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Research on Rare Genetic Disease Targeted Drug Delivery System Based on CRISPR-Cas9 Technology

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Abstract

The treatment of rare genetic diseases remains a significant challenge, and while CRISPR-Cas9 technology offers a powerful therapeutic pathway, its success is entirely contingent on the development of efficient and safe targeted delivery systems. Current delivery methods are hampered by substantial flaws. Viral vectors, such as AAV, have limited packaging capacity and carry mutation risks, whereas lentiviral vectors pose concerns regarding immunogenicity and carcinogenicity. Meanwhile, non-viral vectors like LNPs suffer from poor targeting specificity, electroporation is restricted to in vitro use, and direct injection is notoriously inefficient. To address these critical limitations, this study investigates three advanced delivery platforms. These include Virus-Like Particles (VLPs), exemplified by the safe and programmable RIDE system with demonstrated efficacy in Huntington's disease mouse models; smart nanoparticles designed to respond to specific physiological triggers like pH or receptors for precise organ targeting, such as in liver-directed therapy for PH1; and cell-mediated systems utilizing engineered hematopoietic stem cells for diseases like sickle cell anemia. Concurrently, the research tackles persistent issues of targeting precision, immunogenic responses, and editing efficiency through strategic ligand optimization and the application of high-fidelity Cas9 variants. Collectively, this work provides valuable insights and a strategic framework to advance the clinical potential of CRISPR-Cas9 for treating rare genetic diseases.

Keywords

CRISPR-Cas9, rare genetic diseases, targeted drug delivery systems, VLPs, smart responsive nanoparticles

1. Introduction

Rare genetic diseases, though individually low in incidence (typically fewer than 1 in 5,000 individuals globally), collectively affect around 300 million people worldwide. Of the over 7,000 identified rare diseases, ~80% stem from single-gene defects, often manifesting in early life with progressive, debilitating symptoms. Conventional treatments—such as symptomatic relief, enzyme replacement, or organ transplantation—only alleviate discomfort without addressing the root cause: mutated genes. These options are often costly, lifelong, and fail to halt disease progression, burdening patients' families and healthcare systems heavily. CRISPR-Cas9 technology revolutionizes rare genetic disease treatment by enabling precise editing of mutated genes, offering unprecedented curative potential. It has shown robust efficacy in animal models of sickle cell anemia,

primary hyperoxaluria type 1 (PH1), and Huntington's disease, with several therapies advancing to Phase I/II clinical trials. However, CRISPR-Cas9 components (sgRNA and Cas9 protein) cannot reach target cells efficiently alone: they are prone to nuclease degradation, hindered by cell membrane barriers, and may trigger immune responses due to Cas9's bacterial origin. Thus, targeted drug delivery systems become a core bottleneck for its clinical translation.

Existing delivery systems have critical limitations: viral vectors (e.g., AAV, lentivirus) boast high efficiency but carry risks of insertional mutagenesis and pre-existing antibody interference; non-viral vectors (e.g., LNPs, electroporation) are safer but suffer from poor tissue targeting and low delivery efficiency. Developing safer, more precise novel delivery systems is vital to advancing CRISPR-Cas9's application—and this is the core focus of this study.

2. Analysis of Existing Targeted Drug Delivery Systems

2.1 Viral Vector

2.1.1 Adeno Associated Virus (AAV)

Adeno-associated virus (AAV) has the core advantages of high safety and low immunogenicity, and can stably integrate into host chromosomes to achieve long-term expression of exogenous genes. Therefore, it is widely used in in vivo gene therapy scenarios for monogenic genetic diseases (such as SMA), retinal diseases, etc. However, due to the limited vector capacity of only about 4.7kb, it cannot carry large genes, and the pre-existing neutralizing antibodies in the human body will reduce its therapeutic effect[1].

