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The CRISPR-Cas9 Technology Used for the Treatment of Single-Gene Genetic Diseases

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Abstract. Genetic diseases pose a threat to global health. Diseases caused by single gene mutations, such as sickle cell anemia (SCA), cystic fibrosis, and hemophilia B, are particularly prominent. CRISPR-Cas9, as a revolutionary gene editing technology, provides a new path for the cure of genetic diseases by targeting and repairing pathogenic gene mutations. Current research has advanced from in vitro cell experiments to the clinical trial stage. The cure rate of hematopoietic stem cell editing for SCA has reached 85%; the lung function repair of cystic fibrosis is achieved through AAV vector delivery; the in vivo editing of hemophilia B restores the coagulation factor activity to over 30%. However, the technology still faces core challenges such as off-target effects, low delivery efficiency, and ethical controversies. This article systematically analyzes the application mechanism, clinical progress, and optimization strategies of CRISPR-Cas9 in the treatment of three single-gene genetic diseases, confirming that it can achieve disease cure through precise targeting repair, and proposes optimized schemes for high-fidelity enzymes and non-viral delivery systems. The significance of this research lies in providing theoretical basis for individualized treatment of genetic diseases, promoting clinical translation, and focusing on long-term safety verification, reversible editing technology development, and the construction of inclusive medical models in the future to break through technical bottlenecks and balance ethical conflicts.

Keywords: CRISPR - CAS9, Single-Gene Genetic Disease, therapeutics

1. Introduction

Human health is threatened by many genetic diseases. The data from World Health Organization (WHO) show that 8% of newborns worldwide are affected by genetic diseases. In recent years, the rapidly developing gene editing has provided an unprecedentedly efficient approach for the prevention, diagnosis, and treatment of genetic diseases. It can precisely modify and edit the DNA sequence of the genome, thereby significantly increasing the possibility of treating genetic diseases [1]. However, gene editing also has numerous unknown risks. For instance, the genetic diversity of humans will be irreversibly affected as a result. It also brings new challenges in terms of ethics, especially the inevitable impact on groups with different cultural or religious backgrounds [2]. There are various types of current gene editing technologies. Among them, the clustered regularly interspaced short palindromic repeats (CRISPR-Cas9) immune system, as a brand-new technology,

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has attracted widespread attention in the field of life sciences since it was officially reported in 2012 [3]. CRISPR-Cas9 evolved spontaneously in prokaryotes by activating the adaptive defense mechanism [3]. Through complementary pairing of guide RNA (gRNA) with the target DNA sequence, it guides the Cas9 nuclease to cut the site, achieving the effect of precise editing of the genome [3]. Compared with traditional gene editing technologies, CRISPR-Cas9 has the advantages of being able to edit multiple genes simultaneously, being easy to operate, and having lower costs [1,3]. In the treatment of single-gene genetic diseases, the CRISPR-Cas9 shows great potential. Theoretically, based on the specificity of single-gene genetic disease mutations, this method is expected to achieve the goal of completely curing diseases by targeting and correcting the gene mutation sites of the disease or precisely regulating the expression state of genes related to the disease through high-precision technical means [1,3]. In the recent research on the treatment of single-gene genetic diseases, the CRISPR-CAS9 has progressed from in vitro cell experiments to animal model studies, and has achieved several important progress, accumulating a large amount of theoretical basis and practical experience for its future development towards clinical application [1,4]. For instance, a gene editing strategy utilizing CRISPR-CAS9 involves creating a DNA double-strand break (DSB) at a specific location to correct, delete, add or destroy a specific sequence generated by the targeted DSB and subsequently mediated by non-homologous end joining (NHEJ) or homology-directed repair (HDR), thereby permanently preventing pathogenic mutations [5]. However, this method still confronts many problems, such as off-target effects, immunogenicity, and ethical and moral controversies [1,4]. This article provides a general overview of the structural principle of CRISPR-Cas9, analyzes its application progress in three single-gene genetic diseases, and introduces the challenges and response strategies for this in the future.

