separately. The first utilizes a chemical ligand-dependent incorporation of SpCas9 to GagHIV. The second relies on Gag-mediated HIV Ψ packaging signal to direct sgRNA flanked by HH and HDV self-cleaving ribozymes into NanoMEDIC. In both cases, Gag is the beacon for recruitment and the dual homing approach synergistically recruits active RNP complexes into NanoMEDIC particles (Gee et al. 2020). The particles are produced by transfecting producer cells with five plasmid DNAs expressing FRB-Cas9, Ribozyme-gRNA-Ribozyme, FKBP12-Gag, Tat, and VSV-G. The inventors also established a straightforward large-scale NanoMEDIC production (Watanabe et al. 2023, Fig.1).

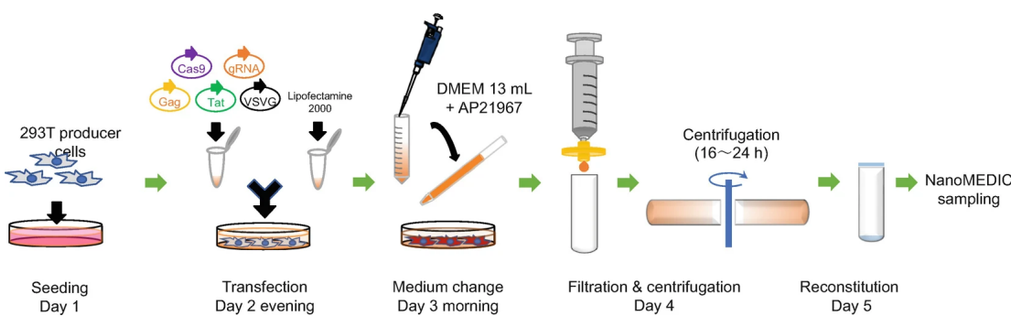

Figure 1. Schematic of experimental procedures for NanoMEDIC production.

➢ Particle application

The researchers then used NanoMEDIC system to target exon 45 in DMD gene to restore out-of-frame dystrophin protein by exon skipping. They delivered two sgRNAs independently without mutual inhibition achieving a high exon-skipping activity. They confirmed the system efficiency in skeletal muscle cells differentiated from DMD patient-derived iPSCs: dystrophin protein expression was restored.
Long-term (160 days) exon 45 skipping was also achieved in mice accompanied by a transient delivery, which was cleared within 3 days. Importantly, off-target cleavage activity was almost eliminated using NanoMEDIC compared with DNA plasmid delivery. Thus, NanoMEDIC system can be used to deliver CRISPR-Cas9 for genome editing into living skeletal muscle tissue. Moreover, NanoMEDIC efficiently induces genome editing in various human cell types, such as T cells, monocytes, iPSCs, and iPSC-derived cortical neurons (Gee et al. 2020).

Technology Readiness Level

- 3

Approach has been validated *in vitro* (various cell types) and *in vivo* (mice)

Potential Applications

- Gene therapy for DMD
- Gene therapy for other diseases

Possible Collaboration Mode(s)

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

Patent No

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Publication(s)

Gee P, Lung MSY, Okuzaki Y, Sasakawa N, Iguchi T, Makita Y *et al.* Extracellular nanovesicles for packaging of CRISPR-Cas9 protein and sgRNA to induce therapeutic exon skipping. *Nat Commun* 2020; 11: 1334.

Watanabe K, Gee P, Hotta A. Preparation of NanoMEDIC Extracellular Vesicles to Deliver CRISPR-Cas9 Ribonucleoproteins for Genomic Exon Skipping. *Methods Mol Biol* 2023; **2587**: 427–453.

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