Engineered exosomes for future gene-editing therapy

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The RNA-guided clustered, regularly interspaced, short palindromic repeats (CRISPR)-associated nuclease protein 9 (Cas9)-based technology is an advanced and popular gene-editing technology, which has shown great potential in treating genetic disorders in both animal models and even in clinical trials.[1, 2] Many transportation strategies are available for delivery of the CRISPR-Cas9 system, among which Cas9 ribonucleoprotein (RNP) delivery has some competitive advantages over other options, such as faster editing onset speed with a reduced immune response and lower off-target activity.[3, 4] However, the low *in vivo* delivery efficiency and poor tissue specificity of RNP delivery have limited further clinical applications[5].

Writing in Science Advances, Wan et al.[6] reported a previously-unidentified genome editing delivery system, named exosomeRNP which transported RNPs within exosomes extracted from hepatic stellate cells via electroporation. The exosomeRNP exhibited effective intracellular and intercellular delivery and accumulation of RNPs to hepatocytes *in vitro* and *in vivo*, resulting in significant therapeutic effects in several hepatic diseases. The system was tested in mouse models of acute liver injury, chronic liver fibrosis, and hepatocellular carcinoma by targeting p53 up-regulated modulator of apoptosis, cyclin-E1, and K(lysine) acetyltransferase-5, respectively, providing a potential strategy for high-efficiency, precise and tissue-specific gene editing in liver diseases.

Apart from exosome-loaded RNPs, many other options are also available and widely used in gene editing, such as the insertion of plasmid vectors or small interfering RNAs. However, the CRISPR-Cas9 system has various competitive advantages over the other methods.[1] First, the high veracity of recognition endows the CRISPR-Cas9 system with better gene-editing efficiency along with reduced off-target effects, resulting in a stable gene-modulating effect. Additionally, editing genes with CRISPR is very convenient and the cost is relatively low. CRISPR-Cas9 edits gene expression at the source and permanently changes the target phenotype. CRISPR technology can also be used to edit several target genes simultaneously. These outstanding advantages make the CRISPR-Cas9 system the favourite technology of gene-editing researchers.

Exosomes are vesicles ranging from 30 to 150 nm in diameter secreted by various cell types.[7] They communicate information intercellularly through surface protein signalling or by transferring contained lipids, nucleic acids, and other biomolecules. The properties of exosomes depend on their cell surface proteins and the biomolecules they carry, which has made them of particular interest in the development of novel transportation approaches (**Figure 1**). In their study, Wan et al.[6] precisely and safely transported the large RNP complex inside exosomes purified from hepatic stellate cells. However, the extraction efficiency of exosomes is not satisfactory. Besides, naturally-obtained exosomes from different cells have different constitutions and batch effects also vary among different batches. In addition, the variation in the diameter of exosomes is not controllable. These drawbacks limit the widespread use of natural exosome vehicles, which makes it necessary to develop better nanocarriers and controllable transportation strategies.[5, 8] Cell membrane-camouflaging nanotechnology is an emerging delivery strategy that might be a better option for nanodrug transportation. Through ultrasonic or extrusion methods, cell membranes extracted from different cell lines can be coated around nanoparticles with controllable size and high rate of output. The membrane-camouflaged nanoparticles have longer circulation time and lower adverse effects on the heterologous antigens that are concealed under the biocompatible membranes. Therefore, by incorporating various

types of cell membranes onto the nanodrugs, engineered nanocarriers with multiple functions and excellent biosafety are developed.[9] As for targeted transportation, homologous or artificially-modified membrane-coated technology has shown potential in intercellular homologous targeting via the ligand-receptor interaction, which could be utilized for enhancing drug accumulation at sites of interest. To further strengthen the targeting capability, some targeting ligands could be introduced onto the surface of membranes, such as cyclic arginine-glycine-aspartate peptides which have been confirmed to increase tumour tissue permeability through active binding of cyclic arginine-glycine-aspartate-integrin $\alpha v\beta 3$[10]. Therefore, modified membrane vesicles can function as advanced targeting drug delivery systems, which will ultimately benefit the therapeutic performance of nanoparticles by lowering the probability of on-target, off-tumour side effects (**Figure 1**).

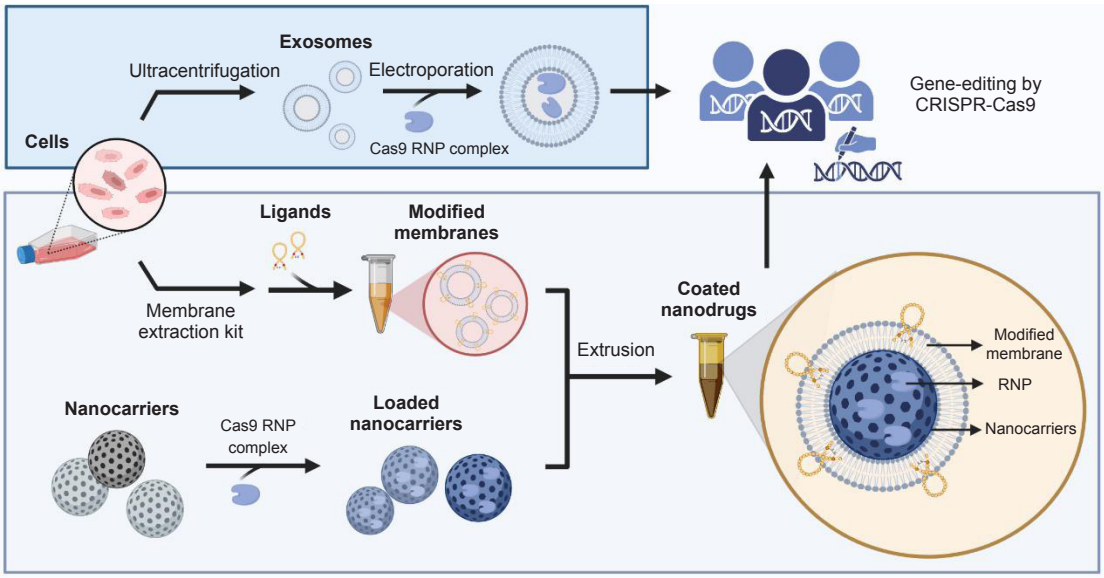

Figure 1. Schematic illustration of exosomes and cell membrane-coated vesicles for targeted delivery of Cas9 RNP for gene-editing therapy. Apart from exosome^{RNP} mentioned by Wan et al.,[6] modified membrane vesicles could also be used to camouflage and deliver the RNPs, which may show greater therapeutic potential due to their controllable size and artificial modular functions endowed by ligands. Cas9: CRISPR-associated nuclease protein 9; CRISPR: clustered, regularly interspaced, short palindromic repeats; RNP: ribonucleoprotein.

In summary, CRISPR-Cas9 is a cutting-edge technology in the field of gene editing which enables gene therapy with better gene-editing efficiency along with reduced off-target effects compared to other methods. The study of Wan et al.[6] sheds light on potential solutions to the problems of low *in vivo* delivery efficiency and poor tissue specificity of delivery of the CRISPR-Cas9 system. Furthermore, other advanced membrane vehicle transportation strategies such as membrane-coating technology might pave the way to further improve transportation efficacy, specificity, and safety by making the properties of the nano-carriers more controllable, which will lead to greater success of future gene-editing therapy and bring consequent clinical benefits.

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Conceptualization, investigation, and writing-original draft: HYG; software, supervision, and writing-review & editing: XH. Both authors read and approved the final version of the manuscript.

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