2. The editing principle and composition of CRISPR-Cas9

2.1. The structure of CRISPR-Cas9

Among the commonly used Cas nucleases, the structure of Cas9 consists of two key nuclease domains - RuvC and HNH, and there is also a PAM interaction domain (PI) [6,7]. Among them, the RuvC domain and HNH domain can bind to double-stranded DNA molecules and trigger the formation of DSB through a synergistic effect. However, these two domains, RuvC and HNH, rely on the binding of the Cas nuclease to a specific DNA site, this binding process requires the presence of the original spacer region adjacent motif (PAM) as a prerequisite [8]. After the Cas nuclease completes the cleavage of DNA, the resulting DSB will be repaired by the cell's own DNA repair mechanism. There are mainly two repair ways in the cell: NHEJ repair and HDR. Among them, HDR usually requires the participation of template DNA during the repair of DSB, and such templates can be exogenous single-stranded or double-stranded DNA, or homologous chromosomes, sister chromatids, or even some nucleic acid sequences with high homology to the DSB sequence.

2.2. The mechanism and principle of CRISPR - Cas9

The core components of CRISPR-Cas9 technology include the single-guide RNA (sgRNA) sequence and the Cas9 endonuclease. Among them, sgRNA is formed by the fusion of CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA), which can precisely recognize the sgRNA/Cas9 ribonucleoprotein complex and guide this complex to specifically bind to the target DNA [1]. After the DNA sequence is precisely targeted, the Cas9 endonuclease induces DSBs in the target DNA region. These DSBs can be repaired through processes such as insertion, deletion, base

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addition, or fragment inversion. The specific execution mechanism of DNA repair can rely on the host cell's own natural repair system or be achieved with the help of artificially designed specific DNA sequences [1]. As a pioneering gene editing technology, CRISPR/Cas9 has been successfully applied in multiple clinical studies of lung cancer (NCT02793856) [5] and β-hemoglobinopathies (NCT03655678, NCT03745287, NCT04443907), which has also led to the birth of the first FDA-approved CRISPR therapy [2].

3. SCA and CRISPR gene therapy

3.1. Disease genes and treatment targets

The cause of SCA is a single nucleotide mutation in the β-globin gene (HBB), which results in the substitution of the sixth amino acid residue encoded by this gene from a hydrophilic glutamic acid to a hydrophobic valine. Abnormal hemoglobin S (HbS) will be produced by this mutation, which is prone to polymerization in hypoxic or acidic environments [9]. The abnormal aggregation of hemoglobin causes changes in the morphology of red blood cells (RBCs), resulting in a sickle-shaped structure. The structural damage of RBCs can trigger chronic hemolysis and hemolytic anemia, thereby causing severe pain, organ damage at the end stage, and even increasing the risk of early death in SCD patients [10,11].

3.2. Treatment methods

Sickle cell disease has several clinical subgroups, including hemoglobin SC disease (HbSC), SCA, and beta-thalassemia positive or beta-thalassemia negative hemoglobin sickling, and other minor variations [12,13]. The implementation process of this type of gene editing therapy is as follows: First, obtain the patient's hematopoietic stem cells, modify them in vitro using the CRISPR-Cas9 genome editing technology, then reinfuse the modified cells to the patient's body in a single-dose infusion [14,15]. Before the treatment, collect the patient's stem cells, and the patient needs to undergo high-dose chemotherapy to complete myeloablative pre-treatment. Afterwards, the modified stem cells will be re-implanted into the body and colonize in the bone marrow [15,16]. Among them, Casgevy (exagamglogene autotemcel) inhibits the production of fetal hemoglobin (HbF) by editing the erythroid-specific enhancer region of the BCL11A gene in CD34+ hematopoietic stem cells and progenitor cells [17]; while Lyfgenia (lovotibeglogene autotemcel) is a cell-based gene therapy method, which uses a lentiviral vector to genetically modify hematopoietic stem cells, enabling them to express HbAT87Q - this protein has a function similar to normal adult hemoglobin (HbA) and can effectively reduce the risk of red blood cell sickling and improve vascular blood flow conditions [17].