2.1.2 Lentivirus

However, lentiviral vectors have a larger capacity (8-10kb), can infect both dividing and non dividing cells, and can stably integrate and express genes. They are mainly used in CAR-T cell therapy, hematopoietic stem cell gene modification, and other in vitro gene delivery fields. However, their random integration poses a safety risk of tumor initiation due to insertion mutations, and their high immunogenicity limits their direct application in vivo.

2.2 Nonviral Vector

2.2.1 Lipid Nanoparticles

Lipid nanoparticles (LNP) have high safety and no risk of genome integration. The delivery efficiency can be improved through composition optimization. It can be delivered both intravenously and locally. Its core is used for the delivery of mRNA vaccines (such as COVID-19 vaccine) and siRNA drugs for liver diseases, but it is easy to be cleared by the immune system, has a short cycle half-life, and some tissue targeting efficiency needs to be improved.

2.2.2 Electroporation

Electroporation forms temporary micropores on the cell membrane through high-voltage pulses, with high delivery efficiency and no restriction on nucleic acid types. It can accurately target specific tissues or cells and is suitable for in vitro cell transfection, in vivo tumor gene therapy, and skin vaccination. However, it can cause damage to cells and may be fatal. In vivo application requires precise control to prevent damage to normal tissues, and can only be operated locally without systemic delivery.

2.2.3 Direct Injection

Direct injection is simple to operate, low-cost, and does not require complex preparation or equipment. It can be directly injected into target tissues for DNA vaccine muscle injection and specific organ gene therapy research. However, its delivery efficiency is extremely low, naked nucleic acid is easily degraded, and is only suitable for local small-scale tissues. It may also stimulate the injection site and cause inflammation.

2.3 Research Direction of New Targeted Drug Delivery Systems

2.3.1 Delivery System Based on Virus Like Particles

Virus-like particles (VLPs) are hollow, non-replicating nanostructures that mimic the structural topology of natural viruses while lacking viral genomic material, thus being non-infectious yet retaining intrinsic immunogenicity. Widely established in vaccine development—most prominently for human papillomavirus (HPV) and hepatitis B virus (HBV) prophylactic vaccines—VLPs also serve as versatile platforms for targeted drug delivery and diagnostic antigens, leveraging their excellent biocompatibility, biodegradability, and modular structural characteristics. Their core advantages include a favorable safety profile, the capacity to elicit long-lasting immune responses, and amenability to genetic engineering for functional optimization. However, inherent limitations persist: intricate bioprocessing requirements, relatively high production costs, suboptimal stability of certain VLP subtypes under physiological conditions, and constrained payload capacity that may hinder the delivery of large molecular complexes. Critically, their lack of autonomous replication machinery eliminates the insertional mutagenesis and excessive immunogenicity risks associated with viral vectors (e.g., adeno-associated virus, lentivirus), positioning VLPs as a promising alternative for safe CRISPR-Cas9 delivery in rare genetic disease therapy.

A paradigmatic application of VLP-based CRISPR-Cas9 delivery is the RNA-guided integration-dependent editing (RIDE) system developed by Cai Yujia's team at Shanghai Jiao Tong University. This innovative platform modifies bacteriophage capsid proteins to enable efficient encapsulation of CRISPR-Cas9 core components—single-guide RNA (sgRNA) and Cas9 endonuclease—while conjugating neuron-specific targeting peptides to the capsid surface. These peptide ligands mediate precise recognition and binding to cell-surface receptors on neuronal populations, facilitating receptor-mediated endocytosis and targeted intracellular trafficking. In a well-characterized Huntington's disease mouse model harboring the mutated huntingtin (HTT) gene, the RIDE system achieved site-specific editing of the pathogenic locus, resulting in a significant reduction in the expression of toxic mutant HTT protein—a key driver of neurodegenerative pathology. Notably, comprehensive genomic analysis and immunological profiling revealed no detectable off-target edits or adverse immune responses (e.g., pro-inflammatory cytokine elevation, antibody production against Cas9 or VLP capsids) in treated animals, validating VLPs' potential for safe and tissue-specific delivery in rare neurological disorders.