3.3. Research status

At present, the technology using CRISPR-CAS9 for treating SCA has made significant progress. On December 8, 2023, the US Food and Drug Administration (FDA) officially approved two therapies, Casgevy (exagamglogene autotemcel) and Lyfgenia, and both had previously received multiple qualifications from the FDA, such as priority review, orphan drug, fast track, and advanced therapy for regenerative medicine [18]. Among them, Casgevy was approved by the FDA for the treatment of patients aged 12 and above with recurrent vascular occlusion crises of sickle cell disease [18]. This marks that the application prospect in the field of therapeutic gene (DNA) editing that people have been expecting has officially entered the beginning of realization.

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4. Cystic fibrosis and CRISPR gene therapy

4.1. Genetic target

Cystic fibrosis (CF) is caused by mutations in the CFTR gene, which encodes the cystic fibrosis transmembrane conductance regulator protein. The most common pathogenic mutation is ΔF508 (the deletion of the 508th phenylalanine), accounting for 70% of global cases [19]. This mutation leads to the misfolding of the CFTR protein, preventing it from being transported to the cell membrane, thereby disrupting the function of the chloride ion channel and causing mucus accumulation and multiple organ damage.

4.2. Treatment methods

CRISPR-Cas9 targets and repairs the CFTR gene through two strategies [20]. The first strategy is in vitro editing. Airway or intestinal stem cells from patients are extracted and corrected for the ΔF508 mutation using CRISPR-mediated homologous directed repair (HDR). The repaired cells are then reinfused. The second strategy is in vivo delivery. Adenovirus-associated virus (AAV) vectors are used to directly deliver the CRISPR components to lung epithelial cells, and HDR or base editing techniques are employed to repair the mutations. A clinical trial in 2024 (NCT04734846) showed that the mRNA-CRISPR system encapsulated in lipid nanoparticles can increase the repair efficiency to 40% [19].

4.3. Research status

In 2023, the team from Cambridge University successfully restored CFTR function in organoid models, with a correction rate of 68%, achieving a breakthrough in preclinical research. In terms of clinical progress, the VX-522 therapy (CRISPR + AAV) developed by Vertex Pharmaceuticals in the United States has completed phase I trials, significantly improving the lung function of patients (the FEV1 indicator increased by 15%). However, the pulmonary delivery efficiency is still limited by the mucus barrier, and the long-term safety needs further verification [21].

5. Hemophilia B and CRISPR gene therapy

5.1. Genetic target

Hemophilia B is caused by a mutation in the F9 gene on the X chromosome, resulting in a drawback of clotting factor IX (FIX). Approximately 80% of patients carry point mutations (such as missense mutations c.676C>T), which reduce the activity of FIX to less than 1% of the normal level [19].

5.2. Treatment methods

The core strategy involves in vivo gene editing, specifically liver-targeted repair. CRISPR-Cas9 is delivered to liver cells via lipid nanoparticles (LNP), and a functional fragment is inserted in the promoter region of the F9 gene, restoring the expression level of FIX to the therapeutic level (>15%). Additionally, attention should be paid to the integration of the safe sites, and the corrected F9 cDNA is inserted into the "safe harbor" site in the genome (such as AAVS1) to avoid random insertion of oncogenic risks [20].

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5.3. Research status

In the 2023 rhesus monkey experiment, a single injection of CRISPR-LNP maintained FIX activity at over 30% for 6 months, demonstrating the success of the animal model. In 2024, the data from the Phase I/II trial of the Chinese ET-001 therapy (NCT05664741) showed that 97% of patients had their FIX activity restored to 30%-50%, without the need for regular infusion of coagulation factors. This can be regarded as a milestone in clinical practice to some extent [19]. However, it also faces some challenges, with significant differences in editing efficiency among individuals (10%-60%), which is related to the rate of liver cell regeneration [21].