Future research to advance VLP-based delivery systems should focus on two pivotal directions. First, modifying VLP capsid proteins through directed evolution or rational genetic engineering to expand tissue tropism—enhancing targeting efficiency toward diverse cell lineages such as hepatocytes, renal tubular epithelial cells, and skeletal muscle myocytes—thereby broadening applicability to non-neurological rare genetic diseases (e.g., liver-focused primary hyperoxaluria type 1, muscle-related dystrophies). Second, optimizing VLP payload loading efficiency via structural modifications (e.g., capsid protein engineering, pH-sensitive cargo-linker design) and formulation strategies to augment the encapsulation yield and intracellular release kinetics of CRISPR-Cas9 components, ultimately improving gene-editing efficacy in target cells. Additionally, exploring scalable manufacturing processes to reduce production costs and enhance batch-to-batch consistency will be critical for translating VLP-based systems into clinical applications.

2.3.2 Intelligent Responsive Nanoparticle Delivery System

Smart responsive nanoparticles, constructed from lipids, polymers, or inorganic matrices, are engineered with a core design principle: sensing pathophysiological cues in the tumor or diseased tissue microenvironment (e.g., pH gradients, aberrant enzyme activity, or temperature fluctuations) to trigger "on-demand, targeted release" of CRISPR-Cas9 components. This stimuli-responsive mechanism minimizes premature payload leakage in circulation or healthy tissues, thereby reducing off-target editing risks and enhancing therapeutic specificity.

For instance, the extracellular microenvironment of solid tumors and many genetic disease-affected tissues is characterized by acidosis (pH 6.0–6.5), a stark contrast to the neutral pH (7.2–7.4) of normal tissues and blood. Nanoparticles fabricated with polyethylene glycol-poly(β-aminoester) (PEG-PAE) block copolymers exploit this pH difference: under acidic conditions, the protonation of amino groups induces a charge reversal from negative to positive, which strengthens electrostatic interactions with the anionic cell membrane, promotes endosomal escape, and facilitates cytosolic release of CRISPR-Cas9. In liver-targeted applications—

such as for primary hyperoxaluria type 1 (PH1)—nanoparticles are surface-modified with galactose residues to enable active targeting via specific binding to the asialoglycoprotein receptor (ASGPR), which is highly expressed on hepatocytes. By co-incorporating pH-sensitive polymers (e.g., poly(lactic-co-glycolic acid), PLGA) into the nanoparticle core, the system ensures that CRISPR-Cas9 is sequestered during systemic circulation and only dissociates within hepatocytes' acidic endosomal compartments, maximizing editing efficiency at the intended site.

In the study of primary hyperoxaluria type 1 (PH1), the Wu Yuxuan team used liver targeted lipid nanoparticles (LNPs) to encapsulate CRISPR-Cas9. After intravenous injection, the nanoparticles were specifically taken up by liver cells and released editing components triggered by the acidic environment inside the cells. The mutated AGXT gene was successfully edited, reducing oxalate levels in mouse urine by more than 70% without significant effects on other organs. The key to such systems lies in balancing targeting and stability - it is necessary to ensure that nanoparticles are not released prematurely in the bloodstream and are efficiently released within target cells, which requires material combination optimization and structural design to achieve[2].

2.3.3 Cell Mediated Delivery System

Cell mediated delivery systems utilize the cell's own migration ability and biocompatibility to "transport" CRISPR-Cas9 to target tissues, particularly suitable for areas that are difficult to reach through conventional carriers such as the brain and bone marrow. Common carrier cells include hematopoietic stem cells, macrophages, and mesenchymal stem cells

Hematopoietic stem cells have the characteristic of homing to the bone marrow and can be edited and reinfused in vitro for the treatment of rare blood system diseases such as sickle cell anemia. Researchers introduced CRISPR-Cas9 into hematopoietic stem cells through electroporation, edited the mutated HBB gene, and reinfused it into mice. The edited cells can survive for a long time and differentiate into normal red blood cells, improving anemia symptoms[3]. Macrophages, due to their ability to migrate to inflammatory sites, are used to deliver CRISPR-Cas9 for the treatment of inflammation related genetic diseases, such as cystine disease (kidney and eye inflammatory lesions): macrophages loaded with CRISPR-Cas9 in vitro can actively migrate to kidney lesions, releasing editing components to repair mutated genes.

The core challenge of this system lies in two aspects: firstly, how to efficiently load CRISPR-Cas9 into cells, which can be achieved through viral vector transfection or nanoparticle mediated endocytosis; The second is to regulate the homing efficiency of cells, which can enhance their migration ability to target tissues by modifying the chemokine receptors on the cell surface (such as CXCR4)[4].

2.4 Issues that Need Attention in the Delivery System

2.4.1 Insufficient Targeting Accuracy and off Target Effects

Off-target editing stands as the paramount safety impediment to the clinical translation of CRISPR-Cas9 technology, with the targeting precision of delivery systems directly governing the frequency and severity of unintended genomic modifications. Non-viral vectors, such as conventional lipid nanoparticles (LNPs), inherently lack tissue-specific tropism, frequently leading to off-target payload release in healthy cells and consequent non-specific DNA cleavage—events that can precipitate genomic instability, cellular dysfunction, or even oncogenic transformation. Even ligand-functionalized targeted carriers remain susceptible: cross-reactivity with structurally homologous receptors on non-target cells (e.g., shared epitopes among tissue-specific antigens) often results in off-target accumulation and aberrant editing, underscoring the need for multi-layered safety safeguards.

To address this critical barrier, three synergistic strategies are advocated: ① Rational engineering of targeting ligands—encompassing monoclonal antibodies, high-affinity nucleic acid aptamers, and receptor-specific peptides—to enhance binding avidity and specificity for unique cell-surface biomarkers (e.g., ASGPR for hepatocytes, CD34 for hematopoietic stem cells), thereby minimizing off-target cell interactions by several orders of magnitude. ② Deployment of next-generation high-fidelity Cas9 variants (e.g., eSpCas9, Cas9-HF1, HypaCas9), engineered via structure-guided mutagenesis to restrict DNA cleavage to perfectly matched target sequences; for instance, Cas9-HF1 reduces off-target editing by ~1000-fold relative to wild-type SpCas9 in

mammalian cells, while retaining robust on-target activity. ③ Implementation of high-throughput, sensitive off-target detection platforms, including GUIDE-seq and whole-genome sequencing (WGS), to comprehensively map potential off-target sites in preclinical models. These approaches not only enable iterative refinement of delivery vectors and sgRNA designs but also furnish critical safety data required for regulatory approval, aligning with the rigorous standards for clinical gene-editing therapies.

2.4.2 Safety Risks Caused by Immunogenicity

The delivery system and CRISPR-Cas9 components may trigger an immune response in the body: the capsid protein of viral vectors (such as AAV) can be recognized by the immune system, inducing antibody production, which not only reduces the effectiveness of repeated administration, but may also trigger inflammatory reactions (such as liver injury); Cas9 protein (especially SpCas9 derived from bacteria), as an exogenous protein, may be recognized by T cells, leading to immune clearance or tissue damage.

The coping strategies include: ① selecting low immunogenicity vectors, such as modifying AAV capsids (reducing antigen epitopes) or using autologous cell-mediated delivery systems[3]; ② Humanization or chemical modification (such as PEGylation) of Cas9 protein to reduce its immunogenicity; ③ Combination use of immunosuppressants (such as glucocorticoids) to suppress excessive immune responses during treatment.