6. Challenges and optimization strategies

6.1. Technical challenges

The gene editing technology faces two core challenges: off-target effects and delivery efficiency. In terms of off-target effects, the non-specific cutting of the Cas9 enzyme may cause mutations at non-target sites in the genome. If such mutations occur in regions of tumor suppressor genes or oncogenes, they will significantly increase the risk of cancer. For example, in the treatment research of SCA, the off-target rate was once as high as 0.1%, posing a potential threat to the safety of the treatment. In terms of delivery efficiency, during the in vitro editing of stem cells, the cell survival rate can only maintain at 30%-50%, severely restricting the therapeutic effect. When delivered in vivo, the system using the adeno-associated virus (AAV) as the carrier is prone to be cleared by the body's immune system. Due to the presence of neutralizing antibodies in some populations of the AAV, the carrier cannot effectively reach the target cells [22].

6.2. Safety and ethical challenges

Reproductive cell gene editing has sparked significant ethical controversies due to its inheritability. Such editing permanently alters the composition of the human gene pool and violates the core principle of the "Oviedo Convention" that prohibits genetic modification of reproductive cells. From the perspective of medical equity, the high cost of gene editing therapies exacerbates health inequality. For instance, the single treatment cost of the SCA therapy Exa-cel exceeds 2 million US dollars, and only a few groups can afford it, making advanced treatment technologies inaccessible to ordinary patients.

6.3. Technical optimization

Regarding the off-target issue, breakthroughs have been made in high-fidelity enzyme variant technology. For instance, the off-target rate of HypaCas9 is 100 times lower than that of the wild-type Cas9 [22], and it has been successfully applied in the research of cystic fibrosis treatment, significantly improving the editing accuracy. In terms of the optimization of delivery systems, non-viral vectors have shown great potential. Among them, ionizable lipid nanoparticles (iLNP) can efficiently break through the physiological barriers of the lungs and increase the editing efficiency by 3 times [20], giving a new path for the treatment of lung-related diseases.

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6.4. Safety and ethical optimization

To regulate the application of gene editing, the "Framework for Governance of Human Genome Editing" issued by the WHO explicitly requires that all related clinical trials must undergo public registration. This aims to enhance the safety of research through transparent regulation and promote the establishment of a unified global regulatory framework [23]. At the technical level, reversible editing technology has become an important breakthrough direction. For instance, the epigenetic editing tool CRISPR off can achieve therapeutic purposes by temporarily regulating the expression state of genes, avoiding permanent changes to the DNA sequence, and having more advantages in terms of ethics and safety.

7. Conclusion

This study systematically summarizes the breakthrough progress of CRISPR-Cas9 in the treatment of three single-gene genetic diseases: SCA, cystic fibrosis, and hemophilia B. It clarifies that by precisely targeting the pathogenic genes such as HBB, CFTR, and F9, it has achieved remarkable results in preclinical and clinical trials, such as enabling patients with SCA to break free from the dependence on blood transfusion, improving the lung function of patients with cystic fibrosis by 15%, and restoring the coagulation factor activity of patients with hemophilia B to the therapeutic level. This marks the transition of gene editing from proof-of-concept to clinical application. The core significance of this research lies in providing a feasible scientific path for the complete cure of genetic diseases. By comparing three treatment strategies for these diseases, it was found that in vitro stem cell editing is applicable to hematological diseases, while in vivo targeted delivery can overcome the limitations of solid organs. The innovative proposals such as "reversible editing" and "layered pricing" align with the argument in the introduction that "gene editing should balance efficiency and fairness", providing new ideas for balancing technical risks and ethical controversies, and also offering a reference for establishing the precision medicine paradigm. However, the current research has limitations. The differences between animal models and human physiology affect the clinical application of some optimization strategies, and the ethical framework does not fully cover global cultural diversity. Future research should focus on three key areas: developing cross-organ delivery systems to expand into multiple genetic disease fields; establishing lifelong follow-up databases to assess long-term safety; and, within the framework of the United Nations, formulating ethical consensus to ensure the inclusiveness of the technology, making gene editing a potent tool for humanity in combating genetic disorders.

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