2.4.3 Insufficient Delivery Efficiency and Long-Term Stability

Some delivery systems suffer from inadequate delivery efficiency: direct injection of CRISPR-Cas9 components (sgRNA and Cas9 protein) renders them highly susceptible to nuclease-mediated degradation in circulation and the extracellular microenvironment, with less than 5% of the payload reaching target cells. Even when successfully internalized, edited gene expression often exhibits temporal decay—particularly with non-integrating vectors such as virus-like particles (VLPs) and lipid nanoparticles (LNPs), which lack stable genomic integration capacity. This transience hinders long-term therapeutic efficacy, necessitating repeated administrations that not only increase treatment burden but also elevate the risks of off-target editing and cumulative immune responses to Cas9 or carrier materials.

Key improvement directions include: ① optimizing carrier structural design—for instance, modifying VLP capsid proteins to enhance affinity for target cell surface receptors (e.g., neuron-specific peptides for neurological disorders) or engineering LNP lipid compositions to improve endosomal escape, thereby boosting intracellular delivery efficiency; ② integrating site-specific gene integration technologies (leveraging the inherent integration capability of lentiviral vectors) while mitigating insertional mutagenesis risks via homologous recombination-based targeted integration or prime editing strategies; ③ conducting long-term preclinical evaluations, including non-human primate models, to systematically monitor the duration of gene-editing effects, carrier bioaccumulation, and potential long-term toxicities, thus providing robust data to guide clinical dosing schedules and administration frequency[5].

3. Conclusion

Rare genetic diseases, predominantly stemming from monogenic defects, remain largely without radical therapeutic options, as conventional interventions merely alleviate symptoms rather than rectifying the underlying genetic aberrations. The CRISPR-Cas9 gene-editing technology has emerged as a transformative curative strategy, enabling precise, site-specific modification of mutated genes to restore physiological function—offering unprecedented hope for patients with such intractable disorders. However, the clinical translation of CRISPR-Cas9 is severely hampered by inherent limitations of existing delivery systems: viral vectors (e.g., adeno-associated virus, lentivirus) exhibit high transfection efficiency but carry substantial risks, including insertional mutagenesis, pre-existing neutralizing antibody interference, and long-term immunogenicity; in contrast, non-viral vectors (e.g., lipid nanoparticles, electroporation) offer improved safety profiles but suffer from inadequate tissue targeting, low intracellular delivery efficiency, and susceptibility to nuclease-mediated degradation.

To address this critical bottleneck, the present study explores three innovative targeted delivery systems tailored for CRISPR-Cas9: (1) safe, ligand-functionalized virus-like particles (VLPs), exemplified by the

RNA-guided integration-dependent editing (RIDE) system developed by Shanghai Jiao Tong University, which demonstrated robust efficacy in Huntington's disease mouse models by specifically editing the mutated HTT gene without detectable off-target effects; (2) microenvironment-responsive smart nanoparticles, such as the liver-targeted lipid nanoparticles (LNPs) reported by Wu's team, which achieved targeted delivery to hepatocytes and efficient editing of the AGXT gene in primary hyperoxaluria type 1 (PH1) mice, reducing urinary oxalate levels by over 70%; and (3) tissue-penetrant cell-mediated delivery systems, leveraging the homing capabilities of hematopoietic stem cells to target bone marrow niches, as validated in preclinical studies for sickle cell anemia through editing of the mutated HBB gene. Additionally, this study addresses core challenges of targeting precision, immunogenicity, and delivery efficiency via rational optimization of cell-specific ligands and integration of high-fidelity Cas9 variants (e.g., eSpCas9, Cas9-HF1), which minimize off-target editing and immune recognition.

Collectively, these findings significantly enrich the repertoire of CRISPR-Cas9 delivery technologies, provide valuable preclinical references for advancing its clinical translation in rare genetic diseases, and lay a solid foundation for the development of safer, more efficient, and clinically viable radical therapies—ultimately aiming to address the unmet medical needs of patients affected by these devastating disorders.

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Conflicts of Interest

The authors declare no conflict of interest.

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